The Enzymatic Dissolution Of Experimental Arterial Thrombi in the Dog by Trypsin, Chymotrypsin and Plasminogen Activators

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THE ENZYMATIC DISSOLUTION OF EXPERIMENTAL ARTERIAL THROMBI IN THE DOG BY TRYPSIN, CHYMOTRYPSIN AND PLASMINOGEN ACTIVATORS [1, 2]

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A number of investigators have undertaken studies on the dissolution of intravascular thrombi and emboli by enzymes capable of effecting the dissolution of blood clots *in vitro* (1–4). Two types of approach have been used; the direct introduction of a proteolytic enzyme capable of digesting fibrin (2, 3), and activation of the naturally occurring proteolytic and fibrinolytic enzyme of mammalian plasma by the injection of a specific kinase (1). This latter approach is based on the observation that plasma contains a proenzyme termed plasminogen (5) or profibrinolysin (6), which, when activated, is converted to plasmin or fibrinolysin, a proteolytic enzyme with an affinity for fibrin (7). The action of plasmin is not limited to fibrin alone (8–11). The natural mechanisms for activation of plasminogen may be through blood (12) or tissue kinases (13, 14). The best known activator of human plasminogen is streptokinase, an extracellular hemolytic streptococcal product (8).

Recent reports have appeared on the successful liquefaction of intravascular clots in rabbit ear veins by the intravenous infusion of large amounts of streptokinase (1); the lysis of venous clots in rabbits and dogs by the intravenous administration of large amounts of trypsin (2); and the dissolution of venous thrombi in rabbits and dogs by injections of purified human plasmin (previously activated *in vitro* by streptokinase) (3).

It is the purpose of this report to describe the effects of the intravenous administration of trypsin, chymotrypsin and activators of plasminogen on the course of an experimentally induced femoral artery thrombosis in the dog. The results obtained suggest that the administration of chymotrypsin, or activation of the animals own plasminogen was associated with a significant increase in clot dissolution. Trypsin administration, however, was observed to have no significant effect on the experimental arterial thrombi.

MATERIAL AND METHODS

Production of experimental arterial thrombus. Femoral artery thromboses were produced in mongrel dogs weighing approximately 10 to 15 Kg. After surgical exposure of the artery a 1 to 2 cm. segment of the vessel was isolated between ligatures. Branches of the vessel were ligated and divided. The isolated segment was then distended with approximately 1 ml. of thromboplastin (Difco) solution.[3] The proximal ligature was removed and blood was allowed to reenter the previously isolated segment, where clotting slowly occurred. After a firm clot formed (usually 5 to 10 minutes), the distal ligature was removed. The artery was observed for another 5 minutes to verify the position of the clot. A black silk marker was loosely positioned about the obstructed vessel to aid in the subsequent identification of the segment. The wound was closed with Michel clips.

Enzyme preparations

Trypsin and chymotrypsin.[4] Preparations of trypsin and chymotrypsin were dissolved in Ringer's solution immediately prior to infusion. For trypsin, the amounts noted in the result section refer to mg. of tryptic activity since the preparations varied in their content of $MgSO_4$. For chymotrypsin, the amounts noted are the actual amounts infused. Infusions of these enzymes were given intravenously at a rate of 1 ml. per minute over a four-hour period using a constant infuson pump.

[1] Presented in part before the 26th Annual Meeting of the Central Society for Clinical Research, October 30th, 1953, Chicago, Illinois.

[2] This study was supported by grants from the Armour Laboratories, and from the Lederle Laboratories Division, American Cyanamid Co.

[3] Prepared as for prothrombin assay (15).

[4] Crystalline preparations of trypsin and chymotrypsin were obtained through the courtesy of Dr. A. H. Holland, Jr. of Armour Laboratories.

Plasminogen activators

Streptokinase or SK.[5] Units of SK are expressed in terms of the Christensen assay procedure (16). Preliminary *in vitro* studies revealed that activation of the plasminogen in dog plasma could be accomplished with approximately 200 units of this preparation of SK per ml. of dog plasma.[6] In the *in vivo* studies to be described, when SK alone was used as an activator, the material was dissolved in 30 ml. of saline and injected intravenously over a 5-minute period.

Human plasminogen preparation plus SK. In vitro studies revealed that the presence of small amounts of preparations of human plasminogen markedly facilitate the activation of animal plasminogen by SK (17). For *in vivo* studies a lyophilized human plasminogen preparation prepared from human plasma Fraction III[7] by the Christensen and Smith technique (18) was used. The preparation contained 4 per cent nitrogen and had a proteolytic assay, following SK activation, of 4.2 casein units per mg. nitrogen (11). Preliminary *in vitro* studies revealed that in the presence of 50 gamma of the above human plasminogen preparation, the plasminogen in 1 ml. of dog plasma was completely activated by only 20 units of SK. For use *in vivo,* the human plasminogen preparation was dissolved in 25 ml. of saline, mixed with 5 ml. of a saline solution containing the appropriate amount of SK, and the mixture injected intravenously over a 5-minute period. Although small amounts of human plasmin (SK activated) were injected with the activator mixture, it was demonstrated, *in vitro,* by adding equivalent amounts of spontaneously activated human plasmin[8] to dog plasma, that the amounts of human plasmin injected into these animals could only account for a very small part of the changes observed.

Other preliminary observations

A number of *in vitro* observations were made on the possible activation of dog plasminogen in plasma by trypsin and chymotrypsin, and on the inhibition of trypsin by plasma. Although trypsin appears to be an activator of partially purified plasminogen (19), studies *in vitro* with trypsin and chymotrypsin failed to reveal any activation of the plasminogen in undilute plasma. The levels of free tryptic activity observed after the addition of trypsin to undilute plasma, was found to represent about 1 to 3 per cent of the enzyme added (up to 2 mg. per ml. plasma), suggesting that the trypsin in undilute plasma may combine reversibly with the plasma inhibitors (20). The type of inhibition observed in undilute plasma is in contrast to the type of inhibition described with dilute plasma or purified systems where specific plasma inhibitors which combine with trypsin in stoichiometric fashion may be demonstrated (21, 22).

Experimental design. For purpose of comparing the effects of each of the preparations used, a standard experimental procedure was followed. Twenty-four hours after the production of the experimental thrombus, treatment with one of the agents was begun, in doses referred to in the results section. Single treatments consisting of 4-hour infusions of trypsin or of chymotrypsin, or an intravenous injection of SK, or SK plus human plasminogen were given on two successive days. Four days following the initiation of therapy the previously thrombosed vessel was exposed, studied and biopsied.

Animals receiving trypsin and chymotrypsin infusions were maintained under pentothal anaesthesia during the infusion period. When injections of SK plus plasminogen, or SK alone were given, no anaesthesia was employed.

Evaluation of results of clot dissolution. Results were classified into successes or failures depending upon whether an effective circulation was restored in the previously thrombosed vessel as judged by gross inspection, presence of a pulse, back bleeding when the artery was divided proximal to the previously thrombosed area, and by gross and histological examination of the biopsied specimen. Failures included all instances in which the lumen remained obstructed by a thrombus even though the thrombi may have shown evidence of partial lysis presumably from the enzyme treatment. Successes included all instances in which adequate circulation was restored even though histological examination occasionally revealed small nonobstructive fragments of thrombus still adherent to the endothelium. No attempt was made to grade the clots for partial lysis since this would require absolutely uniform clots.

Biochemical studies. Biochemical studies, carried out on venous blood removed *before, during,* and *after* the treatment with each agent included the Lee White clotting time, and the subsequent lysis time of the clotted specimen; fibrinogen (23); prothrombin (15);[9] proteolytic activity (24); plasminogen (16); and the presence of an active fibrin lysing system in the dogs plasma (25). On a number of instances the presence of circulating plasmin or trypsin was tested for by the hydrolysis of p-toluene sulfonyl l-arginine methyl ester (26, 27), and the presence of circulating chymotrypsin tested for by the hydrolysis of acetyl l-tyrosine ethyl ester (27).

[5] A commercial preparation (Varidase) Lot No. 7-1089-297A, containing only small amounts of contaminating inhibitor, was obtained through the courtesy of Dr. J. M. Ruegseggar of Lederle Laboratories.

[6] We refer here to that amount of SK which when added to 1 ml. of dog plasma, prior to clotting the mixture with thrombin, produced clot lysis within 30 minutes. The test system consisted of 1.0 ml. dog plasma plus 0.1 ml. SK solution plus 0.1 ml. Bovine Thrombin solution containing 1 NIH unit.

[7] Obtained from the American Red Cross, and through the courtesy of Dr. T. D. Gerlough of E. R. Squibb & Sons.

[8] Obtained through the courtesy of Dr. D. M. Surgenor, Harvard Univ. Lab. of Physical Chemistry (Fraction III_s).

[9] Modified by the addition of 0.1 ml. of 2.5 per cent clottable fibrinogen in saline to test system.

RESULTS

Natural course of the experimental thrombus

Femoral artery thromboses were produced in 29 animals which received no form of therapy. Upon examination 5 days later, 26 of the clots were still *in situ* and exhibited the earliest stages of organization. Three (10 per cent) of the clots had spontaneously disappeared. In several other animals surgical exposure of the vessels was not carried out until two weeks after the formation of the thrombus. At this time the clots were still present and exhibited evidence of advancing organization.

Trypsin treated animals

In 22 instances trypsin was infused at a dose of 16 to 33 mg. per Kg. Only two of these clots dissolved (9 per cent of the total).

Typically, a trypsin infusion caused a prompt rise in circulating proteolytic activity, which per-

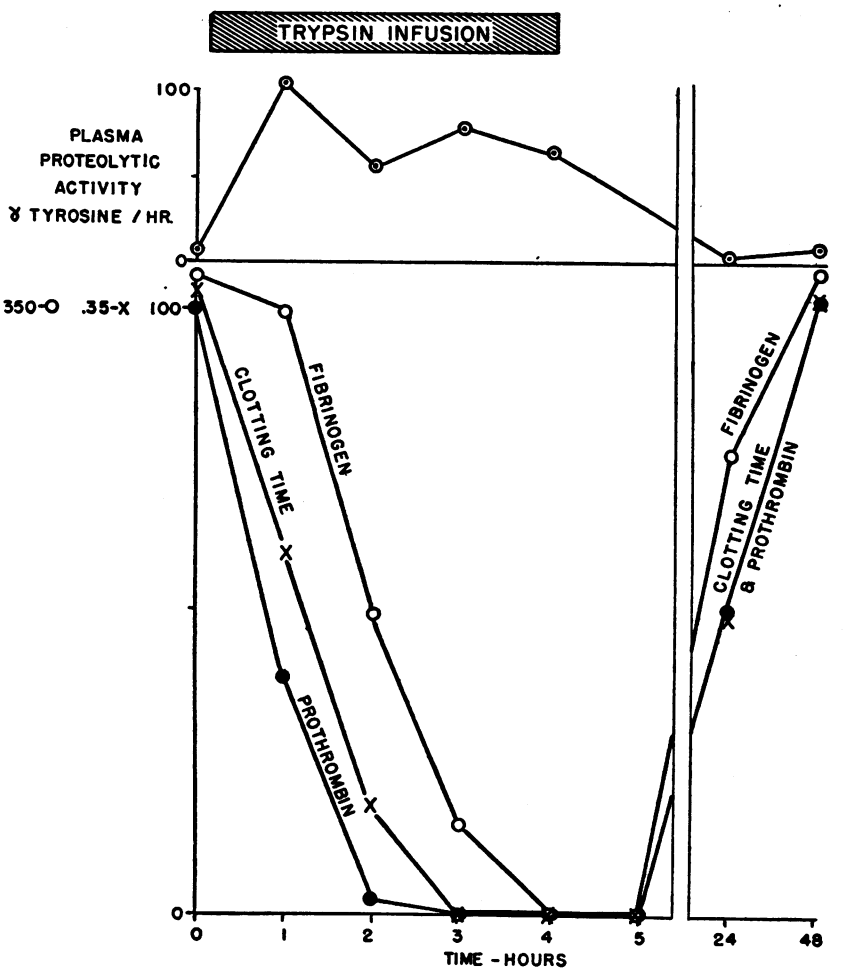

FIG. 1. THE EFFECTS OF A FOUR-HOUR INTRAVENOUS INFUSION OF 300 MG. OF TRYPTIC ACTIVITY INTO A 12 KG. DOG

The abscissa represents time in hours. The ordinate is divided into two parts; at the top is shown the concentration of plasma proteolytic activity (gamma acid soluble tyrosine released per hour); in the lower section is shown the reciprocal of the whole blood clotting time (a measure of the clottability of the blood) X—X, the prothrombin concentration (per cent control) ●—●, and fibrinogen concentration (mg. per cent) O—O. The ordinate is designed so that at 100 per cent prothrombin, the reciprocal of the clotting time is 0.35, and the fibrinogen concentration is 350 mg. per cent.

sisted during the period of infusion and returned to the control value by the next day (Figure 1). Prothrombin rapidly fell to zero and the blood became incoagulable. Fibrinogen also disappeared from the circulation but at a somewhat slower rate. Although these latter effects were of a transient nature, 48 hours were required for a complete return to the control value.

Activation of the animals plasminogen *in vivo* could not be demonstrated. Plasminogen levels were found to remain constant throughout the infusion periods and for the following 24 hours. When the blood became coagulable, subsequent lysis of the clotted blood was not observed

Three animals were infused with small doses of trypsin (0.4 mg. per Kg.). These amounts are higher than those recently recommended by Innerfield, Angrist, and Schwarz for clinical use in man (28) but were not accompanied by any measurable proteolytic activity or changes in fibrinogen concentration, and had no effect on the experimental thrombi.

To exclude the possibility of dissolution of the clot and then subsequent reformation, three animals were infused with 25 mg. per Kg. trypsin in the usual manner, but were also given heparin injections every six hours from the onset of trypsin therapy. In all three instances a firm obstructing thrombus was found at examination.

Chymotrypsin treated animals

Chymotrypsin infusions at a dose of 12 to 30 mg. per Kg. restored the circulation in 13 of 25 thrombosed vessels (55 per cent). In the majority of failures, the thrombus appeared to be much softer than that in the control or trypsin treated animals.

Characteristically, circulating proteolytic activity rapidly rose at the beginning of the chymotrypsin infusion, and then tended to fall slowly during the remainder of the infusion period (Figure 2). The prothrombin concentration was not affected. The fibrinogen concentration fell to about 50 per cent of the control value and was maintained at about that level during the infusion. The clottability of the blood was similarly affected. Restoration to the control values was complete within 24 hours.

No evidence of plasminogen activation could be obtained either by plasminogen analyses, or by observations on the lysis of clotted blood removed during or after the infusion period.

The dissolution of the experimental thrombus with chymotrypsin appeared to bear a relation to dosage. In 10 animals treated with chymotrypsin at a dose level of 3 to 10 mg. per Kg., less profound changes in the fibrinogen concentration and coagulation times were noted, and only three of the clots dissolved.

Animals treated with activators of plasminogen

A. *Streptokinase plus human plasminogen preparation.* In 28 animals treated by intravenous injections of 60 mg. (2.4 mg. nitrogen) of the partially purified plasminogen preparation mixed with 25,000 units of streptokinase, 14 (50 per cent) clots dissolved. The majority of the undissolved clots were noted to be very soft and small and had apparently been altered.

Immediately following the injection of the activator mixture, a burst of circulating proteolytic activity occurred (Figure 3). Proteolytic activity progressively decreased over the next few hours and returned to the control level by the following day. With the appearance of the circulating proteolytic activity, fibrinogen rapidly disappeared from the circulation and the blood was rendered incoagulable. About four hours after the injection of the activator mixture, the fibrinogen level had returned to approximately 50 per cent of the control and the clotting time was almost back to normal. At 24 hours, return of the fibrinogen concentration to the control level had been completed. A moderate and very transient fall in plasma prothrombin occurred shortly after the injection. More detailed study has shown that this apparent prothrombin fall is due to a disappearance of Ac globulin, and that true prothrombin remains intact.[10]

Assays of plasminogen revealed a striking disappearance of the major portion of this proenzyme at the time of the appearance of the circulating proteolytic activity, and the slow reappearance of this plasma constituent to control levels by the next day. The plasma of animals treated with the activator mixture were able to dissolve standard

[10] We are indebted to Dr. Helen I. Glueck for these studies. That Ac globulin is attacked by plasmin has been previously reported (10).

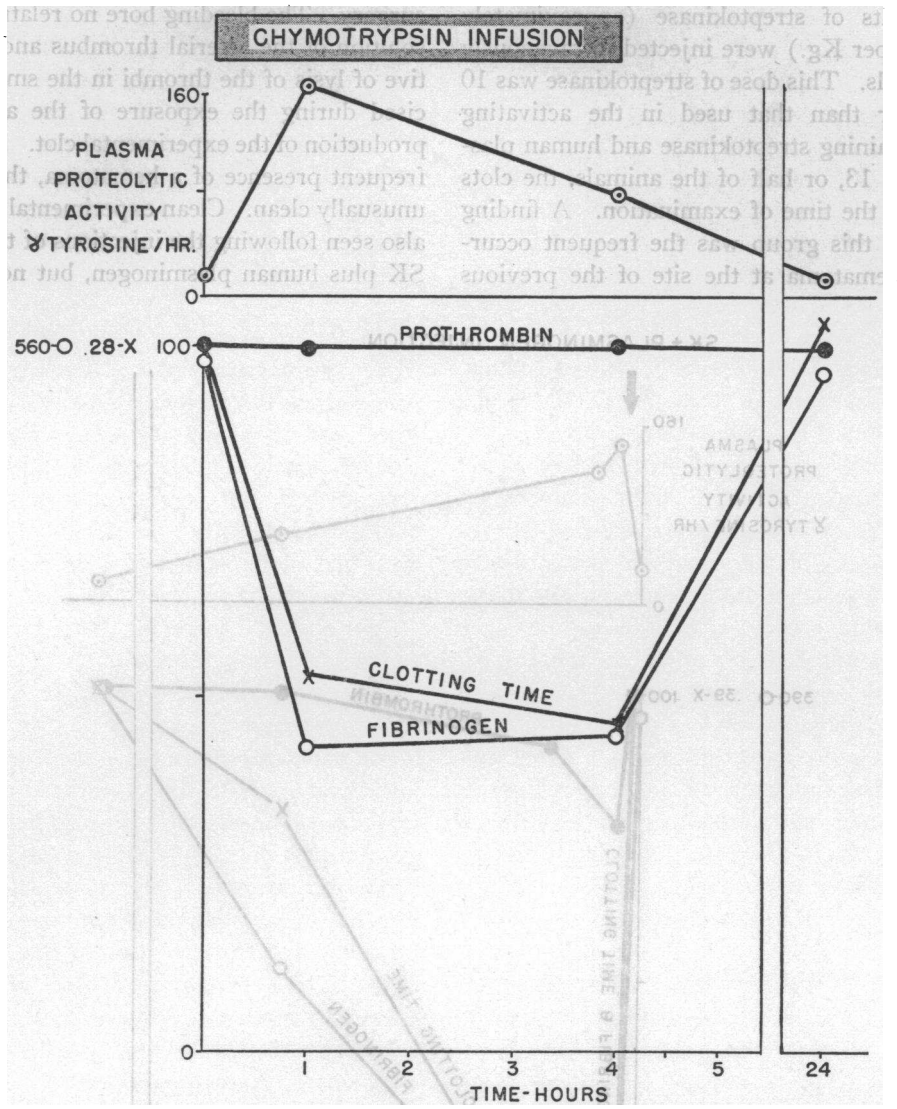

FIG. 2. THE EFFECTS OF A FOUR-HOUR INTRAVENOUS INFUSION OF 200 MG. OF CHYMOTRYPSIN INTO A 10 KG. DOG.

The abscissa represents time in hours. The ordinate plots plasma proteolytic activity (gamma acid soluble tyrosine released per hour) in the top section of the figure; and the prothrombin concentration (per cent control) ●—●, the reciprocal of the whole blood clotting time ×—×, and the fibrinogen concentration (mg. per cent) ○—○, in the bottom section of the figure. The ordinate is designed so that at 100 per cent prothrombin, the reciprocal of the clotting time is 0.28, and the fibrinogen concentration is 560 mg. per cent.

bovine fibrinogen-bovine thrombin clots within 30 minutes for periods of one to two hours following the injection. Observations of this type could not be demonstrated in the trypsin and chymotrypsin treated animals.

No attempts were made to vary the amounts of each of the components of the activating mixture. In a small series of animals, either 60 mg. of the partially purified plasminogen mixture, or 25,000 units of SK were injected alone, without evidence of activation of plasminogen or of clot dissolution.

B. *Streptokinase alone.* Two hundred and fifty

thousand units of streptokinase (approximately 20,000 units per Kg.) were injected intravenously into 26 animals. This dose of streptokinase was 10 times greater than that used in the activating mixture containing streptokinase and human plasminogen. In 13, or half of the animals, the clots were gone at the time of examination. A finding of interest in this group was the frequent occurrence of a hematoma at the site of the previous surgery. The bleeding bore no relation to the dissolution of the arterial thrombus and was suggestive of lysis of the thrombi in the small vessels incised during the exposure of the artery for the production of the experimental clot. In spite of the frequent presence of a hematoma, the wound was unusually clean. Clean experimental wounds were also seen following the injections of the mixture of SK plus human plasminogen, but not in the con-

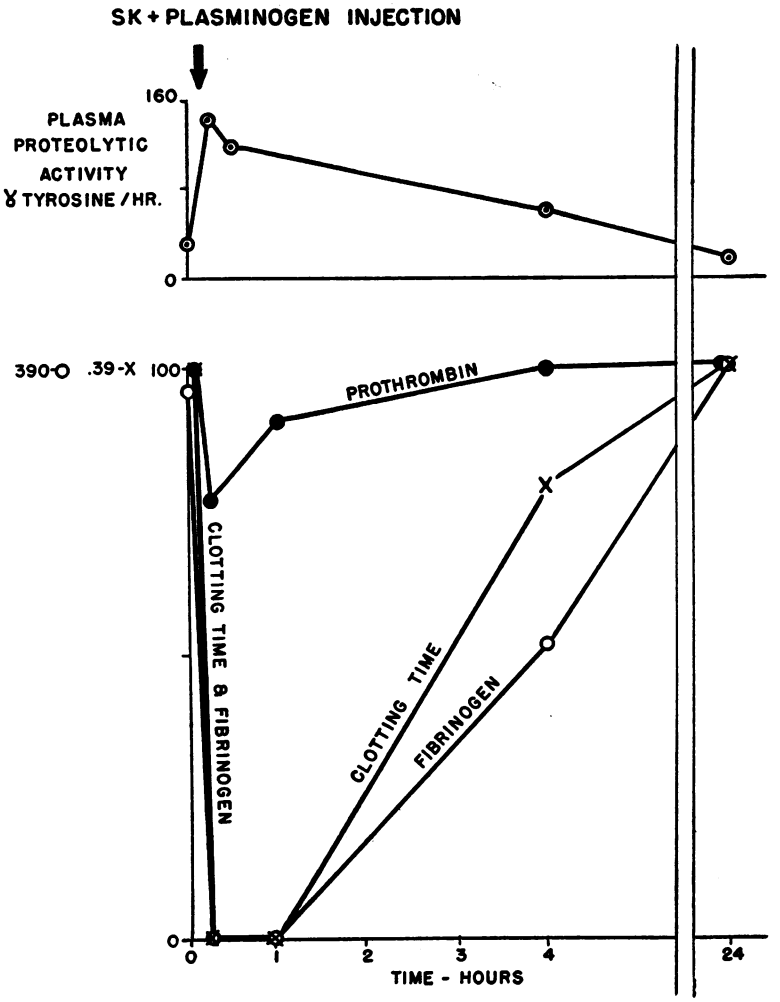

FIG. 3. THE EFFECTS OF A SINGLE INTRAVENOUS INJECTION OF A MIXTURE OF 25,000 UNITS OF STREPTOKINASE PLUS 60 MG. OF A PARTIALLY PURIFIED HUMAN PLASMINOGEN PREPARATION IN A 13 KG. DOG

The abscissa represents time in hours. The injection was given immediately following the removal of the control blood sample. The ordinate plots plasma proteolytic activity (gamma acid soluble tyrosine released per hour) in the top section of the figure; and the prothrombin concentration (per cent control) ●—●, the reciprocal of the whole blood clotting time X—X, and the fibrinogen concentration (mg. per cent) O—O, in the bottom section of the figure.

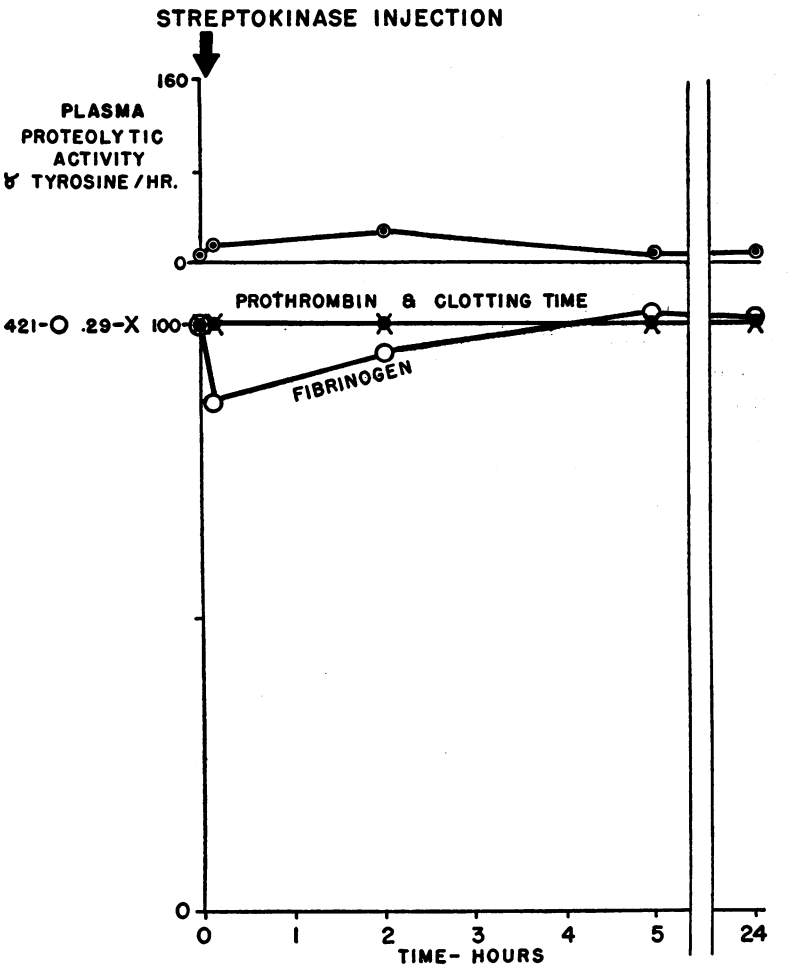

FIG. 4. THE EFFECTS OF A SINGLE INTRAVENOUS INJECTION OF 250,000 UNITS OF STREPTOKINASE INTO A 12 KG. DOG

The abscissa represents time in hours. The injection was given immediately following the removal of the control blood sample. The ordinate plots plasma proteolytic activity (gamma acid soluble tyrosine released per hour) in the top section of the figure; and the prothrombin concentration (per cent control) ●—●, the reciprocal of the whole blood clotting time ×—×, and the fibrinogen concentration (mg. per cent) O—O, in the bottom section of the figure.

trol, trypsin or chymotrypsin treated animals. Following the injection of SK, there was typically a very slight increase in circulating proteolytic activity (Figure 4). This increase was maintained for at least two hours but had completely disappeared by the fifth hour. The plasma prothrombin concentration, and the clotting time of the whole blood remained unaffected by the injection. A relatively minor, but significant, fall (15 per cent) in the fibrinogen concentration of short duration occurred with restoration to normal by the fifth hour.

Although only a slight increase in proteolytic activity, and no alteration in clotting time, was observed, the specimen of blood removed ten minutes following the SK injection promptly lysed within 30 minutes of coagulation. No lysis in 48 hours was noted with any of the other specimens removed prior to, or at later intervals, following the injection of SK. Plasminogen assays revealed a

25 per cent fall in this proenzyme titer in the 10-minute specimen, and a return to normal at two hours. These results suggested a limited activation of plasminogen following the injection of SK at the dose level employed.

Table I summarizes the observations on clot dissolution for each of the various agents utilized.

Trypsin apparently had no effect above that of the spontaneous rate of clot dissolution. Significant increase in the per cent of clots dissolved appears to have been produced by infusions of chymotrypsin and by injections of large amounts of SK, or by injections of an activating mixture of SK plus human plasminogen. In general, the results obtained on clot dissolution at the doses employed with the latter three agents, were approximately the same.

Toxicity

No significant toxic manifestations were observed with any of the agents at the dose levels, rate, and route of administration employed. When the blood was rendered incoagulable by enzyme treatment, a tendency to ooze blood from the sites of needle puncture was noted during the duration of the incoagulable state. Animals injected with the mixture of SK plus human plasminogen were up and about even though their blood remained incoagulable for several hours.

The question of embolization following clot dissolution could not be adequately studied since the clots were on the arterial side of the circulation, and the dog has an extensive collateral circulation in his lower extremities. Although emboli were searched for in the distal segment and branches of the femoral artery, none were found. However, this examination was limited in extent.

TABLE I

Summary of results on the dissolution of intravascular clots in vivo by enzymatic agents

Agent and dose	Number of clots	Number of clots dissolved	Number of clots remaining	Percentage of clots dissolved
Control	29	3	26	10
Trypsin				
0.4 mg./Kg.	3	0	3	—
16–33 mg./Kg.	22*	2	20	9
Chymotrypsin				
3–10 mg./Kg.	10	3	7	30
12–30 mg./Kg.	25	13	12	55
SK (25,000 units) plus human plasminogen (60 mg.)	28	14	14	50
Streptokinase (250,000 units)	26	13	13	50

* Does not include three instances where heparin therapy was given in conjunction with trypsin therapy, with failure of the clots to dissolve.

Rapidity of clot dissolution

Because of the design of the experiment, quantitative data on the speed of clot dissolution with these various agents was not obtained. In each of the groups treated, occasional observations were made of the previously thrombosed artery within six to eight hours after the therapeutic agent had been administered. In several instances, in each of those groups where a significant increase in clot dissolution occurred, disappearance of the thrombus had already occurred.

DISCUSSION

An attempt has been made in this report to evaluate, in the dog, a number of agents which, *in vitro,* have the capacity for either directly or indirectly effecting the rapid dissolution of fibrin and clotted blood.

Crystalline trypsin did not appear to be an ideal agent for clot dissolution since it readily converts prothrombin to thrombin (29). When added to blood in insufficient quantity to destroy all the fibrinogen, it is an excellent blood clotter (29). In addition, it is extensively inhibited by plasma (21, 22), and in partially purified systems appears to attack fibrinogen more readily than fibrin (30). The results observed in the dogs were consistent with this view. Prothrombin was destroyed at a faster rate than fibrinogen and no significant clot lysis or activation of plasminogen was observed. Although most of our biochemical findings agreed with those previously reported by Innerfield, Schwarz and Angrist (2), their observations on clot dissolution were not confirmed. Perhaps the fact that the thrombi in the present experiments were arterial rather than venous may account for some of the differences noted. When small amounts of trypsin were used, no evidence of any increased circulating proteolytic activity was observed.

Chymotrypsin, the other major proteolytic enzyme of the pancreas, rapidly splits fibrinogen and

fibrin. It does not destroy prothrombin or clot blood, and would appear to be more suitable than trypsin for intravascular clot dissolution. Chymotrypsin is extensively inhibited by plasma (31) but comparatively little is known about this inhibition. In partially purified systems chymotrypsin appears to act on fibrinogen more readily than fibrin. The results obtained *in vivo* indicated that the major portion of the infused enzyme was rapidly inhibited since only moderate fibrinogenolysis was observed. Activation of plasminogen could not be demonstrated. Surprisingly, however, a large percentage (55 per cent) of the thrombi were dissolved. It has been suggested that chymotrypsin may facilitate clot dissolution by lowering the antifibrinolytic activity of blood (32).

On theoretical grounds plasmin would appear to be the enzyme of choice for clot dissolution. It is naturally occurring, is inhibited less than the pancreatic enzymes, does not clot blood, has restricted activity in plasma, and in purified systems appears to split fibrin as readily, if not more readily than fibrinogen. The *in vitro* observations of the activation of dog plasminogen by SK in the presence of partially purified human plasminogen (17), allowed us to activate dog plasminogen *in vivo* with small doses of SK. Although this method of activation may be applied to the dog, rabbit, and cow, and perhaps other animals as well, it does not facilitate the activition of human plasminogen (17). The per cent of clots which dissolved following the injection of the activation mixture are not as high as reported by Cliffton, Grossi, and Cannamela (3), in a much smaller series of dogs. Their studies were confined almost entirely to venous thrombi, and earlier treatment of the experimental thrombus was frequently instituted.

The results obtained with injections of streptokinase alone as an activator, contrast quite sharply with the SK plus human plasminogen injections in so far as fibrinogenolysis and circulating proteolytic activity are concerned, yet are similar in their effect on fibrin and the dissolution of arterial clots. This special fibrinolytic activity seen after *in vivo* injections of streptokinase alone has been previously noted by Johnson and Tillett (1). In purified *in vitro* systems, plasmin appears to attack fibrinogen and fibrin at a similar rate (8). The *in vitro* studies of Ratnoff (33) would suggest that the explanation for this disparity may be an accelerated activation of plasminogen on the fibrin clot. Recent *in vitro* studies, suggest that, in the presence of SK, fibrin extracts plasmin from a reversibly inhibited system existing in plasma. Such a mechanism would also explain the differences noted between the native plasma as compared to purified systems. In contrast to the observations with SK alone, the presence of the mixture of SK plus human factor probably removed the plasma inhibition and allowed for a more complete activation of the plasminogen in the dog plasma, resulting in extensive fibrinogenolysis and other evidences of proteolysis. The special fibrinolytic activity observed after the injections of streptokinase alone should serve as a stimulus for further purification of this activator.

In each of the groups where significant clot dissolution was observed, approximately half the clots still remain undissolved. In the majority of these undissolved clots, evidence of partial dissolution was present. Modifications in treatment which may, or may not, have produced a higher percentage of clot dissolution were not investigated.

SUMMARY

1. Experimental femoral artery thrombi have been produced in dogs, and a study made of their dissolution by the intravenous administration of trypsin, chymotrypsin, and two types of plasminogen activators.

2. Trypsin in doses sufficient to produce a significant increase in circulating proteolytic activity and to destroy all the circulating prothrombin and fibrinogen, did not enhance the dissolution of the experimental arterial thrombus.

3. Chymotrypsin, at a dose range similar to that of trypsin, produced similar increases in circulating proteolytic activity but only a moderate fibrinogenolysis, and no effect on circulating prothrombin, but was associated with a dissolution of 55 per cent of the clots. No activation of plasminogen was demonstrable, and the mechanism of the action on the thrombi is not apparent.

4. Following the injection of a mixture of moderately small amounts of streptokinase plus a partially purified human plasminogen preparation, rapid activation *in vivo* of dog plasminogen resulted. The activation of dog plasminogen was accompanied by a significant increase in circulating proteolytic activity and rapid disappearance of

the plasma fibrinogen with a resultant incoagulable state. The activation of plasminogen in the plasma by this method was associated with a dissolution of 50 per cent of the clots.

5. Injections of streptokinase alone promoted clot dissolution (50 per cent) and the rapid liquefaction of normally clotting blood specimens, but produced only moderate changes in the plasma fibrinogen and plasminogen levels. Minor increases in circulating proteolytic activity were demonstrable. The data are consistent with the view that, *in vivo,* injections of streptokinase alone are associated with greater fibrinolytic, as compared to fibrinogenolytic, activity.

6. No toxicity was noted with any of the agents studied with the dose, rate and method of administration employed.

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