LYSIS OF THROMBI PRODUCED BY SODIUM MORRHUATE IN THE FEMORAL VEIN OF DOGS BY HUMAN PLASMIN (FIBRINOLYSIN)*

EUGENE E. CLIFFTON, M.D., CARLO E. GROSSI, M.D. AND DOLLY CANNAMELA, B.A.

NEW YORK, NEW YORK

FROM THE ANDRE AND BELLA MEYER SURGICAL PHYSIOLOGY LABORATORIES OF THE DIVISION OF EXPERIMENTAL SURGERY, SLOAN KETTERING INSTITUTE AND THE DEPARTMENT OF SURGERY, MEMORIAL CENTER FOR CANCER AND ALLIED DISEASES, NEW YORK

THROMBOSIS AND embolism remain one of the serious complications and sources of morbidity and mortality in patients subjected to surgery, and in the many diseases of the older age groups, despite extensive studies of their mechanism of production and methods of control.

To the present time, treatment of intravascular thrombosis has consisted of methods of preventing embolism or further thrombosis by ligation of veins or by the use of chemical agents such as heparin, dicumarol, tromexan and others. No method of lysis of thrombi already formed has as yet been established, although recent studies in experimental animals with streptokinase,[8] and in animals and patients with trypsin,[7] have been reported.

With the preparation and partial purification of human plasminogen (Profibrinolysin) from Fraction III of human plasma,[9] a material theoretically ideal for this purpose has been made available for investigation. We have found this material, in very low concentrations, to be actively fibrinolytic *in vitro;*[1] and *in vivo* studies in rabbits and dogs[2] have shown it to be relatively nontoxic and nonlethal in doses much larger than those producing marked physiological effects. Previous studies have shown conclusively that injection of this material into the general circulation will result in lysis of intravascular thrombi in the veins of the rabbit's ear.[6] The results of our studies on the lytic effect of this material on experimentally produced thrombi in the femoral vein of dogs are reported in this paper.

In the study of intravascular lysis of thrombi in animals, one of the problems has been the establishment of an effective reproducible method of producing thrombi. In general, it may be said that in experimental animals, no satisfactory method of production of thrombi, other than by severe mechanical or chemical trauma to blood vessels, has been reported. Although Wessler recently reported production of small thrombi in isolated venous segments,[16, 17] these were not solid, adherent thrombi. Thrombosis has been produced in experimental animals by mechanical trauma,[11, 12] suppurative infection,[14] chemical irritants,[5, 12, 18, 19] and by the injection of thromboplastic substances.[3, 12] Of these, sodium morrhuate has been most consistent.[5, 18]

In previous experiments with rabbits,[6] we were able to produce thrombi with bovine thrombin, but this was not 100 per cent successful. Thrombosis was produced in distant organs and the thrombi were not sufficiently persistent. In dogs, we have been unable to produce intravenous clots

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FIG. 1

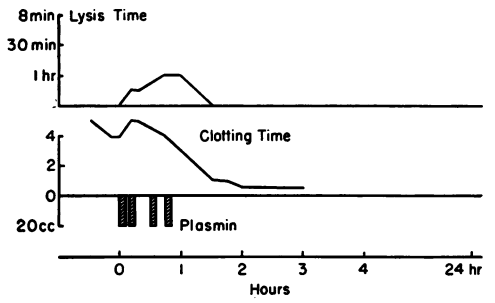
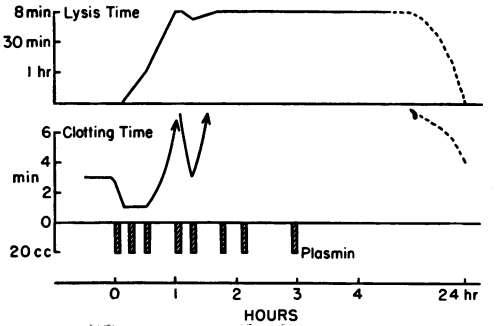


FIG. 2



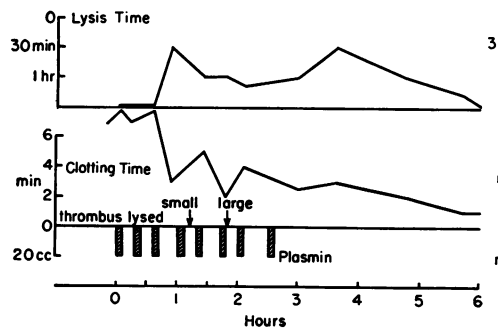


FIG. 3

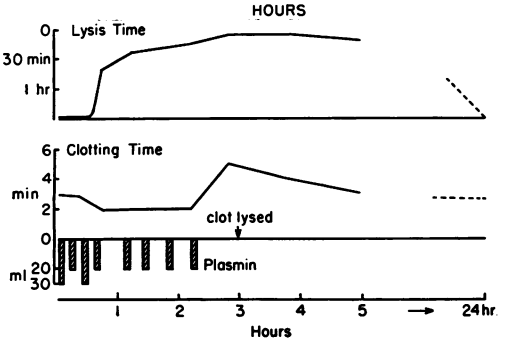


FIG. 4

FIG. 1. Changes in the clotting time and the spontaneous *in vitro* clot lysis of whole blood in normal dog No. 8, to which 80 ml. (2.8 mg./Kg.) Plasmin were given. In this, as in all subsequent graphs, the abscissa represents the time in hours, and there are three sets of ordinates: One is the amount (0 to 20 ml. inverted) of plasmin in milliliters given (each full column indicates 20 ml. of plasmin); the second is in minutes for the clotting time (Lee White method); and the third is the time in minutes in which spontaneous *in vitro* lysis of the blood clot appeared. In this latter (upper) graph the time is inverted, since the shorter the time of lysis, the greater the lytic activity.

FIG. 2. Changes in the clotting time and the spontaneous *in vitro* clot lysis observed in normal Dog No. 9, to which 160 ml. of plasmin were given. Note that the clotting time initially dropped, then as the lytic activity of the blood increased markedly, the blood became incoagulable. This occurred after one hour. The next day the clotting time and lytic activity were normal.

FIG. 3. Changes observed in Dog No. 2. Two thrombi had been formed, one in the femoral vein and one in a tributary. The abscissa and the ordinates are similar to the previous graphs. The time of lysis of the clots are indicated by the arrows marked small and large. The small thrombus lysed in 30 minutes and the large one in the common femoral vein in one hour after the appearance of an active lytic system in the blood.

FIG. 4. Changes observed in the clotting time and the *in vitro* spontaneous lytic activity of the blood in Dog No. 12, in which plasmin was given 24 hours after the formation of a thrombus in the femoral vein. The abscissa and ordinates are as in the previous graphs. Note that the clotting time changed very little in this animal. A total of 180 ml. of plasmin were given and the thrombus lysed in three hours. As in the previous graphs, the arrow indicates the time of clot lysis.

with thrombin with any consistency. With sodium morrhuate, however, we have produced adherent clots in 100 per cent of the rabbits[6] and the dogs treated as described in methods to follow.

Many studies have been made of the effect of known drugs in causing lysis of preformed clots in experimental animals. Heparin has been shown to have some thrombolytic effect in early thrombi.[11, 13, 14] Tromexan, administered over a long period of time, has been found to promote recanalization of thrombi in rabbit's ear veins,[18] in the femoral vein of dogs,[5] and in the femoral artery of rabbits.[19] Trypsin, administered over a long period of time, has been

shown to lyse thrombi in veins of rabbits and dogs.[7] This is difficult to explain, since it is common experience that trypsin produces intravascular thrombosis.[4, 15] It is possible that this lytic effect of trypsin is due to its activation of plasminogen, since this is a known effect of trypsin.[10] Streptokinase, the best known activator of human plasminogen, has also been reported to produce lysis of clots in rabbits when used in large doses over a long time interval.[8]

TABLE I. *Number of Animals Used and Maximum Amount of Human Plasminogen/Kilogram of Body Weight Given Intravenously During Experiment.*

	Number	Maximum Amount Plasminogen/Kg. Body Weight	Mortality
Normal Dogs.....	6	4.9 mg./Kg.	0
Dogs with Thrombus.	9	5.1 mg./Kg.	0

MATERIALS AND METHODS

Animals

Fifteen adult mongrel dogs in good health and kept under routine laboratory conditions.

Materials

1. Nembutal Veterinary: 65 mg./ml.
2. Sodium Morrhuate: 5 per cent solution
3. Streptokinase (SK-SD)*: Streptokinase 100,000 units, Streptodornase 25,000, dissolved in 10 ml. of sterile saline. Two ml. of this solution were added to every 20 ml. of plasminogen used. Therefore, 20,000 units streptokinase and 5000 units streptodornase were present in each 22 ml. of plasmin solution.
4. Plasminogen: Prepared in our laboratory by a previously reported method[1] from Fraction III of human plasma. Concentration used: 0.35 mg./ml. activated with 1 ml. SK-SD to 10 ml. plasminogen.

Methods

The femoral vein was exposed in the dog by a standard incision parallel to the vein. Under direct vision, bulldog clamps were placed on the vein and its tributaries, isolating a 3 cm. segment. The blood was aspirated and 0.3 ml. of 5 per cent sodium morrhuate was then injected into the lumen. Ten minutes later the distal clamp was removed to permit filling of the vein with blood, and then reapplied. The clamps were left in place for two hours and then a partial flow of blood was permitted by releasing the distal clamp. A firm adherent thrombus, which completely occluded the vein and distended it, was formed in all animals. In eight dogs, this occurred within two to three hours, and in the other, within 24 hours. The thrombosis extended well beyond the area treated to include distal tributaries, with a total thrombus length of at least 6 cm. In several animals two thrombi were formed, a large one in the common femoral vein and a small one in a small tributary of the superficial femoral vein.

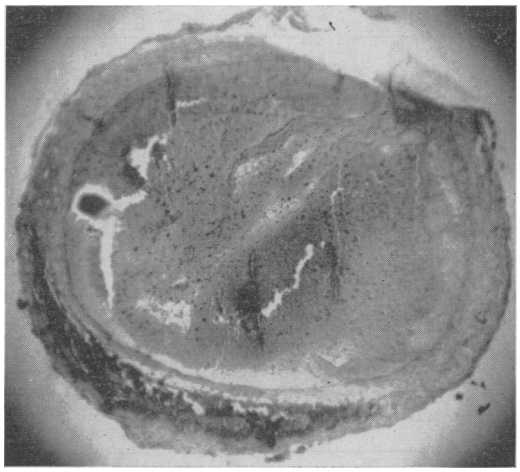


FIG. 5. Femoral vein of a control dog in which a thrombus had been produced with sodium morrhuate three hours before.

At varying times after the thrombus formation, 30 minutes in two animals, one hour in two others, and 24, 48 and 72 hours in five others, plasmin was injected into a foreleg vein. Plasmin (active fibrinolysin)

* Varidase (Lederle Laboratories).

TABLE II. *Dogs in Which Thrombus Was Formed in the Femoral Vein.**

No. Dog	Wt. Kg.	Thrombus Length	Plasmin Administration: Time after formation thrombus	Plasmin Administration: Amount	Plasmin Administration: Duration of Admin.	Lysis Thrombus Time	Plasmin per Kg.	Side Effects
2	11	Small 1 cm. Large 2 cm.	30 min.	49 mg.	2.5 hrs.	55 mins. 1 hr. 50 min.	4.9 mg.	None
3	10	Small 1 cm.	30 min.	7 mg.	8 min.	None	0.7 mg.	None
7	12	Large 3 cm.	1 hour	42 mg.	3 hrs. 20 min.	3 hrs. 15 min.	3.5 mg.	Apnea Temporary
10	11	Small 1 cm. Large 1 cm.	1 hour	40 mg.	2 hrs.	1 hr. 2 hrs. 15 min.	3.5 mg.	None
11	12	Large 8 cm.	72 hours	35 mg.	1 hr. 30 min.	3 hrs. Complete temporary	2.8 mg.	None
12	15.4	Large 8 cm.	24 hours	63 mg.	2 hrs. 30 min.	3 hrs. Still patent by X-ray 24 hrs. later	4.0 mg.	Wound ooze temporary
13	6.8	Large 7 cm.	24 hours	35 mg.	2 hrs.	2 hrs.	5.1 mg.	Wound ooze
14	11.8	Large 6 cm.	24 hours	42 mg.	3 hrs.	3 hrs.	3.5 mg.	None
15	11.3	Large 6 cm.	48 hours	49 mg.	4 hrs.	4 hrs.	4.4 mg.	Wound ooze

*List (by number) of dogs given Human Plasmin after a thrombus had been formed in the femoral vein. The body weight in kilograms, the time and lengths of the thrombus formed in the femoral vein, the time after formation that the Plasmin was given, the amount and rate of plasmin administration, the amount in milligrams of plasmin per Kg. body weight and the time it took for thrombus lysis to occur are recorded. Side effects such as oozing from the wounds are also noted.

TABLE III. *Dogs Showing Occurrence of Lysis of Thrombus.**

	Plasmin Given in mg.	Time Plasmin Given in Hrs.	Time needed for lysis	Plasmin per Kg. body weight	Mortality
Maximum	63 mg.	4 hrs.	4 hrs.	5.1 mg.	0
Minimum	35 mg.	1½ hrs.	55 mins.	2.8 mg.	0
Average	44 mg.	2½ hrs.	2¾ hrs.	4.0 mg.	0

*List of the maximum, minimum and average amounts of plasmin in mg. given to the eight dogs where lysis of the thrombus occurred in the femoral vein. The time period of administration, the time needed for lysis and the amount of plasmin per Kg. body weight are also recorded.

was used in the concentration of 0.35 mg. per ml.

Before injection of plasmin was begun, the wound in the thigh was reopened to observe the extent and type of thrombus. In four animals, phlebography, using Urokon (20 ml.) or 35 per cent Diodrast (20 ml.), was done before and after lysis. In two other animals, sections were taken before treatment and after the lysis in the vein for histological demonstration. A tributary vein was used for the preliminary section, and the main trunk for the final section. A vein from a control animal was used for comparison.

The condition of the wounds was observed and any unusual bleeding was noted and described.

The coagulation time of the blood drawn from a distant vein was determined by the Lee-White method, and the spontaneous whole blood clot lysis time was also determined at standard intervals.

The healing of the wound in the thigh after treatment was observed daily until complete. No abnormalities of healing were noted.

RESULTS

The maximum quantity of plasmin used in this series of experiments was 5.1 mg./Kg. in the animals in whom thrombi had been produced (Table I). None of these animals showed any serious reaction other than mild to moderate oozing of blood from the fresh wounds. All animals

survived in good health until the time they were sacrificed, up to six months after treatment.

Studies of Clotting and Lysis of Clots in Vitro. In the group of six animals, where no sodium morrhuate was injected and no thrombus was produced, the effect of activity of the blood. Spontaneous lysis appeared within 30 minutes of the beginning of the administration of plasmin in the usual quantities, remained elevated for at least one hour after the last dose of plasmin, and returned to normal activity within two to 24 hours.

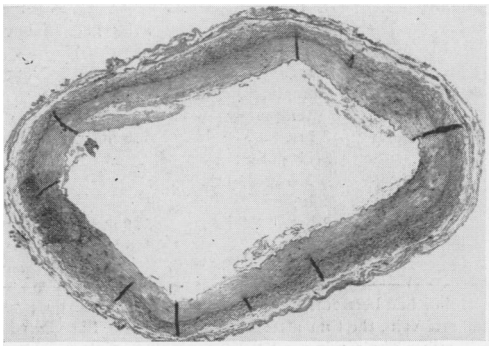


FIG. 6

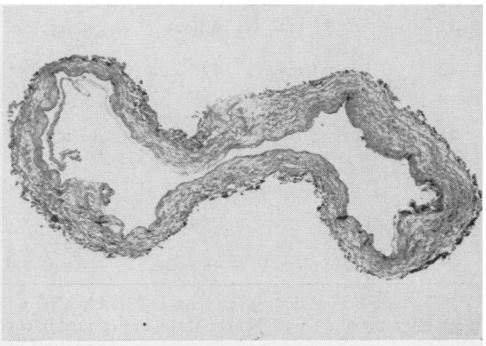


FIG. 7

FIG. 6. Tributary of the femoral vein of Dog No. 10, in which a thrombus had been produced with sodium morrhuate one hour before the administration of plasmin. Biopsy of vein taken after lysis had been produced by the plasmin. Note the small fragment still attached to the intima.

FIG. 7. Femoral vein of Dog No. 10, in which a thrombus had been produced with sodium morrhuate one hour before the administration of plasmin. Biopsy of vein taken after lysis of the thrombus.

plasmin on the clotting mechanism of the blood was studied. The anesthetised animal did not show any unusual reaction or toxic effect of the plasminogen. However, in those animals where there was a fresh operative wound in the femoral area, created for the purpose of venipuncture or observation of the thrombosed vein, there was a mild to moderate oozing of blood from the raw surface when over 3 mg. of plasmin/kilogram had been administered. This ooze was temporary, ceased when the wound was sutured, and in all instances the wound healed well without hematoma formation or infection.

In most animals the clotting time lengthened soon after the administration of plasmin, and remained elevated for the duration of the experiment. In a few animals the clotting time was shortened. The *in vitro* spontaneous whole blood lysis time was used as an index of the fibrinolytic

Observations on two dogs are given in detail, as an example of the results in all animals. Dog No. 8 (control) was given 80 ml. (2.8 mg./Kg.) of plasmin. A marked drop in the clotting time from 5 minutes to 30 seconds was noted and there was a concomitant appearance of spontaneous lytic activity in the blood. This spontaneous lytic activity appeared within 15 minutes of the onset of the first injection of 20 ml. of plasmin, and persisted until about 45 minutes after the administration of plasmin was discontinued (Fig. 1). A mild ooze of blood from the femoral incision was noted, which ceased within 45 minutes, and the dog was well afterwards.

The second control animal (No. 9) was given 160 ml. (4.9 mg./Kg.) of plasmin. In this animal, the clotting time was first shortened as in dog No. 8, then with rapid injection of a larger quantity of plasmin, this trend was reversed. After 60 ml. of

plasmin had been given in 40 minutes, the clotting time rose very sharply and the blood became incoagulable and remained so for the remainder of the experiment except for one drop to a normal clotting time (Fig. 2). The spontaneous lytic activity of the blood increased very rapidly, and after one hour the lysis of the clot was immediate. This high fibrinolytic activity persisted for the remainder of the experiment, but the next day it had returned to normal, as had the clotting time. This animal also recovered in a normal fashion and appeared alert, active and normal.

These findings confirm our previously reported results of a marked increase of fibrinolytic activity of the eu-globulin fraction of the blood of dogs receiving plasmin intravenously.[2]

The same studies of clotting time and clot lysis time were made on several animals in which thrombi were produced by sodium morrhuate.

Figure 3 represents the changes observed in an animal (Dog. No. 2) where two thrombi had been formed in the femoral vein and a tributary. The small thrombus lysed in 30 minutes and the large one in one hour after the appearance of an active lytic system in the blood. This animal also showed a shortened clotting time, as had Dog No. 8, at the same time as the increased lytic effect was observed.

The effect of plasmin in Dog No. 12, which had a 24-hour old thrombus in the femoral vein, is shown in Figure 4. The clotting time was first shortened slightly, then rose to above normal. The thrombus lysed in three hours after the animal had received 4.0 mg. plasmin/Kg. body weight. There was oozing of blood from the wound in the thigh of this animal, but this ceased spontaneously, and the next day his clotting time was normal and spontaneous lysis of the *in vitro* clot was absent.

Lysis of Thrombi in the Femoral Vein. In all eight dogs, where an adequate amount of plasmin was used, lysis of the thrombus in the femoral vein occurred (Table II). From this table one can see that when there were two thrombi present, one small and one large, the small one lysed first. The average amount of plasmin used was 4.5 mg. per kilogram of body weight. The minimum amount of plasmin which resulted in lysis of a clot was 2.8 mg./Kg., and the maximum needed was 5.1 mg./Kg. Lysis occurred in one to four hours, depending on the rate of injection,

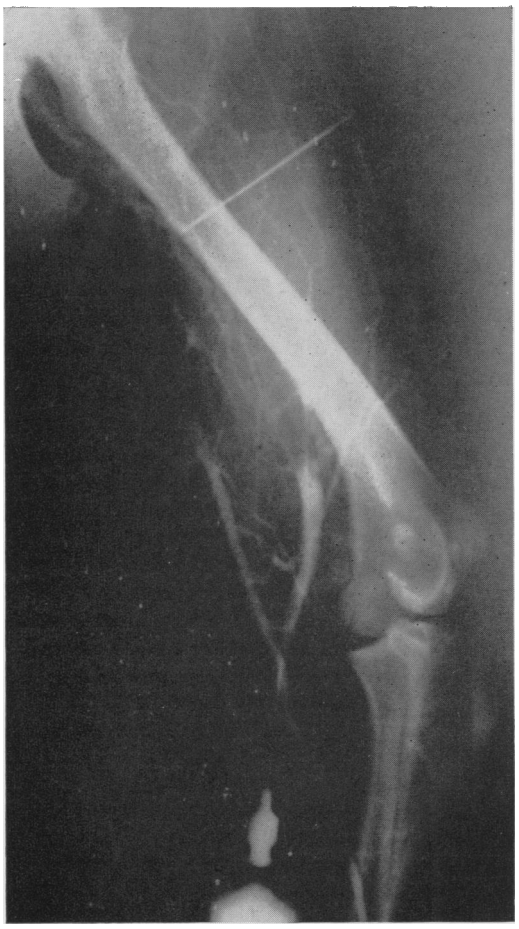


FIG. 8. Phlebogram of the thigh of Dog No. 12. (Contrast medium: Urokon.) This phlebogram was taken 24 hours after the production of the thrombus in the femoral vein. Note the complete obstruction to the flow of contrast medium in 2 veins and the many collateral channels. Hypodermic needle marks the site of the main thrombus in the femoral vein.

and on the age and size of the thrombus. In the one animal where the thrombus did not lyse, only 0.7 mg. per kilogram of body weight was given, an amount of plasmin obviously insufficient to effect a clot lysis *in vivo*. Incidentally, this was one of the two short term (30 min.) thrombi in which lysis was attempted. From these few experiments, there would appear to be little difference between thrombi one hour old, and thrombi 24, 48 or 72 hours old, so far as their response to plasmin is concerned. Actually the 72 hour old clot was lysed completely, albeit only temporarily, by the smallest amount (2.8 mg./Kg.) of plasmin to produce complete lysis of a thrombus.

In Table III are given the general statistics for the eight animals in which intravascular lysis occurred.

In one animal a thrombus (incidentally the oldest [72 hours]) was lysed with as little as 2.8 mg. plasmin/Kg., while the maximum amount needed for a 24-hour-old clot was 5.1 mg. plasmin/Kg. The thrombi in the small veins, 4 mm. in diameter and 1 to 2 cm. long, lysed in about one hour, while those in the common femoral vein 8 mm. diameter and 5 to 8 cm. long required 2 to 4 hours. There was no mortality from the use of the enzyme in these animals.

The patency of the femoral veins before and after lysis of the clot was determined by direct observation of blood flow through the vein in all cases. In three cases this was supported by biopsy of the vein and in four animals by phlebograms. Phlebograms were done before and after lysis of the thrombus,

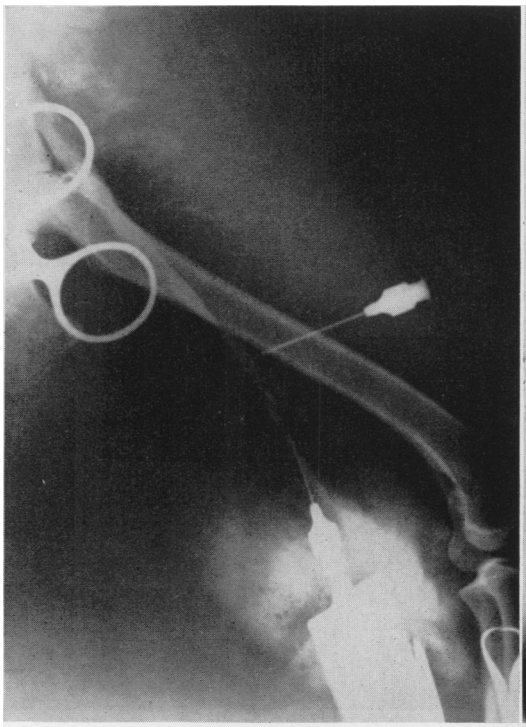


FIG. 9

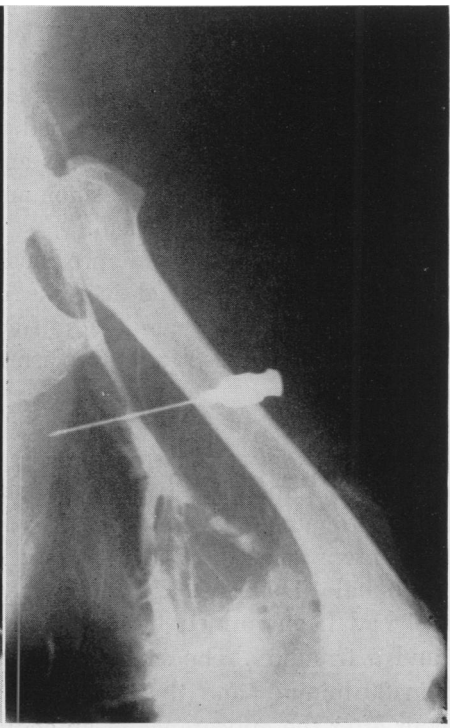


FIG. 10

FIG. 9. Phlebogram of the thigh of Dog No. 12, taken two hours after lysis had occurred in the femoral vein. Note the free flow through the vein. Unfortunately the needle had been inserted higher than in the control, so this was repeated 24 hours later (Fig. 10).

FIG. 10. Phlebogram of the thigh of Dog No. 12, taken 24 hours after the lysis of the femoral vein thrombus had occurred. Note persistence of the lysis and the free flow through the vein.

and 24 hours and two weeks after lysis, in order to determine whether a thrombus had reformed.

Figure 5 is a photomicrograph of a control vein with a thrombus formed by the use of sodium morrhuate. Figure 6 is a photomicrograph of the small vein in Dog No. 10 after lysis had taken place following the use of plasmin. A photomicrograph of the common femoral vein, taken after the lysis of the thrombus in this larger vein of the same animal, is shown in Figure 7. In both these sections it can be seen that the vein has become completely patent, with a few fragments of thrombus still adherent to the intima.

The use of phlebograms gave a clear demonstration of the state of obstruction or patency of the femoral vein. Figure 8 is a phlebogram of the femoral vein of Dog No. 12, 24 hours after production of the thrombus in the left femoral vein. The complete block of flow of contract medium in the common femoral vein is clearly demonstrated. This animal was then given 4 mg. plasmin/Kg. over a period of two and one-half hours, and the thrombus appeared lysed at the end of three hours. A phlebogram was made two hours later, and the free flow of contrast medium through the previously obstructed area can easily be seen (Fig. 9). The wound was then sutured, and 24 hours later another phlebogram was taken, which demonstrates that the femoral vein was still patent and that the thrombus had not yet reformed, in spite of the absence of spontaneous lysis of the *in vitro* blood clot (Fig. 10). A fourth phlebogram in this animal two weeks later showed obstruction of the femoral vein at the site of the original thrombus formation. This dog was sacrificed, and autopsy revealed that the femoral vein lumen was patent, but compressed by dense scar tissue around it, the result of the sodium morrhuate injected.

In Dog No. 13, plasmin was given when the thrombus in the femoral vein was 24 hours old. This thrombus was 7 cm. long and extended down into the branches of the femoral vein. A phlebogram showed complete occlusion of the common femoral vein before the administration of plasmin (Fig. 11). The animal was given 5.1 mg. plasmin/Kg. body weight over a period of two hours, and at the end of this time the thrombus lysed. The only side effect was some mild oozing from the wound, but this stopped as soon as the wound was sutured, and the animal recovered in normal fashion. The phlebogram, taken after the lysis had occurred, clearly demonstrated the free flow of contrast medium through the previously obstructed femoral vein (Fig. 12).

Most of the animals were sacrificed one to three months after the experimental lysis of thrombi, and only one of these showed any evidence of old hemorrhage or any other unusual findings. In this animal (Dog No. 12) an old hematoma (4 cm. in diameter) was found in the gastric wall, along the greater curvature in the midportion of the stomach. A few focal hemorrhagic areas 3 mm. in diameter were noted in the myocardium of this same animal. This animal had been asymptomatic and clinically well. In none of the animals was there any evidence of embolic phenomena or pulmonary infarcts, either in the course of the experiments, during the survival periods of the animals, or in the complete autopsies.

Lysis of an Arterial Thrombus. In one animal a thrombus was formed in the femoral artery which had been cannulated for the collection of blood samples. This thrombus lysed when 3.5 mg. plasmin/Kg. body weight had been administered to the dog over a period of two hours. This is the only instance of an intra-arterial thrombus in this series. One other animal treated in another laboratory had a femo-

ral artery thrombus produced with sodium morrhuate, which lysed after the administration of plasmin.*

DISCUSSION

The results reported in this paper, lysis of thrombi up to 72 hours old within the femoral vein of the dog, in from one to four hours, support the previously reported results with thrombi in the marginal vein of the rabbit's ear. In addition, it has been shown that arterial thrombi react in the same way to systemic administration of activated human plasminogen (plasmin).

Complications were few and not serious. One might expect that bleeding would be a serious complication, since the blood collected lyses very rapidly and, with large doses, becomes incoagulable. However, on only one animal was there evidence at postmortem of any significant hemorrhage, in this case, into the wall of the stomach. The fact that rather heavy wound ooze ceased immediately upon closure of the wound leads one to the probable explanation that this lack of bleeding into tissues is due to the presence of inhibitors in the tissue juices themselves.

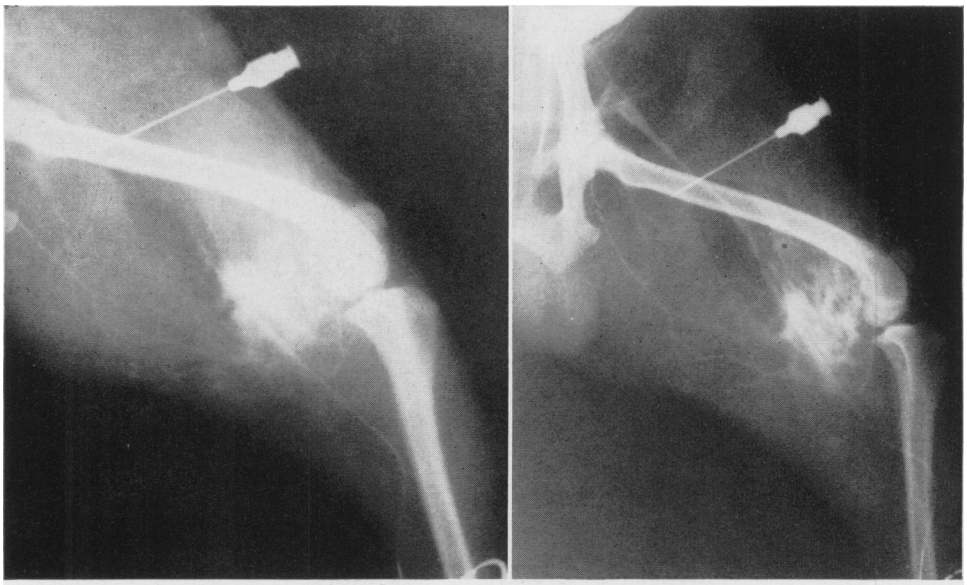


FIG. 11 FIG. 12

FIG. 11. Phlebogram of the thigh of Dog No. 13. This phlebogram was taken 24 hours after the formation of a thrombus in the left femoral vein. The hypodermic needle marks level of the cephalad end of the thrombus. Note the complete obstruction of the femoral vein.

FIG. 12. Phlebogram of the thigh of Dog No. 13, taken after the lysis of the thrombus in the femoral vein. Note the free flow of the contrast medium through the previously obstructed vein. This thrombus was 24 hours old when it was lysed with plasmin.

Another serious difficulty that might be expected with rapid lysis of clots would be the freeing of emboli into the blood stream: this has been shown not to occur. If the material were injected directly into a vein distal to the clot, this could conceivably happen, but with the injection into the general blood stream, the lytic effect must act from the periphery of the clot. This is beautifully illustrated by observing the clots as lysis is taking place. First, the tip of the clot softens and begins to disappear, then the distention of the vein lessens and

* The Surgical Research Laboratory, Cornell University School of Medicine–New York Hospital. This work was supported by a grant from the New York Hospital–Cornell University Medical School joint research funds.

the whole clot becomes softer. Oozing of blood from the needle wounds in the vein now occurs. Then in a few minutes the clot melts away completely, and a free flow of blood is observed in the vein. Even if a small segment of clot should be broken off, the marked lytic activity in the blood, probably greater than at the interface with the main clot, would make quick work of its dissolution.

The re-occlusion of the veins following the loss of the plasmin, we feel, is to be expected, because of the serious trauma to the vein, both chemical and mechanical. The surprising fact is that some stayed open for more than 24 hours, as shown by venograms. The fact that at postmortem several weeks later the lumen of some of these veins was free of clot, with the obstruction being due to compression of scar from the outside, is to us even more unexpected. In practice, reformation of clots would be less likely because of the absence of severe chemical and mechanical trauma, and further protection could be furnished by the use of anticoagulants.

The fact that this material remains active in the animal organism for only a few hours at most would be an additional safety factor in possible clinical use, in case hemorrhage were a complication. Since no chemical inhibitors are known at present, this ability of the body to inactivate or inhibit the material would have to be depended upon to control its activity. In all animal species studied (dog, rabbit, cat and monkey), this control has been more than adequate.

In all animals studied to date,[2, 6] and in studies of local use of this material in human patients, no serious allergic or anaphylactic manifestation has been observed.

SUMMARY

Thrombi were produced in the femoral veins of nine dogs by the use of sodium morrhuate. In eight of these dogs, the thrombi were completely lysed by general systemic injection of 2.8 to 5.1 mg./Kg. of human plasmin. The one animal in which lysis did not occur received only 0.7 mg./Kg., an obviously inadequate dose. Lysis occurred in one to four hours, and the veins remained patent for at least 24 hours in some animals.

No deaths occurred in 15 dogs studied, and no serious complications occurred. A slight to moderate ooze of blood was evident in fresh wounds, but this ceased with closure of the wounds, and in only one instance was there evidence of bleeding into other tissues. No emboli were apparent.

A marked increase in spontaneous lytic activity was observed in the whole blood of all animals studied. In most animals there was a prolongation of clotting time, although some receiving the smaller amounts showed a shortening of the clotting time.

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