Behavior in the Circulation of the Radioactive Pulmonary Embolus and Its Application to the Study of Fibrinolytic Enzymes *

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Introduction

WHEN the early experimental evidence of clot lysis in living animals was reported, using true fibrinolytic agents,[3, 10, 16] application of these agents to clinical thromboembolism was supplied with an experimental basis. Elimination of impurities in the active agents has yielded a product that can be tolerated without serious side effects, and it is now possible to compare several reports in which these agents have been given an early clinical trial.[4, 14, 17, 18] However, preparations of plasmin have yielded satisfactory results in only about one-third of the cases in which they have been used. Improvement was not permanent or not observed in about two-thirds. The reason for failure to achieve lysis in this majority is, at the present time, obscure.

Inability to predict which cases will be benefited by these enzymes may give rise to reluctance to employ them in clinical situations which can be treated safely by other means. For example, the effect of heparin on the clinical course and complications of thrombophlebitis is proven and predictable. Heparin can be relied upon to terminate clot propagation and to reduce the incidence of pulmonary emboli. The tendency to thrombosis usually subsides if anticoagulants are continued for several days. Although successful use of a fibrinolytic agent should accomplish the first two of these effects, if unsuccessful, the agent would not be as good as heparin. Although there appears to be no objection to combining anticoagulation with the fibrinolytic enzyme for the present, this sort of double therapy will not simplify the task of evaluating the effectiveness of the fibrinolytic agent. Certain types of arterial thrombosis which cannot now be satisfactorily treated by surgery or anticoagulants represent an area in which the fibrinolytic agents will probably be exhibited even though their effectiveness is not thoroughly tested. The possibility of salvaging life or limb would justify their use even before their effect can be predicted. Even here the value of anticoagulants to prevent clotting in important collateral vessels still will need to be considered.

Increasing clinical experience with the fibrinolytic agents may demonstrate patterns of successful lysis and show situations that are unlikely to be benefitted. Thus it is already apparent that the greatest likelihood of success exists early in the course of the thrombosis, and that after 72 hours lysis of the original thrombus is seldom seen. Still, with our present meager knowledge of the cause of thromboembolism, the prospect of finding the key to successful clot lysis in a large number of clinical cases of thromboembolism presents an awesome task. Here, indeed, one is confronted by a disease with a capricious course, vague signs and inconstant symptoms and by a great void in our laboratory acquaintance with the thrombotic state, incipient throm-

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bosis or the thrombotic tendency. This present situation has been likened to evaluating the therapy of anemia without a means for measuring the hemoglobin or hematocrit.

Recourse to experimental methods under these circumstances is especially attractive. Sherry *et al.* have developed new concepts of the fibrinolytic system based on *in vitro* demonstration of lytic activity.[17] Johnson has induced thrombosis in the superficial veins of human volunteers to permit a controlled experiment in which factors influencing lysis could be evaluated.[12] Among various animal preparations a radioactive blood clot has several unique qualities to recommend it and has been employed by ourselves[9] and others.[2] Besides the evident advantage of animal experimentation, this method offers the special attraction of demonstrating clot lysis as it progresses by observed changes in radioactivity. A large part of the present study has been given over to a consideration of factors affecting the stability in the circulation of such experimental clots. A summary of some of these observations will serve to introduce the present method.

1. The use of the arterial circulation was discarded early because of total fragmentation of the clot. The use of the venous circulation for an experimental pulmonary embolus proved satisfactory in that the radioactivity could be detected externally, the clot remained well localized and recovery at necropsy was not difficult.

2. Spontaneous decrease in the mass of clots introduced into the venous circulation has been demonstrated previously by others.[5, 15] It was hoped that this factor could be allowed for, if the rate of change in weight was known or if it could be inferred from untreated controls. Measurement of clot weight before and after the experiment defined the rate of lysis more precisely and appeared to be a natural advantage of a technic using an experimental clot made outside of the circulation, introduced intact as a pulmonary embolus and then recovered from the branches of the pulmonary artery.

3. Clots prepared in an identical manner to the experimental embolus but incubated in a physiologic solution maintained their initial weight well during the first 48 hours. In general it was observed that a clot of acceptable stability would remain within 5 per cent of initial weight in buffered saline, while an identical clot in the circulation would be reduced 75 to 80 per cent of initial weight. If the *in vitro* clot was reduced to 85 to 90 per cent, the clot in the circulation was likely to be reduced to 50 per cent of initial weight. This much change in weight was not considered acceptable stability after some of the factors involved were appreciated. The cause of this spontaneous weight change is not entirely understood. Apart from the mechanical action of the circulation, some hemolysis of erythrocytes in the clot occurs, there is autolysis of protein after several days, and the action of bacteria may have a variable influence. Merthiolate 1 : 100,000 added to the buffered saline solution inhibited the growth of molds and of bacteria.

4. It was demonstrated that clots made from the blood of different animals were not equally firm. It was found valuable to select the donor animal from among several dogs on the basis of the retraction of a clot of the blood of each and a rough estimate of clot firmness and the tendency for erythrocytes to become detached and settle to the bottom of the clotting tube.

5. The experimental clot tends to be less firm if the ratio of the radioactive solution was large in proportion to the blood with which it was mixed before clotting. Fortunately, it has not proven difficult to keep the specific radioactivity of the fibrinogen high while keeping its volume small. If the fibrinogen was kept to one-tenth of the total volume, the clots were as firm as those made from blood only.

6. A thorough mixing of fibrinogen, blood and thrombin in the clotting tube was found to require that the materials and equipment be chilled to delay coagulation. The use of an anticoagulant was not desirable and was avoided by drawing the blood into siliconed equipment.

7. Complete clot retraction occurred within 15 hours at 37° C. Apparent changes in the weight of the clot could be avoided by waiting for completion of clot retraction before weighing.

8. A part of the radioactivity of the I_{131}. fibrinogen is not clottable on the addition of thrombin, this amount varying from 7 to 20 per cent. Of the total radioactivity only two per cent is not precipitable with trichloracetic acid. Efforts to remove from the experiment any radioactivity not bound to the fibrin mesh were directed along the following lines: 1) After retraction, the clot was gently washed with saline on a Buchner filter funnel. 2) After washing, the clot was placed in cold buffered saline for 48 hours to permit unbound radioactivity to diffuse into the bath solution. 3) After insertion into the circulation a period of several hours was permitted to elapse until determination of the radioactivity over the clot indicates that a steady state has been reached. Only after this were changes in radioactivity considered to represent changes in the clot itself.

Materials

Buffered saline:

Sodium phosphate dibasic (Na_2HPO_4)	141.98 Gm.
Sodium chloride	90.00 Gm.
Merthiolate, 1:1,000	100 ml.
Water, ad	10,000 ml.
Hydrochloric acid, 1 N, adjust to pH	7.8

Thrombin:

Topical Thrombin (Upjohn)

Plasminogen:

Human plasminogen prepared under the direction of Dr. D. L. Kline[13] from Fraction III of human plasma, released through the courtesy of Dr. J. N. Ashworth of the American National Red Cross.

Plasmin:

Partially purified plasmin prepared under the direction of Dr. D. L. Kline.[6] This preparation contains some activator which probably accounts for its fibrinolytic effect.

Streptokinase:

Varidase. Supplied by Lederle Laboratories division of American Cyanamid Company through the courtesy of Dr. J. M. Ruegsegger.

Fibrinogen:

Purified bovine fibrinogen supplied by Warner-Chilcott Company through the courtesy of Dr. Charles Scott, and Mr. John Doczi.

Ash (NaCl)	63.3%
Soluble protein	36.0%
Proportion of protein clottable	94.0%

$Chromium_{51}$:

Sodium radiochromate (Abbott)

$Iodine_{131}$

Sodium $iodide_{131}$ (Oak Ridge National Laboratory)

Mannitol infusion:

Mannitol, USP	75.0 Gm.
Sodium chloride	14.61 Gm.
Water, ad	1,500 ml.

Method

Cr_{51} labelling of erythrocytes: 40 ml. of dog blood was drawn into 10 ml. 0.1 M sodium citrate. 100 microcuries of Cr_{51} was added to one-half of this amount, the mixture incubated 30 minutes at 37° C. with occasional shaking and the plasma removed from the other aliquot of blood and set aside. After 30 minutes the blood containing isotope was centrifuged, the plasma discarded and the erythrocytes washed three times with cold saline. The cells were resuspended in the fresh plasma set aside previously, and introduced into the clotting tube.

I_{131} labelling of fibrinogen: This method is adapted from a technic [1] based on the method of Eisen and Keston. Purified fibrinogen 0.3 Gm. is dissolved in 15 ml. buffered saline. Carrier-free I_{131} 5 to 10 millicuries is evaporated on an electric hotplate to a volume of about 0.2 ml. pH should be alkaline to indicator paper. To the isotope is added the following reagents

in the amounts indicated at room temperature and as rapidly as possible:

1. Potassium iodide	0.02	M	0.05 ml.
2. Hydrochloric acid	2	N	0.2 ml.
3. Sodium nitrite	1	M	0.05 ml.
4. Ammonium sulfamate	1	M	0.05 ml.
5. Sodium hydroxide	2	N	0.2 ml.

pH is checked with paper and the fibrinogen quickly delivered into the iodinating solution. After 30 minutes the fibrinogen is passed three times down a column of Amberlite resin (IRC 400, 40 mesh, chloride form). A small amount of buffered saline is used to rinse the column. Eighty-five to 90 per cent of the activity is retained on the resin. Ninety-nine per cent of the radioactivity of the solution is protein bound. Eighty to 85 per cent of the radioactivity is thrombin clottable. Ninety-eight to 99 per cent of the radioactivity is precipitable with trichloracetic acid. These values do not change appreciably during a four-week period. The specific activity of the fibrinogen solution is about 4 to 8 microcuries per mg. on the day of preparation.

Determination of fibrinolytic action of plasma: This method is adapted from a technic using preformed trace labelled human plasma clots.[1] Plasma (0.4 ml.) taken from outdated bank blood is mixed with 1/100 volume of I_{131} labelled fibrinogen and placed in a 10 × 75 mm. test tube containing a short helix of stainless steel wire with a handle long enough to remove the clot. Thrombin (0.1 ml.) is added (10 units) and the clot incubated overnight, rinsed and stored in cold buffered saline at least 48 hours before use. When incubated with fluids containing activator, release of radioactivity from such clots reaches a maximum in about four hours' incubation at 37° C. and does not change greatly if incubated longer. The release of radioactivity in solutions having no activator is negligible. Because the plasma being tested for fibrinolytic activity contains some radioactivity from the experimental embolus, double counting is necessary to determine how much radioactivity has been released from the human plasma clot.

Preparation of the experimental embolus: Several glass tubes of 12-mm. internal diameter are scored to indicate 10-ml. volume. Using chilled siliconed syringes and needles, and chilled plastic containers for mixing the blood and isotope, but not using sterile precautions, dog blood is obtained by jugular puncture, carefully mixed with one-tenth volume of I_{131}-fibrinogen and 10 ml. aliquots transferred to the glass clotting tubes. One hundred units of thrombin is added,* the tubes stoppered, inverted several times and incubated overnight at 37° C. Each clot is removed and washed in a Buchner funnel with normal saline, then placed in cold buffered saline for 48 hours to allow unbound radioactivity to diffuse into the bath solution. Each clot is then weighed and is either used as an *in vitro* control or introduced into a curved cannula for insertion as the experimental embolus. The control clots are either immediately digested in 1 N NaOH or placed in buffered saline and incubated at 37° C. for the duration of the experiment. At the completion of the experiments all clots are weighed and hydrolyzed in sodium hydroxide, 1 N, to determine specific radioactivity.

Human plasminogen was added to the blood-fibrinogen mixture in some experiments before clotting. The final concentration of the plasminogen varied from 0.1 to 10 mg. per ml. of blood-fibrinogen mixture.

Insertion of the experimental embolus: Under Nembutal anesthesia (or dial with urethane) the inferior vena cava is exposed transperitoneally through a midline incision and a segment 6 cm. long just below the renal veins isolated by ligating the cava and lumbar veins. Proximally a vascular clamp provides temporary occlusion of the cava and a short curved glass cannula is

* Thrombin has been omitted in recent experiments.

then inserted in a proximal direction. This is connected to the cannula containing the weighed clot, which is then washed into the circulation as the vascular clamp is briefly released. The cava is ligated and the wound closed.

Demonstration of the fate of the experimental embolus: External radioactivity is detected with a probe-type scintillation detector, scaler, rate meter and continuous charting recorder. A straight tube collimator on the detector limits the area monitored to a portion of the thorax, and the detector is located over the point of maximum activity and secured in place. At least three hours elapse before radioactivity detected over the clot reaches a steady state. This period is longer if monitoring is begun immediately after the embolus is introduced. A steady level of anesthesia is desirable because changes in radioactivity recorded will result from restlessness or a large change in respiratory rate during the experiment. A small diameter polyethylene cannula is inserted as a catheter in the bladder and the urine collected by gravity in hourly fractions. An infusion of 5 per cent mannitol in hypertonic saline (166 meq/l) is given at a rate of one ml. per minute with a constant speed infusion pump. Blood samples are taken at intervals. Radioactivity of urine and blood samples is measured in a well-counter at the conclusion of the experiment and is expressed as net radioactivity after subtracting background. Necropsy is performed on the heart and lungs. Heparin sulfate 3 mg. per kg. body weight is given immediately before sacrifice to permit certain identification of remaining fragments of the embolus. These are collected, weighed, and hydrolysed as described above.

Results

Long-term Clot Stability. The spontaneous loss of weight observed in control clots in the previous study[9] suggested that the radioactive pulmonary embolus be applied to a long-term study of the rate of disappearance of intravascular clots. Chromium$_{51}$-tagged erythrocytes as a source of radiation was selected for this part of the experiment because its half life is longer than that of iodine$_{131}$. In five experiments the radioactivity from a pulmonary embolus measurable at the skin level over three sites was recorded and correlated with changes in the clot at necropsy. The decline of radioactivity in such an experiment reaches a steady state in a few weeks, after which the rate of decline parallels the decay of the isotope. Necropsy done after this point revealed very small fragments of thrombus in the pulmonary arteries or none at all. It was inferred from these observations that when the external activity began to parallel a decay curve for the isotope, spontaneous lysis had progressed to a point beyond which further changes could not be recognized with the methods employed. Figure 1 demonstrates the changes in radioactivity during one of these long-term experiments. A curve has been freely drawn through the mean of the radioactivity values. The intersection of this curve with the decay curve for the isotope gives a point of reference of clot lysis that could be compared among the experiments and with necropsy findings. These data are summarized in Table I.

Propagation of New Thrombus. Although clots in the circulation were observed to decrease in weight more rapidly than similar clots incubated in buffered saline, close inspection of clots removed from the animal after 24 hours or more demonstrated suggestive evidence of fresh thrombus propagating on the experimental embolus. Inspection of the original clot, or the *in vitro* control clot, reveals a smooth dark shiny surface and even diameter throughout its length. After 24 or more hours in the circulation the surface of the experimental embolus is opaque, shows fine striations (lines of Zahn) and gives rise to spurs or projections extending be-

tween trabeculae or into branches of the pulmonary artery, sometimes for 2 or 3 cm. Since the change in the surface of the clot must have occurred within the circulation, it is inferred that fresh thrombus has been deposited on it. This hypothesis was tested by dissecting the embolus and analyzing separately the spurs and projections for specific radioactivity. In Figure 2 the result of such a dissection and analysis is compared with a control clot incubated in buffered saline and with a second embolus taken from an animal given sufficient heparin to keep the clotting time longer than two hours during the experiment. It would be anticipated that any new thrombus deposited during the experiment would have a specific radioactivity that was less by some amount than the radioactivity of the original embolus. In this and two similar experiments the specific activity of the spurs and projections dissected from the clot has been consistently less than the specific activity of the main portion of the clot, the *in vitro* clots or the clot removed from the animal given heparin. This difference reinforces the evidence gained from inspection of the clot that fresh thrombus does propagate upon the surface of the experimental embolus. These changes suggesting the deposit of new thrombus were observed from 24 to 48 hours after the experimental clot was introduced, without regard to the changes in weight of the embolus that occurred while it was in the circulation. Only heparin, in sufficient amount, would prevent these changes.

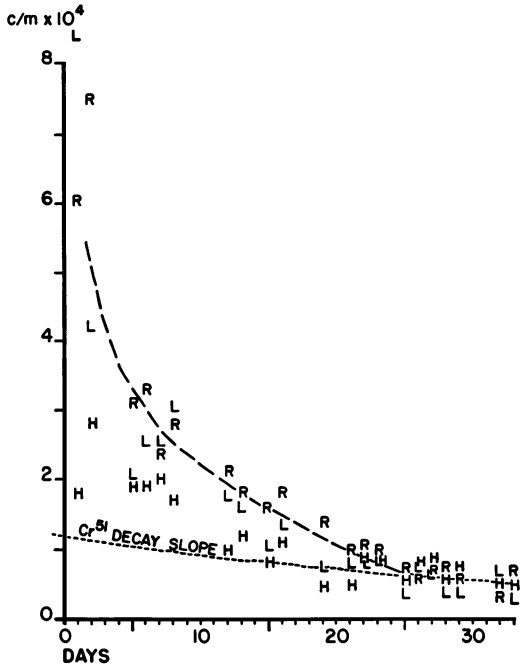

FIG. 1. A 10.14-Gm. Cr_{51} labelled clot was inserted at day zero as a pulmonary embolus in this experiment. Detection of external radiation is recorded at intervals in counts per minute as "L" if monitored over the left side of the thorax opposite the apex impulse, "R" at a corresponding point on the right side of the chest and "H" at the costal margin on the right over the liver. Frequent blood samples were drawn and activity determined in a well counter. Except for the first two days they were uniform and only slightly above background. They have not been recorded. The dashed line is drawn freely through the determinations over the thorax (R and L). In the latter part of the experiment a mean of all measurements (R, L and H) parallels the decay slope of Cr_{51}. A dotted line carried through these mean points back to the time of origin of the experiment is a decay curve for Cr_{51} plotted for this period. The intersection of these two lines is interpreted as indicating a point at which spontaneous lysis has so reduced the experimental embolus that it can no longer be distinguished from background by the monitoring method used. In this experiment this intersection occurred 25 days after embolization (Text and Table 1).

Clot Integrity in the Circulation. Figures 3 through 6 are characteristic of experiments in which clot lysis could be inferred from analysis of the changes in radioactivity. A prolonged decline in the activity over the thorax was the single most reliable evidence of clot lysis. This is illustrated in Figure 3 in which nearly complete lysis occurred in an embolus that contained a large amount of human plasminogen. The embolus was reduced to 18 per cent of starting weight. Obviously, an embolus containing this much human plasminogen is too unstable in the circulation to be well suited to evaluating a fibrinolytic enzyme.

Figure 4 demonstrates nearly complete lysis after plasmin had been given. The embolus was reduced to 10 per cent of initial weight during this experiment.

In Figures 4 and 5 the rapid rise in blood

TABLE 1. *Fate of Cr_{51} Labelled Emboli Monitored Externally*

Exp. No.	Clot Weight when Inserted (Gms.)	Intersection with Cr_{51} Decay Slope (days)	Sacrifice (days)	Necropsy Findings
57 A	5.19	16	17	2 intimal plaques, LLL Largest 2 mm. in diam.
57 B	10.14	25	63	Intimal plaque, RUL
58 A	5.59	17	23	No evidence of clot
58 B	7.87	17	21	2 mm. fragment on chorda, fibrous strand in LLL, org. frag't, 1 × 4 mm., RML
58 C	8.31	19	22	0.15 Gm. frag't RML

radioactivity after administration of partially purified plasmin is conspicuous and by comparison with the previous baseline appears to offer a compelling demonstration of clot lysis induced by this enzyme. The failure to obtain samples in the later part of these experiments makes it impossible to know how long the rise in blood activity was maintained but in other experiments using streptokinase, the increase in the blood radioactivity often showed a brief high initial spike and then a later more gradual increase. Figure 6 illustrates this type reaction in an embolus to which plasminogen had been incorporated to a final concentration of 0.1 mg./ml. blood-fibrinogen mixture. This embolus was reduced to 79 per cent of initial weight while

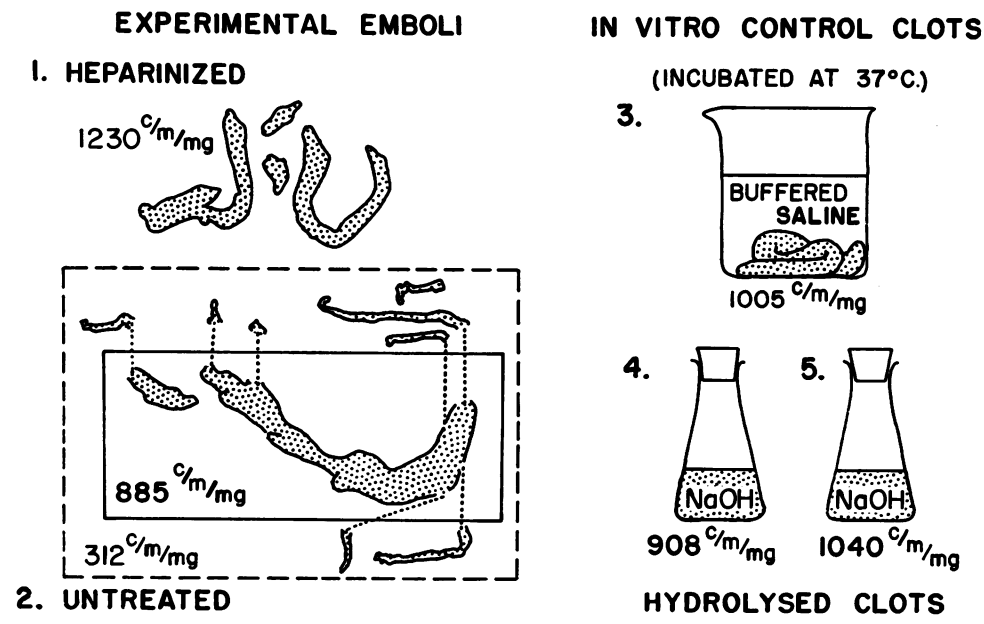

FIG. 2. After weighing, the clots illustrated in the diagram were disposed as follows: 1. As an experimental embolus in a dog heparinized from immediately before the embolus until necropsy. The four fragments recovered from this animal had a dark shiny surface, no lines of Zahn and no spurs or projections. 2. As an embolus in a dog given no heparin. At necropsy the surface of the clot was gray, marked with transverse lines, and gave rise to several spurs or projections extending into the branches of the pulmonary artery. These projections were dissected from the clot and analysed separately as shown. The remaining clots were incubated at 37° C. as *in vitro* controls. 3. In buffered saline, had a dark shiny surface. Clots 1, 2 and 3 were followed for the same length of time, about 24 hours, before second weighing and digestion; 4 and 5 were hydrolysed immediately after initial weighing. The specific activity of the whole and dissected clots is expressed as counts per minute per milligram of wet weight at the time of hydrolysis.

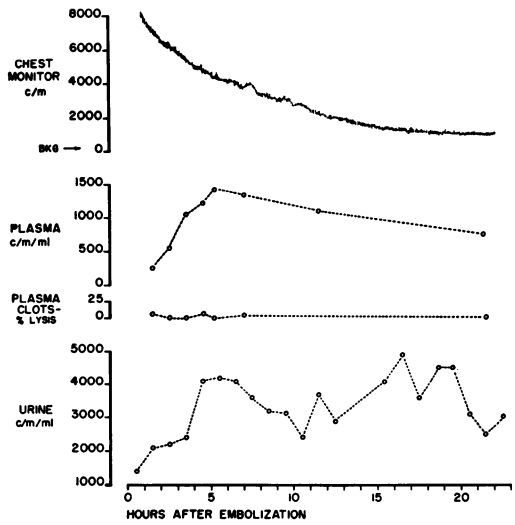

FIG. 3. Human plasminogen was incorporated into the blood-I_{131} fibrinogen mixture to a concentration of 10 mg./ml. before clotting. The embolus weighed 3.17 Gm. when inserted and 0.57 Gm. when recovered (18% of initial weight). Brisk clot lysis is demonstrated in changes in precordial radioactivity, urine and blood radioactivity. No more than 6 per cent of radioactivity was released from human plasma clots incubated with samples of plasma drawn from the experimental animal. An identical clot in another animal (not illustrated) underwent lysis until 7 per cent of initial weight remained, during the same period, and two *in vitro* control clots maintained 103 and 106 per cent of initial weight respectively in buffered saline.

an *in vitro* control clot maintained 104 per cent of initial weight. The effect of samples of plasma drawn at intervals to release radioactivity from preformed clots of human plasma trace-labelled with I_{131} fibrinogen demonstrates the presence of circulating activator during the streptokinase infusion.

Radioactivity in the urine presents an index of clot integrity of variable value. Eighty-nine per cent of the radioactivity in the urine is not protein bound, and is therefore presumably a concentration of inorganic radioactive iodide filtered by the glomerulus. Thus it is subject to the various factors influencing urine flow as well as factors affecting the level of plasma radioactivity. At its best, with a fairly constant hourly urine volume, changes in urine radioactivity can be related to the other parameters demonstrating clot lysis. Figure 6 is an example of good correlation of urine radioactivity with other evidence of partial clot lysis. A diuretic and slightly hypertonic saline solution has been useful in maintaining steady urine flow, and the constant speed pump has proven essential in maintaining an even hourly urine flow and to insure that enzymes are administered at a constant rate. The 12 other experiments in which lytic agents (plasmin, streptokinase) were used did not show more evidence of lysis than the foregoing experiments. In nearly all, some changes in radioactivity indicating partial lysis occurred. In none did complete lysis occur.

Discussion and Summary

The present distressing lack of objective criteria by which thromboembolic disease can be described seriously hampers an evaluation of fibrinolytic enzymes based

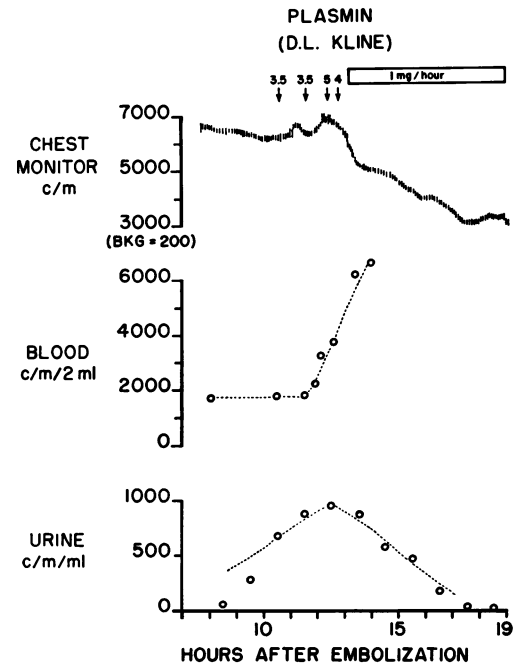

FIG. 4. The embolus weighed 5.24 Gm. when inserted, and 0.53 Gm. when recovered (10% of initial weight). Plasmin given as four injections of 3.5, 3.5, 5 and 4 mg., respectively, and infused at a rate of 1 mg./hr. as indicated resulted in the lysis of the clot shown in changes in precordial radioactivity, urine and blood radioactivity. No human plasminogen had been incorporated into this embolus.

on clinical observation. An experimental method of evaluation consequently assumes greater importance to this field. The experimental method presented demonstrates one approach to this problem.

The spontaneous lysis of experimental clots is a recognized phenomenon that must be considered in assessing their usefulness in evaluating fibrinolytic enzymes. An intimate knowledge of the behavior of such clots in the circulation becomes of considerable importance for this reason. It has been shown that clots injected into the venous circulation decline in mass steadily until by four weeks very little if any of the original clot remains.[5, 15] The age of the clot at the time of injection affects the rate of removal, young red clot being removed more rapidly than two-week-old thrombus.[8] In none of these experiments had any demonstration been made of new thrombus, although it was usually sought and its absence noted.

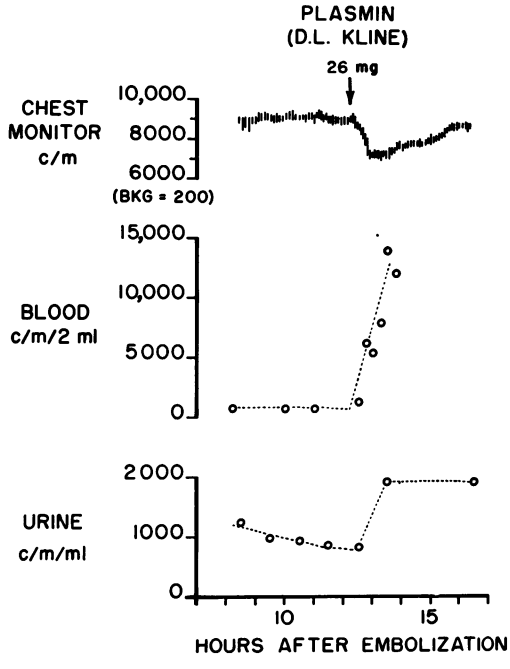

FIG. 5. Changes in precordial, blood and urine radioactivity subsequent to a single injection of plasmin are illustrated. The animal expired without heparin, voiding comparison of clot mass before and after insertion into the circulation. No human plasminogen was combined with the clot when it was made.

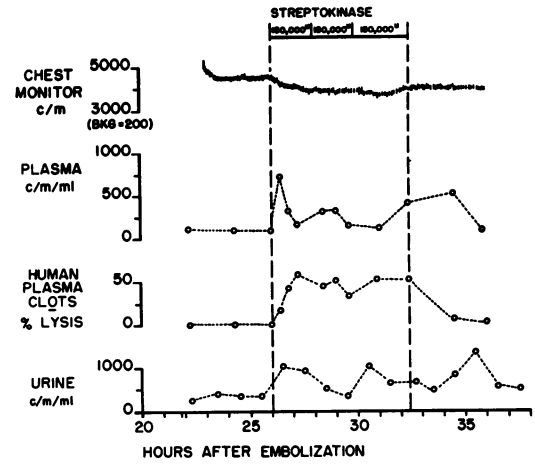

FIG. 6. Human plasminogen was incorporated into the blood-I_{131} fibrinogen mixture to a final concentration of 0.1 mg./ml. before clotting. The embolus weighed 4.50 Gm. when inserted and 3.53 Gm. when recovered (79% initial weight). Streptokinase (Varidase) was infused as indicated, to a total amount of 450,000 units. Changes in precordial radioactivity, urine and plasma radioactivity and plasma activator level are demonstrated.

Observation of the radioactive embolus confirms previous demonstration of spontaneous clot lysis and supplies additional information on the rate of clot removal. There is also suggestive evidence that new thrombus may be deposited even as the mass of the experimental clot is being reduced in the circulation. Weighing the clot permits a somewhat more precise estimate of the rate of change in clot mass and has aided the evaluation of several details which bear upon the stability of the clot in the circulation. Identical clots that could serve as an external control have been used and it has been observed that if the weight of these clots is well maintained *in vitro*, the clot in the circulation also demonstrates an acceptable stability. Observation of these control clots, when compared with the experimental embolus, is of some value as an indication of clot lysis in those experiments in which fibrinolytic enzymes were used, but even more reliance can be placed upon a demonstration of changes in radioactivity occurring after a steady baseline has been reached. The failure to demonstrate complete lysis in these experi-

ments indicates the complexity of this new therapeutic field. It is anticipated that subsequent experience will show how crude an attempt to effect lysis is represented by the introduction of a simple excess of a potent lytic agent or activator. The importance of inhibitors, antibodies and the plasminogen level has already been demonstrated.[12] Probably there are few areas in physiology in which a system of checks and balances makes a more involved mechanism for homeostasis than in the fibrin-fibrinolytic system. It is most likely that some of these interacting systems must be taken into account in effecting complete clot lysis. It is to be hoped that in evaluating the fibrinolytic agents as they become available, more basic information about the fibrinolytic system and its inhibitors will be forthcoming.

Acknowledgment

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