

THE LYSIS IN RABBITS OF INTRAVASCULAR BLOOD CLOTS
BY THE STREPTOCOCCAL FIBRINOLYTIC SYSTEM
(STREPTOKINASE)*

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The purposes of the present study have been to explore the possibility of producing a strongly active fibrinolytic system in the circulating blood of rabbits by intravenous injections of streptokinase and to observe its effect on artificially induced intravascular clots.

In developing the study, a number of quantitative estimations have been made pertaining to the streptococcal fibrinolytic system as it occurs in rabbit's blood. The determinations have included streptokinase and plasminogen levels, streptokinase-activated plasmin (SK-plasmin), serum inhibitor (anti-plasmin), fibrinogen, and acid soluble N. Measurements have been made before, during, and after the intravenous injection and protracted infusion of solutions containing SK.

These findings have been applied to the development of a method for the lysis of intravascular clots by creating an effective lytic system in the general circulation through the intravenous use of SK.

Materials and Methods

Streptococcal Concentrate.—Streptokinase (SK) and streptococcal desoxyribonuclease (SD) were together contained in *partially* purified concentrates as a dry, sterile powder which also contained sodium chloride and phosphate buffer.¹ Two preparations were used, lot 37A and 73A. The SK-SD titers per vial, as measured by the methods developed by Christensen (1) were 75,000 SK—770 SD and 80,000 SK—47,000 SD respectively. 37A had 100 units of SK per gamma of N, while 73A had 51 units of SK per gamma of N.

Inasmuch as SK was the principal enzyme under consideration, and because the results to be described were not observed when the streptokinase-activated proteolytic system was specifically inhibited, the concentrates that were employed will be described in terms of their content of SK without special consideration of other contained substances except their possible significance for toxicity. The preparations of SK were brought to the desired volume in 5 per cent glucose. When high concentrations of the preparation were used and the resulting

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¹ The streptococcal concentrates were supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., under the trade name of varidase.

solution was in consequence hypertonic owing to the presence of large amounts of buffer salts the solutions were made isotonic by dialyzing against a measured volume of distilled water.

Assay Methods.—Methods for the assay of SK and plasminogen (1), as well as of serum inhibitor (antifibrinolysin) (2-5) depend upon the lysis of standard clots within a given period of time (1-5). For obvious reasons, the standard clot must be stable and as reproducible as possible. Since the reagents available for these assays were not in a pure state, it was necessary to modify techniques to afford carefully standardized conditions. In order to insure the reproducibility of the results, as well as their accuracy, dilutions as closely spaced as 0.05 units were employed rather than twofold serial dilutions as previously described. Otherwise the SK assay was performed as described.

In the plasminogen assay, plasma was used for the sample rather than sera because a small amount of plasminogen was found to be adsorbed on the fibrin clot during the process of coagulation. The amount of SK was increased about 40 times (4000 units of low inhibitor SK (6) per tube), in order to minimize the action of the inhibitors normally present in the reagents and sample. The plasminogen assay was further modified by plotting the clot lysis time and the reciprocal of the dilution of the sample on log-log paper. One unit of plasminogen was defined as the dilution lysing in 30 minutes, as interpolated on the lysis time curve. When the slope of the curve was markedly abnormal, it usually indicated deterioration of the fibrinogen.

Because of the fact that measurements of serum inhibitor presented special problems the method is presented in detail. Serum inhibitor titrations were performed by analyzing the residual activity of a standard solution of fibrinolysin after prolonged incubation with various dilutions of serum or purified inhibitor (2-4). One unit of fibrinolysin (plasmin), according to Loomis (7) and Seegers (3), was defined in terms of a clot lysis time of approximately 120 seconds. Because the unit of SK used in this study was based upon the lysis of a standard clot in 10 minutes, at 35°C., by SK-activated plasmin (1), it proved desirable to use a 10 minute end-point at 35°C. for inhibitor titrations. Using bovine fibrinolysin,² it was found that one-half as much of the material was needed to lyse a standard clot in 10 minutes as in 5 minutes. Therefore, a unit was defined as the amount of fibrinolytic activity required to lyse a standard clot in 5 minutes, *i.e.*; 1 ml. of a 0.1 per cent fibrin clot at 35°C. in saline phosphate buffer at pH 7.5. When logarithmic dilutions of rabbit and human sera, as well as of purified serum inhibitor were incubated for 1 hour with 1 unit of bovine fibrinolysin at 28°C. and the resulting mixture incubated at 35°C. with the standard clot, the logarithm of the time of lysis of the standard clot was inversely proportional to the logarithm of the reciprocal of the inhibitor dilution in this range. A unit of serum inhibitor was defined as the quantity of inhibitor which will inactivate 50 per cent of the fibrinolysin in 1 ml. of a standard solution containing 1 unit at 28°C., at pH 7.5. In practice, the clot lysis time and the reciprocal of the dilution of the inhibitor were plotted on log-log paper as in the plasminogen assay, and a lysis time of 10 minutes, as indicated by interpolation on the lysis time curve, was considered to contain 1 unit of inhibitor.

Collection of Plasma and Sera.—When plasma was desired it was obtained from an ear vein on the side opposite to the site of insertion of the cannula. About 30 mg. of sodium citrate per ml. of plasma was used as the anticoagulant. Calculated amounts of a 48 per cent solution of sodium citrate were used to prevent clotting of the whole blood as the sample was taken. However, it was found to be necessary to dilute the concentrated plasma further to prevent clot formation. This was effected by bringing the plasma to a final dilution of 1:2 with an aqueous solution of 1.8 per cent sodium citrate. All samples were stored at -20°C. and subsequent dilution for assay performed in an ice bath. Since excessive blood loss is poorly toler-

² The bovine fibrinolysin was supplied by Dr. E. C. Loomis of the Research Laboratories, Parke, Davis & Company, Detroit.

ated by rabbits under stress, the number of the samples and amount of each were kept to a minimum.

The *coagulation time* was determined by a modification of the Lee-White method (8). Ten drops of undiluted venous blood were allowed to flow from the vein of a siliconed³ ear a 150 × 750 mm. test tube in about 30 seconds. A stopwatch was started with the first drop. The normal coagulation time for rabbits by this method was found to be about 5 minutes. The *lysis time in vitro*, (representing the SK plasmin) was determined on the same sample and was calculated from the same initial time as the coagulation time because SK-activated plasmin appears to attack fibrinogen and fibrin at the same rate (9). Furthermore, the coagulation time varied widely in rabbits, following the injection of SK, and therefore did not seem appropriate as a base line for the lysis time. Complete lysis of the clot usually occurred in about 12 minutes with a normally active fibrinolytic system.

Fibrinogen Assays.—Classical methods (10, 11) for the assay of fibrinogen by the determination of clottable nitrogen were modified to prevent the SK-activated plasmin from attacking the fibrinogen and fibrin in the plasma as the assays were being performed. Each step was performed in the cold, with ice-cold reagents. Crystalline (soy bean) trypsin inhibitor was added since it has been shown to inhibit the SK-plasmin system (12). Preliminary experiments in this laboratory have indicated that trypsin inhibitor gave excellent protection against clot lysis at 35°C. for more than 5 hours when equivalent quantities of SK-plasmin and soy bean inhibitor (1) were used.

Approximately five times this amount of trypsin inhibitor was added to the solutions used in the determination. Citrated rabbit plasma was diluted 1:25 in isotonic saline in a siliconed test tube, clotted with thrombin,⁴ and allowed to remain in the icebox for 1 hour. At the end of this time, the clot was broken up by rotation of the tube and subsequent centrifugation. The supernatant was then decanted and the formed clot washed with distilled water and centrifugation five times. Trypsin inhibitor was not added for the final wash. The fibrin was dissolved in 1 N NaOH and incubated for 3 hours at 56°C. The solution was neutralized and the "tyrosine" was then determined colorimetrically on the incubation mixture, using trisodium phosphate in the place of sodium carbonate (13). Trisodium phosphate was used because of the increased sensitivity with this reagent (14). The fibrin concentration was calculated from standard curves in which "tyrosine" color was plotted against dried, purified fibrin as well as fibrin nitrogen.

Acid-Soluble Nitrogen.—Acid-soluble nitrogen was determined on 8 per cent trichloroacetic acid filtrates by micro-Kjeldahl and nesslerization.

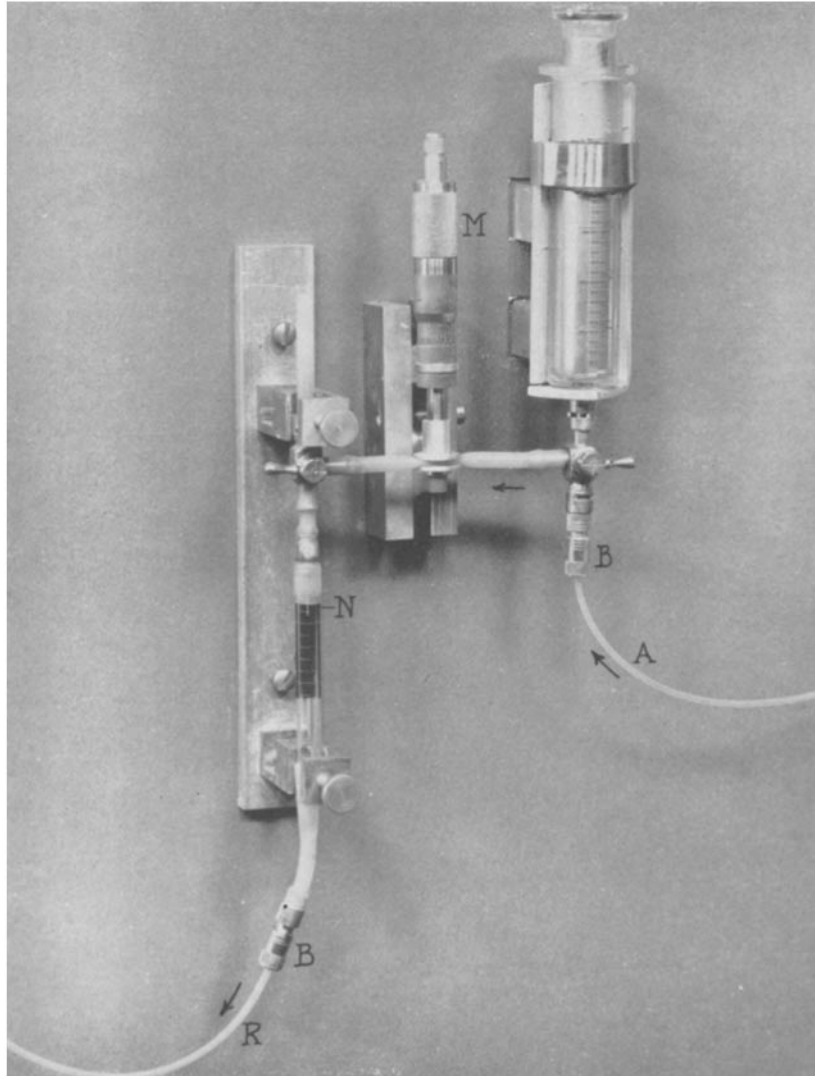
Urine.—Samples were collected by indwelling Foley catheters and were analyzed for SK and SD by the methods previously referred to.

Infusion Apparatus.—The micro-infusion apparatus used in these studies is illustrated in Text-fig. 1. The flow of liquid was regulated primarily by a stainless steel micrometer that applied appropriate pressure on rubber tubing between two three-way stopcocks. A separatory funnel was elevated by a pulley approximately 7.5 feet above the micrometer to obtain a suitable pressure head for large volumes of fluid, and a size 24 needle with the bevel cut off, was used to obtain a small, uniform drop. A 30 ml. syringe was mounted in the system to facilitate the withdrawal or addition of limited amounts of fluid. Intermediate hydrostatic pressures, for very fine adjustments of the flow, were obtained by raising or lowering the separatory funnel above or below the height already mentioned.

The handle of the 30 ml. syringe was filled with mercury to provide a pressure system for a small volume of fluid. Small bore polyethylene tubing was used wherever feasible. The in-

³ 2 per cent solution of Dow Corning silicone, DC 200, in ether.

⁴ Thrombin, topical (bovine origin), Parke, Davis & Company, Detroit.



TEXT-FIG. 1. Micro-infusion apparatus for intravenous introduction of SK.

A—polyethylene tube from separatory funnel; *B*—adapter to connect polyethylene tubing; *M*—micrometer; *N*—drip needle (against black background) in drip apparatus; *R*—polyethylene tube to rabbit ear and cannula.

fusion apparatus reproducibly delivered as much as 20.0 ml. per minute in a continuous stream through the needle, or less than 0.02 ml. per minute at about 1 drop every minute.

The same micrometer setting did not give exactly the same rate of flow on different occasions, but the rate could be reproduced exactly by using the number of drops per minute as the criterion for minor changes in the micrometer setting.

The SK infusion was given at a rate of 0.31 ml. a minute for most of the experiments in order to standardize any increase in the rabbit blood volume. Hematocrit determinations on control rabbits showed that hemodilution was not severe at this rate, even though the experiment was continued for many hours.

EXPERIMENTAL

Determination of the Streptococcal Fibrinolytic System in Rabbits

The studies were made on plasma and serum obtained from rabbits before, during, and after injections of the enzyme concentrates. The methods of injection ranged from the rapid introduction of large amounts in a few minutes to as long as 24 hours of continuous infusion. Since the method of protracted intravenous infusion has yielded the most satisfactory results, most of the data contained in this article were obtained from the latter type of experiments.

Plasminogen.—Using the method previously described, with different batches of low inhibitor SK, approximately 300 to 350 units per ml. has been found to represent the average content of plasminogen in the plasma of normal rabbits. (This method has shown about 7000 units in the plasma of normal man.)

The data for plasminogen recorded in Text-fig. 2 were obtained from serial determinations made during the intravenous infusion of SK over a 24 hour period. It can be noted that the level of plasminogen progressively decreased until it reached a value of 30 units per ml. of plasma approximately 24 hours after the beginning of the infusion.

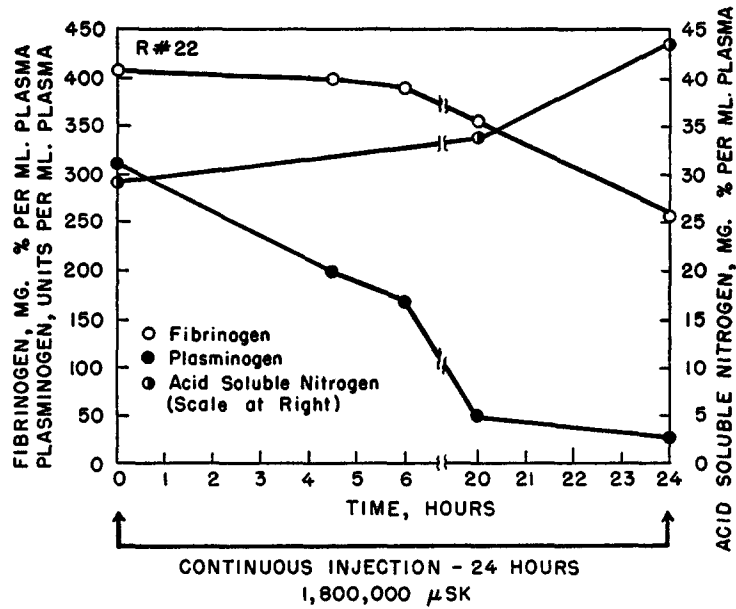
According to current theory (12), this was due to the progressive conversion of plasminogen to plasmin by SK. Indeed, the level of plasminogen in the plasma has been found to be one of the most dependable and sensitive biochemical measures of the streptococcal fibrinolytic system *in vivo*.

On the basis of a large number of experimental results comparable to that shown in Text-fig. 2 it appears likely that the rate of formation of plasminogen in the rabbit is insufficient to keep pace with its activation by SK.

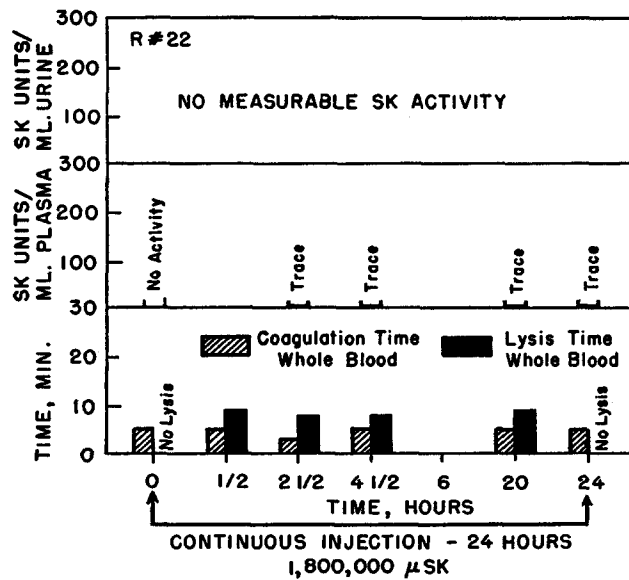
Additional studies to be presented subsequently (Text-fig. 4) have also indicated that the restoration of the plasminogen to the preinjection level usually occurs within 24 hours after the infusion of SK has ceased.

SK-Plasmin.—The presence of an active lytic system in the circulating blood during and after the injection of SK was demonstrated, as indicated under Materials and Methods, by the lysis of samples of undiluted whole blood taken during the experimental period and allowed both to clot spontaneously in a test tube and to undergo lysis subsequently (Text-figs. 3 and 5).

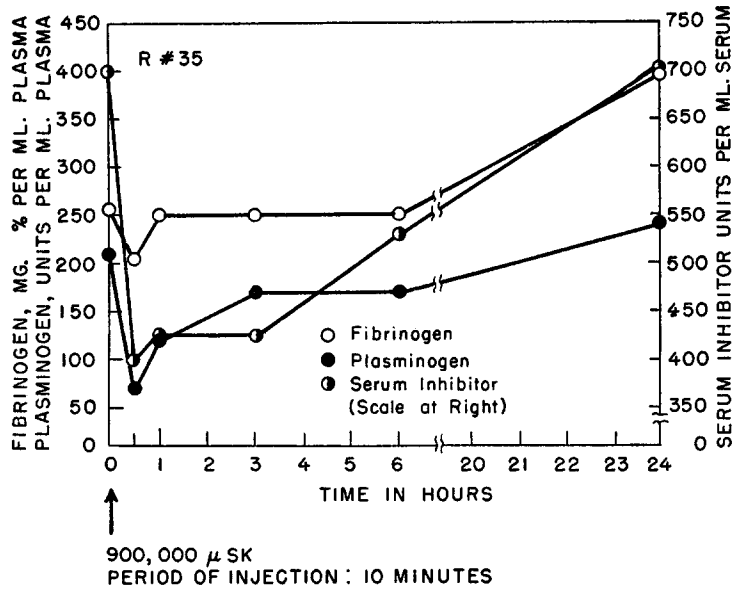
The effect of the SK-plasmin on the coagulation time seemed to be independent of the amount of SK infused but varied, within 1 to 2 minutes, according to the lot number of SK used. Unlike trypsin (15-17) and papain (15), however, the coagulation time was not uniformly shortened in over 350 samples from 48 rabbits inasmuch as the mean of the postinjection coagulation time (5.3 minutes) was about the same as the preinjection coagulation time (5.2 minutes). On the other hand, the lysis of these clots was the principal criterion



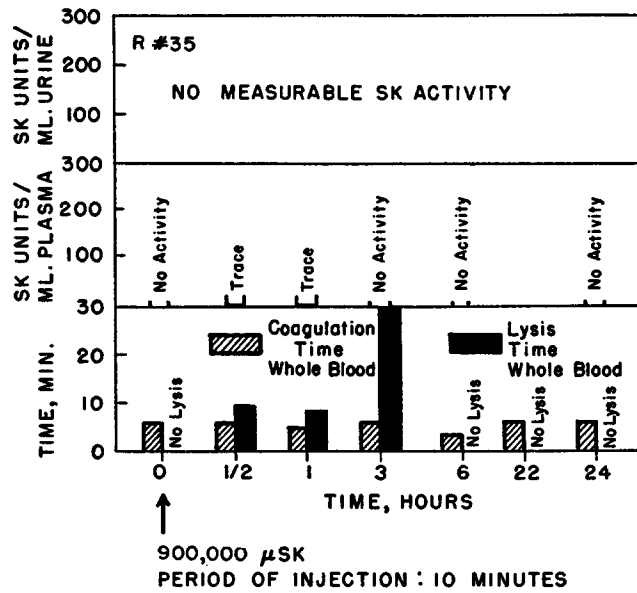
TEXT-FIG. 2. Effect of continuous intravenous injection of 1,800,000 units of SK in a rabbit over a 24 hour period.



TEXT-FIG. 3. Effect of continuous intravenous injection of 1,800,000 units of SK in a rabbit over a 24 hour period.



TEXT-FIG. 4. Effect of a single intravenous injection of 900,000 units of SK in a rabbit over a 10 minute period.



TEXT-FIG. 5. Effect of a single intravenous injection of 900,000 units of SK in a rabbit over a 10 minute period.

for the *presence* of SK-plasmin and the time of lysis was the best indication of the *amount* of SK-plasmin. The mean lysis time of the samples referred to above was 12.3 minutes. In contrast to the data of Macfarlane with dilute plasma (5), complete lysis of the undilute, clotted whole blood occurred in less than 30 minutes, or it did not occur at all. The difference did not appear to be due to the presence of the red cells but is thought to be due to the concentration of inhibitor in the undiluted blood together with the very mild proteolytic system developed by the SK *in vivo*.

During the infusion of adequate SK doses, an active lytic system was observed to be present within 30 minutes following the beginning of the experiment. It remained present for periods of time ranging from 1 hour to 20 hours, depending upon the concentration of SK and the duration of the infusion as well as the amount of activatable plasminogen and the level of the serum inhibitor.

In Text-fig. 3, for example, it can be seen that the active lytic system endured for approximately 20 hours during a period of infusion of 1,800,000 units of SK for 24 hours. The fact that an active lytic system was not present at 24 hours although free SK was found in the plasma at this time (Text-fig. 3), would seem to indicate that the very low plasminogen mentioned above was probably responsible for its absence.

In the experiments recorded in Text-fig. 5, a single injection of 900,000 units of SK was given over a period of 10 minutes. The die-away curve of SK plasmin can be readily visualized as the clot-lysis time extended to its maximum of 30 minutes, at 3 hours postinjection. This die-away can also be seen in the free SK (Text-fig. 5), and its consequences mirrored by the concordant fall and subsequent rise in the plasminogen, fibrinogen, and serum inhibitor levels (Text-fig. 4).

In spite of the interplay of a number of different factors, the large amounts of SK that were introduced intravenously appear to have created an effective lytic system for individual rabbits in which the special factors, plasminogen and inhibitors, varied. These findings will again be discussed in connection with the actual lysis of the intravascular clots to be described.

Free SK.—In general, very small amounts of SK were detectable in the plasma during or after the infusion of SK *in vivo* (Text-figs. 3 and 5). Inactivation, inhibition, or removal of SK from the blood occurs rather promptly. Striking evidence of this is shown in Text-fig. 5 where only trace amounts of SK were detectable in the rabbit plasma 30 minutes after the injection of 900,000 units of SK. When SK was added to rabbit whole blood and plasma respectively, in amounts comparable to the calculated *in vivo* blood levels, assay of the samples accounted for about 10 per cent of the SK. It appears, therefore, that while a considerable loss of SK activity occurs *in vitro*, a significantly greater loss occurs *in vivo*. This suggests that another mechanism or mechanisms may play a part in the *in vivo* loss of SK activity. The mechanism does

not appear to be urinary excretion since SK was very rarely found in the urine (Text-figs. 3 and 5). It is not known whether this was associated with the very low plasma levels noted above, or due to a specific renal block.

Serum Inhibitor.—The mean *serum inhibitor* in rabbits as measured by the method previously described, was about 500 units per ml. with a range of 250 to 750 units per ml. Although a mild decrease in the serum inhibitor was usually noted during and immediately following the injection of SK, the inhibitor level rapidly returned to normal within 24 hours (Text-fig. 4). The striking changes observed by Lewis and Ferguson (18) on the injection of fibrinolysin and staphylokinase in dogs were not observed in these experiments. Further work on this and other inhibitors to SK and SK-plasmin is in progress.

Fibrinogen.—In view of the fact that the active SK fibrinolytic system attacks fibrinogen at about the same rate as fibrin *in vitro* (9), determinations have been made of changes in the amount of circulating fibrinogen occurring during infusions of SK (Text-figs. 2 and 4). The rate of reduction in fibrinogen was very slow in spite of the constant infusion of SK and the presence of an active fibrinolytic system. These findings suggest that the rate of fibrinogenolysis *in vivo* may be slower than anticipated from the *in vitro* studies, or that the formation of fibrinogen by the animal nearly paralleled its destruction. In some of the animals, the level of fibrinogen rose considerably above normal 24 hours after the injection of SK (Text-fig. 4). It was not known whether this was due to a specific response to fibrinogenolysis or a non-specific response to the injection of foreign protein.

Acid-Soluble N.—Moderately consistent elevations (15 to 30 mg. per cent) have been found in the level of the plasma acid-soluble nitrogen following the infusion of SK. This has been interpreted as further evidence of proteolysis induced *in vivo*, by the SK fibrinolytic system, and was thought to be due to the products of proteolytic digestion.

From Text-fig. 2 it can be seen that the acid-soluble N rose about 15 mg. per cent during the period of infusion with SK. It is of interest that this occurred in the face of a mild hemodilution effected by the infusion. From this and other experiments, it was concluded that the rise in acid-soluble N seemed to correlate with the apparent lytic activity of the system, and was not present after the infusion of SK in two instances when an elevation in serum inhibitor prevented the formation of an active lytic system. The rise usually started about 3 to 6 hours after the beginning of the infusion and returned to normal within 12 hours after the infusion was terminated.

Lysis of Previously Prepared Intravascular Clots in Vivo by the Systemic Administration of Streptokinase

A standard blood clot about 10 to 20 mm. long was formed in the medial or marginal ear veins of gray chinchilla rabbits by means of a sclerosing agent, sodium morrhuate.

Through and through suture ligatures were used to isolate a segment of the vein after a single intravenous injection of nembutal had been given. The blood was then extracted from the isolated segment with a 26 Huber point hypodermic needle and the segment washed with 0.5 per cent procaine in saline or with saline alone. 0.1 to 0.05 ml. of a 5 per cent solution of sodium morrhuate in 2 per cent benzyl alcohol⁵ was introduced through the needle, allowed to remain in contact with the vein wall at least 5 minutes, and the vein segment was then washed free of the sodium morrhuate with saline. The needle was withdrawn, the suture ligatures cut, and the venous flow allowed to continue uninterrupted. A firm occluding clot was formed in less than 15 minutes (Fig. 1 a).

Patency or obstruction of the vein was determined by transillumination of the ear with a 200 watt microscope illuminator, after the validity of this method had been established in preliminary experiments by dissection of the vein following the application of morrhuate, and the lysis of *in vivo* clots, respectively.

Gross and histological examinations of the segments of vein made at varying intervals following the introduction of sodium morrhuate revealed complete obstruction by the freshly formed clot within 15 minutes. Eventual fibrosis of the area followed after 2 to 4 weeks in all the control animals. In contrast, spontaneous recanalization of a significant percentage of clots occurred with all other agents tested, including thrombin, hypertonic glucose, and sodium psylliate,⁶ as well as trauma to the walls of the veins.

No anesthetic was required to keep the rabbits quiet during the infusion period. They were placed in a conventional rabbit box (painted white) without the top. A domestic type 250 watt infrared light was placed about 4 feet away from the box and the very mild beam directed over the box proved to be effective in keeping the animals in the box. Small quantities of food and water were given to many of them during the long infusion periods.

SK-SD was injected intravenously in the ear contralateral to the one containing the clot. The total dosage of SK per rabbit varied from 410,000 units to 2,080,000 units. The preparations of concentrate containing SK also contained 100 to 2,000,000 units of streptodornase (SD). The findings with respect to intravenously injected SD are not included in this article but will be given in a separate communication. The average effective dose of SK proved to be 40,000 units per kilo per hour, although in some instances the animals developed a lytic system with 15,000 units per kilo per hour.

The methods of injection of SK were: single massive injections, smaller intermittent injections, and finally, the most efficient and easily controlled technique, continuous infusion, which was employed in about 80 per cent of the experiments. The average duration of the continuous and intermittent infusions was 8 hours, with a range of 6 to 12 hours. In order to insure a firm, well consolidated clot the beginning of the infusion was deferred for 6 to 72

⁵ Sodium morrhuate 5 per cent with benzyl alcohol 2 per cent, G. D. Searle and Company, Chicago.

⁶ Sodium psylliate (slynasol) in benzyl alcohol 2 per cent, G. D. Searle and Company, Chicago.

hours after the clot had formed. Figs. 1 *a* and 1 *b* are photographs of the ear of a rabbit which was treated in the following manner:

A clot (Fig. 1 *a*) was formed in the marginal vein⁷ of the left ear with 0.05 cc. sodium morrhuate and a cannula inserted in the marginal vein of the opposite ear. 8 hours later, the SK infusion was started at a dosage level of 25,000 units SK per kilo per hour. 1 hour later, samples were taken and allowed to clot and lyse in test tubes. They did not lyse completely, however, and the dosage was elevated to 50,000 units per kilo per hour. At this level, an active fibrinolytic system was established and complete lysis of the samples occurred. 4 hours and 30 minutes after the infusion was begun, the clot in the ear vein appeared lysed, and subsequently remained lysed. The infusion was discontinued 2½ hours later. The total quantity of SK given was 880,000 units.

TABLE I
Lysis of Intravascular Clots in Rabbits as Result of Intravenous Infusion of SK

Lot No. of SK	No. of rabbits	Complete lysis effected			No lysis effected	Survived	Died*
		Lysis persisted	Clot re-formed	Total			
37A	6	4 (1 ACTH)	2 (2 ACTH)	6	0	3 (3 ACTH)	3
73A	19	15	2 (1 ACTH)	17	2	14 (1 ACTH)	5
Total	25	19	4	23	2	17	8

Average dose SK: 35,000 to 40,000 units per kilo per hour.

Mean total dose: 7.8×10^6 units. Range: 4.1×10^6 to 20.8×10^6 .

Average duration of infusion 8 hours. Range: 6 to 12 hours.

Infusion began 6 to 72 hours after the clot had formed.

* See text for details.

A number of these experiments are summarized in Table I.

Following liquefaction of the clot, lysis was permanent in 19 of 23 animals (Table I). No re-formation of the thrombus occurred for a period of observation of at least 6 months among the animals that survived (Table I). An analysis of the duration of the SK infusion in these experiments revealed two general principles. (*a*) It was found to be necessary to maintain an active lytic system for 3 to 7 hours in the circulating blood of these animals in order to lyse their clots. (*b*) 3 to 4 additional hours of an active lytic system were required to pre-

⁷ The large veins at the base of the ear were generally chosen for clot formation, but were found to be very difficult to photograph. It was felt that clot lysis in a large vein represented a more significant test system because a thrombus in a large vein with very few tributaries simulated closely a thrombus in a human vein. As might be predicted, the lysis time of a clot in the small, distal ear vein with its many tributaries was considerably shorter than the lysis time of a clot in the large veins.

vent re-formation of the clots. Thus, 6 to 12 hours of constant infusion with SK produced clot lysis *when the infusion was accompanied by demonstrable activation of the SK-plasmin*. The previously clotted vein remained patent for the life of the animal if these conditions obtained.

The clot re-formed at the original site in four of the animals (Table I). Three of them received ACTH,⁸ however, and comment on them will be made subsequently in connection with the administration of ACTH. Re-formation of the clot in the fourth animal of this group probably occurred because the intravascular lytic system was not maintained for a sufficiently long time. In this instance, progressively increasing doses of SK failed to activate the SK-plasmin *in vivo* for longer than 2½ hours. Samples of plasma from this animal, subsequently tested, were observed to inhibit SK-plasmin markedly *in vitro*.

Two experiments are included in Table I wherein no clot lysis was effected. No SK-plasmin was demonstrable in either of these at any time despite the administration of massive doses of SK. Samples of plasma from these animals also exhibited striking inhibition of SK-plasmin when tested *in vitro*.

It is significant that the minimum requirements for clot lysis were not satisfied in the animal with high inhibitor whose clot re-formed, and in the two whose clots did not lyse.

Inasmuch as clot re-formation was prevented by continuing the infusion for 3 to 4 hours after the clot appeared to be lysed, it was of interest to determine whether the duration of this infusion could be shortened and an ancillary anticoagulant used in its place.

In four instances, this was done, and heparin⁹ was given after the SK infusion was terminated and the lytic system was no longer present. In one preliminary experiment the heparin was given for only 6 hours and a clot promptly re-formed in the same place after the heparin was discontinued. A tendency toward clot re-formation was also evident after heparin was given for as long as 14 hours. Less tendency toward clot re-formation occurred in two instances when heparin administration was prolonged (24 hours or more).

These experiments seemed to indicate that the mechanism for the prevention of clot re-formation by SK was not that of an anticoagulant, although prolonged use of an anticoagulant could be substituted for it. Additional studies are in progress.

Toxicity.—Because of the unusually large amounts of SK required to effect clot lysis, *in vivo*, over half a gram of protein was present in the partially purified enzyme concentrates injected intravenously in each experiment. On the basis of present information it has been estimated that these preparations were less than 10 per cent pure SK (19). As a result of the large amount of contaminating protein, some of it in the form of other enzymes, the toxicity of the SK itself has been very difficult to evaluate.

⁸ ACTH (adrenocorticotropin), The Armour Laboratories, Armour and Company, Chicago.

⁹ Heparin, Connaught Medical Research Laboratories, Toronto, Canada.

It was our impression that ACTH minimized the secondary toxic effects of these injected concentrates. ACTH was administered to 4 of the 25 animals in this series (Table I), and all the rabbits treated with ACTH and SK survived. Eight of the 21 animals not receiving ACTH died within 48 hours after the infusion had been started (Table I), although none died after this time. There was no evidence to indicate that ACTH interfered with the formation of an active lytic system in these or other animals so treated.

In the 8 animals that died, special interest has centered around whether or not pulmonary infarction occurred. Detailed gross examination of the lungs has failed to reveal any evidence of pulmonary infarction that may have arisen from the partial liquefaction of the clot with subsequent release of a fibrinous embolus.

On examination of the dead animals, it was observed that 2 had extensive preexisting coccidiosis of the liver and lungs, respectively, with abscess formation. In one rabbit, trauma to the psoas muscle resulted from the struggling of the animal under partial anesthesia. In two other instances, marked damage was found in the psoas as a result of the *local* effect of intraperitoneally injected metrazol.¹⁰ (The latter was used as an analeptic agent, and was injected after the anesthesia was terminated and at least 6 hours before the SK infusion was started.) In all 3 animals there was evidence of massive hemorrhage into the previously traumatized area.

It appears highly probable that when the fibrinolytic system was developed intravenously there ensued increased bleeding at sites where small blood vessels had been injured. This type of bleeding has also been observed when the urethra has been traumatized by the insertion of a catheter into the bladder to collect urine for assays of SK and SD excreted in the urine.

In the 3 remaining rabbits which died, no significant contributing cause, such as coccidiosis or hemorrhage, was found and death is ascribed to an as yet, undetermined effect of the streptococcal concentrates. In this connection, lot 37A of SK-SD was found to be far more toxic than lot 73A even though lot 37A was twice as "pure" as lot 73A as measured by units of SK per gamma of N. This was further emphasized by the fact that the only animals that survived infusion with 37A had also received ACTH, while only 1 of the 14 animals that received lot 73A and survived had also been given ACTH.

The subject of the toxicity of the preparations is being given detailed study with respect to which one or more of the several different enzymes or extraneous materials may be of special significance.

Previous mention has been made of the antitoxic effect of ACTH. ACTH has proved to be of limited use in these experiments, however, because after lysis had occurred a clot re-formed at the original site in 3 of the 4 animals receiving ACTH (Table I) whereas re-formation of the clot occurred in only one of the 19 animals not receiving ACTH. In one of the animals in which a clot re-formed, the first dose of ACTH was not administered until 10 hours after lysis of the clot had been accomplished. These observations are in accord with

¹⁰ Metrazol (pentamethylentrazol), Bilhuber-Knoll Corp., Orange, N. J.

the work of Cosgriff in man (20-21) wherein he has shown that there is an increase in the coagulability of the blood in patients treated with ACTH and cortisone, together with an increased incidence of thromboembolic phenomena.

SUMMARY

1. Based upon quantitative estimations of the factors that promote or retard the development and activity of the streptococcal fibrinolytic phenomenon, an active lytic system was developed within the circulating blood stream of rabbits following the intravenous infusion of streptokinase. During the infusion of adequate doses of SK, an active lytic system was observed to be present within 30 minutes following the beginning of the experiment and remained present for periods of time ranging from 1 to 20 hours depending primarily upon the concentration of SK and the duration of the infusion. Some of the biochemical changes accompanying the lytic system *in vivo* were: (a) a striking fall in the plasminogen; (b) a moderate fall in the serum inhibitor and the fibrinogen; and, (c) a rise in the acid-soluble nitrogen. These changes were usually self-terminating within 24 hours following the infusion of SK. In earlier studies similar trends were observed in the chest fluid of patients with hemothorax and empyema treated locally with streptokinase (22).

2. Intravascular clots, induced artificially by local applications of sodium morrhuate within the ear vein of the rabbits, were observed to liquefy and disappear in 23 of 25 rabbits during the intravenous infusion of SK into the opposite ear.

The average quantity of SK necessary to effect an active lytic system was found to be about 40,000 units per kilo per hour.

The clot in the ear vein was observed to be lysed completely in periods of time ranging from 3 to 7 hours after the infusion of SK was begun. Maintenance of an active lytic system for 3 to 4 additional hours was required to prevent re-formation of the clots at the original site. In 3 of 4 rabbits in which clots did reform, ACTH had been given to combat the toxic effects of the streptococcal concentrates.

3. The toxicity of the unusually large dose of the streptococcal concentrates, containing measured amounts of SK, along with other identifiable and unknown substances, was considerable, inasmuch as 8 animals eventually died. No evidence of pulmonary infarction was observed at autopsy. The administration of ACTH appeared to prevent a fatal outcome in at least 3 of the rabbits. Further studies of toxicity are in progress.

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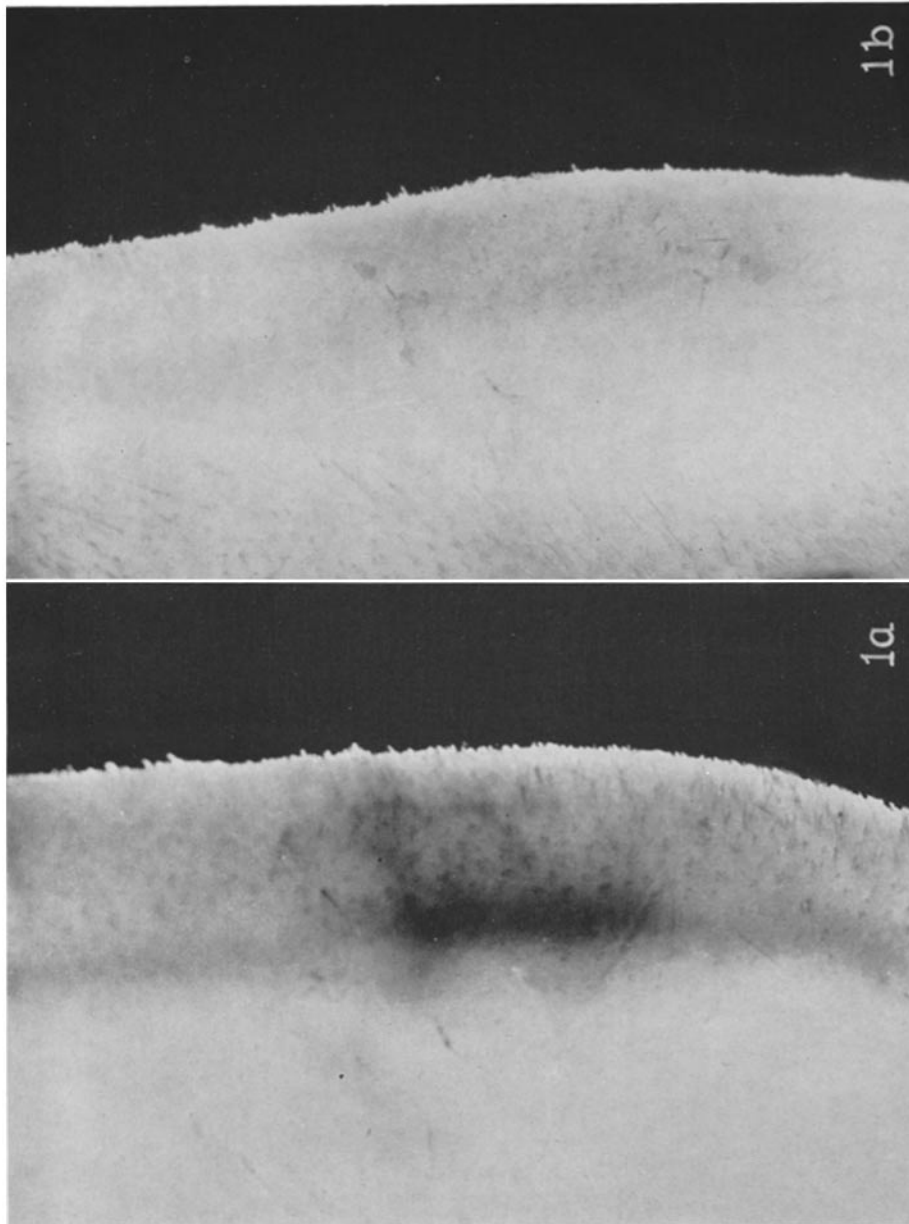
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EXPLANATION OF PLATE 45

The photographs were made by John Goeller.

FIG. 1 *a*. The photograph of the ear of rabbit 1 was made 5 hours after the clot had been formed and 3 hours before the SK infusion was started. $\times 2\frac{1}{2}$.

FIG. 1 *b*. The photograph of the ear of rabbit 1 was made about 48 hours after the photograph in 1 *a*, and about 41 hours after the clot had been lysed. $\times 2\frac{1}{2}$.



(Johnson and Tillett: Lysis of intravascular blood clots by streptokinase)