Quantitative Method for the In Vivo Testing of Fibrinolytic Agents: Effect of Intravenous Trypsin on Radioactive Thrombi and Emboli

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Quantitative methods for testing fibrinolytic agents in vivo are described. Methods based on the incorporation into the clot of P32 labeled blood cells or Cr61 labeled erythrocytes were found to be unsatisfactory. Clots were produced with 111I labeled fibrinogen. Dissolution of a clot resulted in decrease of radioactivity. This was recorded by placing segments of blood vessels in a special lead shield containing a scintillation detector head, connected to a radiation rate meter and an Esterline recorder. Pulmonary, peripheral and coronary emboli were produced by injecting radioactive clots produced and ground, in vitro. Using these methods it was found that maximal tolerated doses of trypsin showed no significant fibrinolytic effect.

Many methods have been used to produce experimental thrombi and emboli in vivo for the study of potential fibrinolytic agents. Most of these methods are based on either the injection into isolated segments of veins or arteries of—sclerosing agents, bacterial cultures, thrombin, thromboplastin preparations or in vitro formed clots; or on mechanical traumatization of the vessels—ligation, alteration of electrical potentials or insertion of various materials into the vessels. Usual criteria of clot dissolution are palpation, visual observation with the aid of transillumination, histologic study, determination of the patency of the vessel by angiography, infrared photography or at autopsy. All these methods are obviously qualitative. It was felt that more quantitative methods were needed. The aim of this study was to develop such methods and to test the in vivo fibrinolytic effect of intravenously administered trypsin. Trypsin has been tested from this point of view by a number of investigators, with greatly variable results. A restudy of this question using quantitative methods was indicated.

### Methods and Materials

#### Experiments in Dogs with P32 labeled Clots

Adult mongrel dogs of 30 to 40 lb. body weight were maintained on Purina laboratory chow, horsemeat and water ad libitum. Anesthesia was with 25 mg./Kg. sodium pentobarbital, i.v.; supplemented with further doses when needed. A section of the femoral or jugular vein or femoral artery was isolated and artery clamps placed on both the distal and proximal ends. All side branches were ligated except one, into which a fine polyethylene cannula was inserted. Most of the blood from the segment was withdrawn through this cannula into a syringe containing 3 mc. of P32-labeled sodium phosphate. This mixture was then rapidly reintroduced through the cannula into the vein segment. The mixture was withdrawn and injected three times to insure thorough mixing. Radioactive phosphorus was rapidly taken up by...
New York, N. Y.
ethylene tube of about the same external diameter as
the vein which was to receive the thrombus. Clotting
a syringe containing P n labeled sodium phosphate.
Drawing approximately 5 ml. of the animal's blood
vessel in order to prevent the escape of the clot
vessel, and prior to the placement of the shield,
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the red and white blood cells. Fifty to one hundred
units of thrombin† in 0.1 ml of physiological saline
was then introduced into the segment. In prelimi-
ary experiments, this procedure was found
to be more satisfactory than the use of thrombo-
plastin.‡ After the polyethylene cannula was
removed and the side branch ligated, the vessel
was placed into the groove of a specially designed
lead shield. This shield contained a rim into which
a shield housing the Geiger-Mueller tube fitted.
The Geiger-Mueller tube was attached to a radia-
tion rate meter. Counts per minute were auto-
matically graphed on an Esterline Recorder at-
tached to the rate meter. Figure 1D shows the
entire apparatus used. Figure 2D shows the shields
and the counters.

Ligatures. Before removing the clamps from the
vein, and prior to the placement of the shield,
one of two types of ligatures was placed on the
vessel in order to prevent the escape of the clot
from the area.

A semiconstricting ligature which was so ad-
justed that if blood flow was possible through the
clot, it would not be impeded by the ligature; yet,
if the clot were to become loose, it would not be
able to pass the constriction. The correct placing
of the semi-constricting ligature appeared to be
important. Disappearance of part or all of the clot
was observed before this method was adopted in
venous and particularly in arterial clots. It is
thought that some positive results reported in the
literature might be due to such escape of the clots;
in many instances this is difficult to detect by routine
autopsy. In our studies, such accidental move-
ments of clots were easily detected with the aid of
the Geiger-Mueller tube. Dislodged venous clots
usually were caught in the pulmonary capillaries,
and arterial clots were detected in the capillary
bed of the corresponding limb. In the first ex-
periments, regular cord was used to make the ligature.
This became loose on arteries, however, and alu-
num wire was later used, both on arteries and
veins. The twisted ends of the wire were enclosed
in a fine polyethylene tube and the latter sealed
with the flame of a match.

A completely constricting ligature placed either
proximal or distal to the clot.

Clots. Two types of clots were made.

In vitro-formed clots, were produced by with-
drawing approximately 5 ml. of the animal’s blood
from the contralateral femoral vein of the dog into
a syringe containing Pn labeled sodium phosphate.
The blood was then rapidly introduced into a poly-
ethylene tube of about the same external diameter as
the vein which was to receive the thrombus. Clotting
was permitted to occur spontaneously. After clot-
ting was completed, the clot was pushed out of the
tube with the aid of a polyethylene rod; its length,
height, and weight recorded, then it was reinserted
into the same polyethylene tube. The external
jugular was prepared above and below its bifurcation,
and artery clamps placed on all three branches of the
Y thus prepared. The internal maxillary vein was
 cannulated with the polyethylene tube, the clot was
pushed into the vein, below the bifurcation. The clot
formed in the tube would be less adherent to the
vessel wall, thus exposing more of its surface to
the effect of the fibrinolytic agent. This type of clot
always was used with a semi-constricting ligature
placed proximal to the clot. At the end of the experi-
ment, the clot was removed and its weight and
size determined. This was thought to give an indica-
tion of the net result of lysis and growth of the clot
by apposition while under the effect of the drugs
to be tested.

Canalized clots were produced in the jugular
vein in the same manner, with the modification
that, before the clot was formed, a polyethylene
rod was inserted into the jugular through one of
the maxillary branches, which was subsequently
ligated. A semiconstricting ligature was placed
proximal to the clot. After the artery clamps were
removed and the infusion of the drug to be tested
started, the polyethylene rod was removed. Im-
mediately after removal of the rod, Thorotrast,*
or in later experiments Diodrast † was injected
distal to the clot and its flow through the canal in
the clot observed on the fluoroscope. The same
procedure repeated 4 hours later as the last
sections of the drug used were being infused. In
the control animals, the canal invariably became
obiterated. Thus, this method served to indicate
the effect of the drug in preventing apposition by
virtue of its anticoagulant effect. Figure 1 shows
flow of the x-ray contrast medium through a nor-
mal jugular vein, A, through a jugular vein with a
caanalized clot immediately after removal of the
polyethylene rod, B, and one hour later, C. It can
be seen that good flow is maintained. After one
hour the canal becomes obliterated, yet a trace of
flow is maintained through the clot.

Phlebography as well as recording of blood pres-
sure after injection of epinephrine revealed that
material injected distal to clots in even com-
pletely ligated vessels is able to arrive rapidly
into the systemic circulation. Backflow of the x-ray
contrast medium took place through collaterals. In
addition, small quantities seem to pass through
clots with semi-constricting ligatures. After com-
pleting the injection of the x-ray contrast medium

† Thrombin of bovine origin was kindly supplied
by Parke, Davis & Co., Detroit, Michigan.
‡ Thromboplastin, Warner-Chilcot Laboratories,
New York, N. Y.

* Thorotrast. Testgar & Co. Inc., Detroit,
Michigan.
† Diodrast. Winthrop-Stearns & Co., New York,
N. Y.
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Fig. 1. Phlebograms of A. normal jugular vein, B. vein with canalized clot and semiconstricting ligature, taken immediately after removal of the polyethylene rod, and C. same vein, one hour after. Note obliteration of central canal.

the latter rapidly disappeared from the area except for small amounts which often appeared to be trapped in the meshwork of the clot. This residual material completely disappeared within half an hour. All these observations indicate that the clot may be in continuous contact with agents injected into the blood stream. (Figures 3D§, 4D§.)

Dogs were prepared with one or several of the above described types of clots. In all cases, artery clamps isolating the thrombosed segments were removed one half hour after production of the clot. Infusion of the drug under test, or corresponding amounts of saline into the controls, was made into a nonthrombosed femoral or jugular vein, or, if both jugular as well as femoral veins were thrombosed, into the cephalic vein. Increase or decrease of the size of the clot was evaluated by the following criteria.

Pallpation. This had to be done with extreme caution so as not to break up the clot. Outside of qualitative evaluation of the presence of the clot, this was not of much value.

Transillumination. The light source of a Metro Photoelectric Tensiometer was dismounted. This consisted of a powerful light at the end of a narrow, bent, stainless steel tube which could be slipped under the thrombosed vessel illuminating the clot. The clot usually appeared as a meshwork of dark shadows with lighter spots in between. Upon touching the clot, liquid material could be identified within this meshwork. Occasionally, a small trapped air bubble was seen. The estimated length on the basis of transillumination was invariably less than the actual length measured at autopsy. The following arbitrary units were used to report these data: 3 = full clot, no fluid phase, 2 = full clot, little fluid phase, 1 = decreased clot size, more fluid phase, 0 = no clot.

Direct Measurement of in vitro-formed Clot. Measurement of length and weight of clot was undertaken before and after infusion in the case of the in vitro-formed and "pushed in" clots. This procedure was found to be rather unsatisfactory and for this reason was later abandoned. The clots were difficult to remove quantitatively from the vessel. It was difficult to determine whether to include or exclude some viscous material which was found at both ends of the clot. Changes in weight were often not parallel with changes in length.

Radiography. This was performed to determine the degree of passability of x-ray contrast media through the thrombosed area.

Radioactivity. As described above, the radioactivity of the clots was continuously recorded. Counts were corrected for natural decay of the isotope, dead time of the tube and background radiation. No corrections were found to be necessary for backscattering and calibration of the instruments. A source of error of this method in chronic experiments appeared to be slight changes in geometry since it was not possible to place the shield exactly in the same position at each daily reading. This error did not enter into acute experiments.

Autopsy and Histopathology. At the termination of each experiment, all the vessels in which clots were formed were removed and subjected to histologic study.

Before starting the infusion of either the test drug or saline, 1 million units of penicillin* and

*Penicillin was kindly supplied by Lederle Laboratories, Pearl River, N. Y.
250 mg. of streptomycin* was injected subcutaneously into the animal to avoid infection. Terramycin powder was applied locally to the wound. Trypsin, was infused in 500 ml. of physiological saline in 4 hours. This infusion rate resulted in no fall of blood pressure with doses up to 35 mg./Kg.

Experiments in Dogs with Cr* labeled Clots

Methods were identical with those described in the previous section, except for the method of labeling the clot. Red blood cells were labeled with Cr*. Since Cr* is not taken up as rapidly as I*, it was necessary to incubate the cells prior to injection into the isolated vein segment. Approximately 10 ml. of blood was removed from the saphenous vein of the dog, placed into a test tube containing sodium oxalate and 40 µ. of Cr*. The mixture was incubated for 2 hours at 37°C. The blood then was centrifuged at 2000 rpm and the red blood cells washed three times with Tyrode solution. Approximately 0.8 ml. of the concentrated, labeled red blood cells was injected into the isolated segment of the vein. This was followed by thrombin as in the previous studies. When the clot formed, the radioactive cells were incorporated into the meshwork of fibrin. Thrombosed segments removed after a period of from one hour to five days showed no radioactivity of the vessel wall.

Experiments Using I* labeled Fibrinogen

I* labeled fibrinogen was prepared on the basis of a modification of the method of Mihalyi and Laki.13 Two grams of commercial bovine fibrinogen was dissolved in 100 ml. of a 0.1 M phosphate buffer at pH 6.4. To this was added 100 µ. of Cr*. The mixture was incubated for 2 hours at 37°C. The blood then was centrifuged at 2000 rpm and the red blood cells washed three times with Tyrode solution. Approximately 0.8 ml. of the concentrated, labeled red blood cells was injected into the isolated segment of the vein. This was followed by thrombin as in the previous studies. When the clot formed, the radioactive cells were incorporated into the meshwork of fibrin. Thrombosed segments removed after a period of from one hour to five days showed no radioactivity of the vessel wall.

Production of Peripheral emboli. Five milliliters of blood was withdrawn and mixed with 1 to 2 ml. of I* labeled fibrinogen and a few drops of bovine thrombin. The clot thus formed was homogenized in a Potter-Elvehjem type homogenizer, allowed to stand for a few minutes, the supernate decanted, and the fibrin fibers suspended in saline. One-half milliliter of this suspension was injected through a cannulated side branch into the femoral artery. The leg was immediately scanned with the scintillation counter and the areas showing approximately equal radioactivity were marked on the shaved skin with fuchsin. Radioactivity of these areas was followed up for at least five days.

Experiments in unanesthetized Rabbits

A segment of the marginal ear vein was isolated between two artery clamps. Using long curved hemostats, all side branches to the vein were compressed. Fifty units of thrombin in 0.05 ml. of saline, and 0.5 µ. of I* labeled sodium phosphate in 0.05 ml. saline were injected through a 27 gauge needle into the distal end of the segment. By withdrawing and reinjecting the material several times, thorough mixing was effected with the blood in the area. If the side branches were not well compressed, and sufficient amounts of thrombin entered the circulation, the animals died. In experiments with I*, 0.05 ml. of labeled fibrinogen alone or together with 0.05 ml. of 5 per cent sodium

* Streptomycin kindly supplied by Sharp and Dohme, Division of Merck & Co.
† Terramycin kindly supplied by Chas. Pfizer & Co., Brooklyn, N. Y.
‡ Bovine fibrinogen, Bios Lab., Inc., New York, New York.
morrhuate* was injected into the marginal ear vein as described above. A half hour after injecting the above material, the distal clamp was removed. Clot size was estimated by transillumination. Radiographic studies were made using Thorotray or Diodrast. Radioactivity was registered with the aid of a shielded Geiger-Mueller counter in the experiments using $^{14}C$ and with a scintillation counter in the studies using $^{131}I$ labeled fibrinogen. The ear was placed on the lead shield. (Figures 2D§, 5D§, 6D§.)

Production of Pulmonary Emboli

Pulmonary emboli were induced by injecting $^{131}I$ labeled fibrin particles (produced in vitro as previously described) into the marginal ear vein. The chest area was scanned with the scintillation counter. It was not possible, however, to definitely circumscribe areas of equal activity as in the case of the peripheral emboli. Thus, this method is regarded as a qualitative one for the indication of disappearance of pulmonary emboli.

Biochemical determinations. Prothrombin activities were determined periodically from oxalated plasma with the aid of Simplastin.$^{14}$ Duplicate determinations were made on both 100 per cent and 50 per cent plasma. Values used in tabulating the results are the means of the determinations with the 100 per cent plasma. Clotting index was calculated as the ratio of the prothrombin activity before treatment and the experimental values. To determine the relative role of changes in fibrinogen level in alterations of the index, an additional series of prothrombin time determinations were made after the addition of 0.1 ml. of 2.5 per cent fibrinogen solution to the test mixture. An equal volume of phosphate buffer, pH 7.2, was added to a control tube. Plasma fibrinogen levels were determined in 100 per cent and 50 per cent plasma. Clotting index was calculated as the ratio of the prothrombin activity before treatment and the experimental values. To determine the relative role of changes in fibrinogen level in alterations of the index, an additional series of prothrombin time determinations were made after the addition of 0.1 ml. of 2.5 per cent fibrinogen solution to the test mixture. An equal volume of phosphate buffer, pH 7.2, was added to a control tube. Plasma fibrinogen levels were determined in 100 per cent and 50 per cent plasma. Clotting index was calculated as the ratio of the prothrombin activity before treatment and the experimental values.

Blood Pressure, Electrocardiogram, and Respiratory Frequency. Blood pressure was recorded from the carotid artery through a mercury manometer. Respiration was taken with a dog pneumograph. A Sanborn-Viso Cardiette was used to record the electrocardiogram.

Autopsy and Histopathologic Examinations. Routine clinical procedures were performed on each experimental animal. Representative samples of all organs and glands were fixed in both formaldehyde 4 per cent and Carnoy fixative. Mounted sections were routinely stained with Delafield's haematoxylin and eosin. Selected tissues were stained with chromatrope 2 R.

Enzymes. Tryptar* was used after recrystallization. Human plasminogen† (fraction A) was prepared by the method of Kline$^{15}$ and activated in vitro with streptokinase-streptodornase‡ as indicated in the tables. Plasmin§ as well as trypsin was assayed by the method of Loomis$^{11}$ and all values for plasmin or plasminogen refer to Loomis units of plasmin activity or potential activity of plasminogen after activation. Tryptin was used on a mg./Kg. basis.

Preliminary Experiments

It appears from the results that most experiments with trypsin were negative. To investigate the sensitivity of the recording methods, experiments were undertaken with plasmin, a fibrinolytic agent, the effectiveness of which has been demonstrated. Figure 2 shows recordings from an acute experiment in which 10 Loomis units of bovine plasmin were incorporated into the clot. Significant lysis is apparent within five hours and almost complete dissolution occurred within 24 hours. Daily readings were recorded of an experiment in which the dog was treated intravenously with 30 units/Kg./day of human plasmin, fraction A, prepared according to the method of Kline$^{18}$ (fig. 7D§). A gradual decrease of radioactivity took place with indications of almost complete lysis at 96 hours. These values are, of course, not corrected for natural decay and other factors mentioned above.

It was necessary to investigate whether uptake of isotopes by the vessel wall in the thrombosed segment would constitute a source of error. At various intervals after forming the clots by the above methods in vivo, the segment was excised, the clot removed, the vessel wall washed in saline, wet ashed and its radioactive content determined. The results were negative.

* Tryptar, lyophilized crystalline trypsin, Armour Laboratories, Chicago, Ill.
† For starting material, generous quantities of human blood and blood fraction III were kindly supplied by the University of Pennsylvania Hospital blood bank, E. R. Squibb and Co. through the American Red Cross and Dr. R. K. Brown of the State of New York Department of Health, Albany, N. Y.
‡ Streptokinase (Varidose) generously supplied by Lederle Labs.
§ Bovine plasmin was kindly supplied by Parke, Davis & Co., Detroit.

* Sodium morrhuate 5 per cent w/v with benzyl alcohol 2 per cent. Endo Products, Richmond Hill, N. Y.
† Warner-Chilcott Laboratories, New York, N. Y.
activity determined. No significant amounts of $^{131}$I (from labeled fibrinogen) or $^{51}$Cr were taken up within five days. On the other hand, $^{32}$P was taken up in the first few minutes, and its presence can represent an important error.

In the method used, labeled fibrinogen was mixed with the dog’s own blood and the mixture clotted by the addition of thrombin. Fibrinolytic agents thus work on both labeled and unlabeled fibrin in the clot. If the distribution of labeled fibrinogen is not homogeneous, the results might be variable. For this reason, in vivo-formed clots were removed immediately after formation. They were approximately halved or quartered. The radioactivity of each was measured as described above. Distribution of radioactivity appeared to be fairly even considering the crudeness of the procedure.

The question arose whether iodide would be liberated if a clot formed with $^{131}$I labeled fibrinogen were lysed by trypsin. If so, registration of radioactivity over the thyroid gland might be a more sensitive method to detect small degrees of fibrinolysis rather than registration over the highly radioactive clot itself. To 4 ml. of labeled fibrinogen, thrombin was added and a clot produced. Fifty milligrams of trypsin was then added and the mixture incubated at 37 C. overnight. By next morning lysis of the fibrin had occurred, with only a few flocules remaining in the fluid. After filtration, 1 ml. of the material was injected intravenously into a dog and 1 ml. into each of two rabbits. The animals were placed in metabolism cages, and urine and feces were separately collected. The animals were periodically scanned with the scintillation counter and blood samples were withdrawn. No radioactivity could be detected in the thyroid and salivary glands or other organs. Blood samples were negative from the tenth minute on. The animals were sacrificed after 24 hours and samples of all organs, and pooled urine and feces samples were wet ashed in alkali and radioactivity determined. High activity was found in the urine and slight activity in the livers. None of the other samples showed counts above background. It appears that in the process of lysis of an $^{131}$I labeled fibrin clot, iodide is not liberated nor are diiodotyrosine or related metabolites split off. Apparently peptides of low molecular weight are produced which are small enough to be excreted in the urine.

Preliminary toxicity studies indicated that
the maximal acutely tolerated dose of trypsin in dogs is 35 mg./Kg.; the maximal chronically tolerated dose is 25 mg./Kg. These doses caused severe hypotension if injected rapidly intravenously, but did not alter blood pressure if infused in 500 ml saline solution over a period of four hours. This infusion rate was used in all experiments. Rabbits tolerated two daily injections of 10 mg./Kg. into the ear vein for five days. A single daily injection of 20 mg./Kg. proved to be fatal to most animals.

**RESULTS**

*Experiments in dogs and rabbits using P³²-labeled clots.* Table 2D§ indicates abbreviations used in all of the following tables. Tables 3D,§ 4D,§ and 5D§ show results and statistical analysis of control and trypsin experiments in dogs using P³² labeled clots. In these experiments, daily infusions of 10 mg./Kg., 25 mg./Kg. and 35 mg./Kg. were given daily for five days. Radioactivity decreased in all clots, while transillumination and phlebography revealed no decrease in clot size. Probably labeled cells were disintegrated and washed out from the clot. Analysis of variance revealed no significant difference in decrease of radioactivity between control and treated groups.

Tables 6D,§ 7D,§ and 8D§ show and analyze statistically similar experiments in treated and control rabbits. Trypsin was given in doses of 10 mg./Kg. twice daily, intravenously. Similar to the above results, decrease of radioactivity was noted in practically all ear vein clots. On the other hand, in some of these instances, decrease of the clot size or restoration of patency as shown by phlebography was also evidenced. Since it was not possible to place ligatures proximal to the marginal ear vein clots it is likely that some of the clots slipped away. Scanning the lungs with the scintillation counter, indicated the presence of pulmonary emboli in the instances marked in the tables.

On the basis of these experiments, and because of the P³² uptake by the vein wall in the area (see preliminary experiments), it was thought that P³² labeling may not be a suitable method for testing fibrinolysis in vivo.

*Experiments using Cr⁶¹ labeled clots.* Tables 9D§, 10D§, and 11D§ show results obtained in control and trypsin (25 mg./Kg. or 35 mg./Kg. daily for five days) treated dogs as well as statistical analysis of the results. It appears that radioactivity decreased in all clots indicating probably lysis and washing out of the labeled red cells. Analysis of variance indicated no significant difference between the control and treated groups. It appears that Cr⁶¹ labeling may have disadvantages similar to P³² labeling.

*Experiments in dogs and rabbits Iⁱ¹ labeled clots.* Table 12D§ shows results in control rabbits with thrombi produced in the marginal ear vein and disseminated pulmonary emboli. Table 13D§ summarizes results in animals treated by two daily injections of 10 mg./Kg. trypsin intravenously. Figures 8D§ and 9D§ graphically illustrate the results in typical control and treated rabbits. It appears that radioactivity of the ear vein clots decreased significantly during the observation period; decrease of the pulmonary embolus values was even more pronounced. In the former the lack of

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**Fig. 3.** Graphs showing radioactivity (ordinates) —counts/min. X 10⁶ (upper) and clotting index (lower) in a control dog (no. II1, 2, 15).
proximal constricting ligatures, in the latter the large surface of exposure may be partly responsible. Analysis of variance, Table 14D§ revealed no significant difference between control and treated groups.

Tables 15D§ and 16D§ show results in control and trypsin treated (25 mg./Kg.) dogs in which I\(^{131}\) labeled arterial and venous clots and peripheral emboli have been produced. Figures 3, 4, 10D§ and 11D§ illustrate results in typical control and experimental dogs, including biochemical findings. In control dogs, decrease of radioactivity during the five day observation period was found to be minimal in contrast to the experiments with P\(^{51}\) or Cr\(^{51}\) labeled clots. No discrepancy was seen between results of transillumination, radiography and measurement of radioactivity in experiments using I\(^{131}\) labeled fibrinogen. Decrease of radioactivity was more pronounced in areas with peripheral embolization. Analysis of variance, table 17D§ indicated no significant difference between the treated and control groups. In all control experiments in which canalized clots were used, the central canals became completely obstructed within one hour. In experiments where infusion of trypsin was started before removal of the polyethylene rod, no obstruction of the canal occurred until after the four hour infusion period. Such experiments are illustrated in figure 5. It thus appears that while trypsin in maximal tolerated, and even in toxic dose ranges, did not exhibit significant fibrinolytic effect, it had a powerful anticoagulant activity.

Biochemical and pathologic studies. Infusion of trypsin produced a marked fall in plasma fibrinogen levels and in clotting index. Decrease of clotting index appeared to be only partially due to fall of fibrinogen level, since addition of excess fibrinogen did not normalize the clotting index (fig. 4). It was frequently
observed that 24 hours after the first infusion of trypsin, regeneration of the above factors was not complete. The subsequent infusions were followed by less and less regeneration, the animals finally dying of multiple hemorrhages.

Histopathologic* studies will be reported elsewhere in detail. In trypsin-treated animals, hemorrhage and sinusoidal congestion was observed in the lungs, liver and in the gastrointestinal tract; occasionally gastric ulceration occurred. In the heart, focal hemorrhage was frequently observed and, in one case, myocardial degeneration was seen. Cerebral hemorrhage was noticed in only one animal.

**Discussion**

Shulman and Tagnon20 were the first to describe the use of I" labeled fibrin for the in vitro testing of fibrinolytic agents. The increase of radioactivity in the supernatant solution was taken as a measure of fibrinolytic activity. It was thought that the method of labeling used in this study may produce higher activity and may thus be more suitable for in vivo experiments. This method involved dissolution of fibrinogen in urea solution. Mihalyi and Laki19 studied in detail the properties of iodinated fibrinogen exposed to urea as compared to unaltered fibrinogen.

It is a weakness of the procedure used that bovine fibrinogen has been employed to produce clots in dogs, and this method has even been used to test the fibrinolytic effect of human plasmin preparations. This was done out of necessity since we had no facilities to prepare sufficient quantities of fibrinogen and plasmin from dogs.

Several methods and procedures have been investigated. Finally the registration of radioactivity over venous or arterial clots or emboli prepared with I131 labeled fibrinogen was found most satisfactory. Results correlated well with the findings obtained by the more commonly used, less quantitative procedures (transillumination, phlebography, palpation, histopathology). The sensitivity of the method is indicated by the fact that the fibrinolytic effect of 10 Loonis units of plasmin incorporated into the clot (fig. 2) or 30 units/Kg. body weight, i.v., figure 7D§ can readily be demonstrated. Methods based on recording of the I131 liberated from the clot into the blood stream or measurement of uptake by the thyroid or other organs were shown to be impractical.

**Summary**

Several methods have been investigated for the quantitative measurement of fibrinolytic activity in vivo. Methods based on incorporation of P53 or Cr45 into blood cells trapped in the meshwork of the clot or based on determining weight changes in preweighed clots were found to be unsatisfactory.

Arterial or venous clots, pulmonary, peripheral, or coronary emboli were produced with I131 labeled fibrinogen. Decrease of radioactivity in these areas as recorded by a scintillation counter connected to a radiation rate meter and an Esterline recorder was found to be a quantitative measure of fibrinolytic activity. Results correlated well with the findings obtained by other methods (transillumination, phlebography, palpation, histopathology).

A method has been described to differentiate between fibrinolytic and anticoagulant effects. This is based on the formation of a radioactive clot with a central canal. Decrease of radioactivity in the area indicates fibrinolysis, delay of obstruction of the canal as shown by phlebography indicates anticoagulant activity. In addition clotting index and fibrinogen levels have been measured.

Trypsin has been administered for five days in the maximal acutely and maximal chronically tolerated doses. No significant fibrinolytic effect has been observed in vivo as tested by the above methods. On the other hand a powerful anticoagulant effect was noticed. Fibrinogen levels and clotting index decreased. Many animals died during the five day treatment period. Autopsy and histopathologic studies demonstrated multiple hemorrhages.

**Summario in Interlingua**

Esseva investigate plure methodos pro le mesuration quantitative de activitate fibrinolytic in vivo. Se monstrava non-satisfactori le
methodos basate super le incorporation de P35 o Cr44 in cellulas sanguinee capturate in le structura del coagulo o super le determination de alterationes de peso in coagulos pre-ponderate.

Coagulos arterial o venose e embolos pulmonar, peripheric, o coronari esseva producite per medio de fibrinogeno etiquettate con I131. Le reduction dc radioactivitate in iste areas—registrate per un contator de scintillation conectite con un velocimetro de radiation e un registrator Esterlinc—se revelava como mesura quantitative del activitate fibrinolytic. Le resultatos se trovava in bon correlation con le constatationes obtenite per altere methodos (transillumination, phlebographia, palpation, histopathologia).

Es describitc un methodo pro le differenciation inter effectos fibrinolytic e anticoagulante. Illo se basa super le formation de un coagulo radioactive con un canal central. Reduction del radioactivitate in le area indica fibrinolyse; retardation del obstruction del canal (demonstrabile per phlebographia) indica activitate anticoagulante. In plus, le indice coagulative e le nivellos de fibrinogeno esseva mesurate.


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Circ Res. 1956;4:430-439
doi: 10.1161/01.RES.4.4.430

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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