

INTRAVENOUS TRYPSIN: ITS ANTICOAGULANT, FIBRINOLYTIC AND THROMBOLYTIC EFFECTS¹

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Fibrinogenolysis and prolonged blood coagulation have been observed following large intravenous doses of crystalline trypsin (1).² The present study was undertaken to evaluate further the effects of intravenous crystalline trypsin upon the protein components of the coagulation mechanism and the fibrin content of artificially induced intravascular thrombi, in 60 rabbits and 25 dogs. A striking observation in this study was the lack of untoward reactions, including shock, when the rate and concentration of injected trypsin were kept within carefully calculated limits.

MATERIALS

1) Sixty rabbits whose average weight was 2 kg. were fed rabbit food pellets and given water *ad libitum*.

2) Twenty-five mongrel dogs, including 5 dogs used as controls, were housed together and fed Purina Dog Chow. Their average weight was between 10 to 12 kg.

3) Crystalline trypsin, *i.e.* Armour Tryptar® preparation,³ was used in each experiment.

4) 0.5 ml. of 10 per cent sodium morrhuate, or 0.2 ml. (125 units) of topical thrombin (Parke, Davis), or 0.5 ml. of thromboplastin (simplastin) were used to produce thrombi in rabbits and dogs.

5) Bovine fibrinogen (Armour), and topical thrombin (Parke, Davis) were used in antithrombin determinations.

METHODS

1) *Experimental production of thrombi in rabbits and dogs.* Experimental thrombi were produced in one of the marginal ear veins of each of 60 rabbits by injecting 0.5 ml. of 10 per cent sodium morrhuate or 125 units

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² On the basis of animal observations crystalline trypsin (Tryptar) was administered intravenously to 101 patients. The only untoward reaction was an occasional mild chill toward the end of an infusion. It appears that, when carefully controlled, intravenously administered crystalline trypsin is a safe procedure. Data on this clinical study constitute a separate report.

³ The trypsin used in this study was kindly supplied by Marvin C. Ziporyn, M.D., of the Armour Laboratories.

of topical thrombin or 0.5 ml. thromboplastin into the vessel. The distal portion of the vein was then compressed for 5 to 10 minutes. Experimental thrombi were produced in the femoral vein of 10 dogs. Under Nembutal® anesthesia, the femoral vein was exposed in the upper third of the thigh without dividing the femoral fascia. Then 0.5 ml. of 10 per cent solution of sodium morrhuate was injected into the vein. During the injection, and for 5 to 6 minutes thereafter, the vessel was gently compressed distal to the point of the injection. A firm thrombus, verified by palpation and dissection of the vessel, formed 10 to 15 minutes following instillation of the thrombogenic agent.

2) *Preparation of trypsin solution.* In experiments on rabbits, 250,000 units of specially prepared trypsin (250 mg. of tryptic activity) were dissolved in 250 ml. of isotonic saline. A vinylite catheter was introduced into the ear vein, and the inflow was regulated at 6 drops per minute. In experiments on dogs, 1,000,000 units of trypsin were dissolved in 500 ml. of isotonic saline, and introduced through the femoral vein. The infusion rate was maintained at 35 to 40 drops per minute.

3) *Laboratory determinations.* All prothrombin determinations were done according to the method of Quick (2). Fibrinogen was determined by the method of Greenberg (3). The Innerfield, Angrist, and Benjamin method (4) of antithrombin titer determination was used. Factor V was measured by the method of Stefanini (5); and fibrinolysis assay was made by the Conley, Ratnoff, and Hartmann method (6). Determinations of coagulation time were made by the Lee-White method (7). Blood volume was estimated following administration of labeled albumin (8). Continuous measurements of clotting viscosities were made with a Rich-Roth Ultra-viscosimeter (9).

PROCEDURE

1) *Rabbits.* Blood samples were obtained from non-anesthetized rabbits by cardiac puncture, before and at varying intervals during infusion of trypsin. Sections of an ear vein thrombus were obtained for histologic study prior to, during, and after completion of each intravenous infusion.

2) *Dogs.* Trypsin infusions were given to dogs who had been anesthetized with 6 per cent Nembutal. Blood samples were obtained prior to, and during the course of the infusion. In securing

TABLE I

Mean effect of increasing increments of small intravenous trypsin dose upon the coagulation mechanism in 6 dogs

Trypsin units 30 drops/ min.	Pro- thrombin time seconds	Factor V units	Fibrino- gen mg. %	Anti- thrombin clotting time —5 Min. Incuba.— seconds	Coagula- tion time minutes
Control	13	750	360	26	7.6
500	13	710	368	27	7.0
1,000	13	690	354	26	7.1
2,000	13.5	705	373	26	7.5
3,000	14	670	349	27	8.1
5,000	12.5	720	370	26	7.6

plasma, nine volumes of blood were added to one volume of 1.85 per cent sodium citrate; the plasma was carefully removed from the cellular elements and determinations were carried out. Sections of thrombi artificially induced in the femoral vein were obtained from 10 dogs.

RESULTS

The data are summarized in Tables I through V. Following the administration of small doses

TABLE IV

Duration of prolonged clotting time following massive intravenous doses of trypsin

Dogs	Trypsin dosage 30 drops/ min.	Clotting time				
		Before treatment	After treatment			
			½ hr.	1 hr.	15 hrs.	24 hrs.
	units	min.	min.	min.	min.	min.
D	300,000	8	42	7	8	8
B	750,000	6	400+	400+	400+	9
G	1,000,000	8	400+	400+	400+	12

of trypsin to 6 dogs, no significant changes were noted in the coagulation mechanism (Table I). Control studies included determinations of coagulation time, prothrombin time, factor V, anti-thrombin clotting time, and fibrinogen. Following the administration of trypsin intravenously in graded doses ranging from 500 to 5,000 units, no appreciable deviation from normal was noted. Following the administration of intravenous trypsin in doses of 25,000 to 250,000 units in rabbits, and 50,000 to 1,000,000 units in dogs, the blood

TABLE II

Mean effect of increasing increments of massive intravenous trypsin dose upon the coagulation mechanism, thrombi, and blood volume in 60 rabbits

Trypsin units 30 drops/min.	Prothrombin time seconds	Factor V units	Fibrinogen mg. %	Antithrombin —5 min. incuba.— seconds	Antifibri- nolysin	Coagulation time min.	Effect upon thrombi	Blood volume %
Control	5	650	350	25	++	3	0	100
25,000	11	610	370	25	++	3.5	0	100
50,000	17	590	340	26	+	2.8	0	98
75,000	22	540	310	24	+	4.6	++	96
100,000	36	410	290	21	0	7.8	++	91
150,000	54	390	210	18	0	15.9	+++	76
200,000	100+	48	55	14	0	60+	++++	51
250,000	100+	31	22	14	0	60+	++++	33

TABLE III

Mean effect of increasing increments of massive intravenous trypsin doses upon the coagulation mechanism, thrombi and blood volume in 25 dogs

Trypsin units 30 drops/min.	Prothrombin time seconds	Factor V units	Fibrinogen mg. %	Antithrombin —5 min. incuba.— seconds	Antifibri- nolysin	Coagulation time min.	Effect upon thrombi	Blood volume %
Control	14	750	350	25	+ to ++	7.6	0	100
50,000	16	670	365	24	++	7.9	0	96
100,000	19	640	220	26	+	8.2	+	93
200,000	29	590	165	23	+	11.1	++	84
300,000	56	270	70	21	0	42.2	+++	91
500,000	300+	110	50	17	0	150+	++++	86
750,000	300+	too	too	16	0	400+	++++	68
1,000,000	300+	low to assay too low to assay	low to assay too low to assay	16	0	400+	++++	44

TABLE V
Fibrinolysis in 15 dogs at increasing levels of
intravenous trypsin

Dogs	Occurrence of fibrinolysis trypsin units					
	0	50,000	100,000	200,000	350,000	1,000,000
1	—	—	—	X	X	XX
2	—	—	X	X	XX	XX
3	—	—	—	—	X	XX
4	—	—	X	X	X	XX
5	—	—	X	X	X	XX
6	—	—	—	—	X	XX
7	—	—	—	X	X	XX
8	—	—	—	—	X	XX
9	—	—	X	X	XX	XX
10	—	—	—	—	X	XX
11	—	—	—	X	X	XX
12	—	—	—	—	XX	XX
13	—	—	X	X	X	XX
14	—	—	—	—	—	XX
15	—	—	—	—	X	XX

— No fibrinolysis.

X Fibrinolysis within the first 24 hours.

XX Fibrinolysis within 20 minutes.

coagulation time was prolonged, protein constituents of the clotting mechanism showed diminishing titers, the blood volume was reduced, and changes in the consistency and microscopic appearance of intravascular thrombi were observed. Quantitatively, these changes roughly paralleled the level of enzyme concentration infused at any given time.

A. Rabbits

1) *Prothrombin*. The magnitude of change in prothrombin time varied from 5 seconds in the control animals to greater than 100 seconds after the administration of more than 200,000 units of trypsin.

2) *Factor V*. The mean level of factor V in control rabbits was 650 units. Appreciable diminution did not occur until 100,000 units of trypsin had been administered. A precipitous fall occurred when the dose exceeded 200,000 units.

3) *Fibrinogen*. There was slight variation from the normal value of 350 mg. per cent following the introduction of 100,000 units of trypsin. When 150,000 trypsin units had been administered, a drop to 210 mg. per cent was observed. A profound fall in fibrinogen occurred following doses exceeding 200,000 trypsin units.

4) *Antithrombin*. Subsequent to the administration of 75,000 trypsin units mean antithrombin titers declined. Marked depression in antithrom-

bin titer occurred when the trypsin dose exceeded 200,000 units.

5) *Antifibrinolysin*. The phenomenon of antifibrinolysis disappeared following doses in excess of 100,000 trypsin units.

6) *Effect upon thrombi*. Changes in thrombi were recorded on a 0 to 4 plus basis, varying from no observable change to increasing thrombolytic changes (Figure 1). No appreciable gross or microscopic alterations in thrombi were noted prior to the administration of 75,000 units. Two to three plus changes occurred in the 75,000 to 200,000 trypsin unit range. Complete clot dissolution and restoration of circulatory integrity consistently occurred at a range beyond 200,000 units of trypsin.

7) *Blood volume*. Significant changes in mean blood volume did not occur prior to the administration of 150,000 units of trypsin. At 200,000 units, however, the blood volume was 51 per cent of control; at 250,000 units the blood volume fell to 33 per cent of control.

8) *Coagulation time*. The coagulation time showed slight variations prior to the administration of 100,000 trypsin units. Following the administration of additional trypsin, the coagulation time became markedly prolonged. When trypsin doses exceeded 200,000 units, *in vitro* blood samples failed to clot throughout an observation period of 72 hours.

B. Dogs

1) *Prothrombin time*. The prothrombin time showed prompt changes following increasing doses of trypsin. After 100,000 units of trypsin the prothrombin time was 19 seconds; after 300,000 units the prothrombin time was 56 seconds; at and above 500,000 units the prothrombin time was greater than 300 seconds. The control prothrombin time was 14 seconds.

2) *Factor V*. The control was 750 units. Marked diminution was observed after 300,000 units of trypsin had been introduced. Following trypsin doses exceeding 750,000 units, the quantity of factor V was too low to determine.

3) *Fibrinogen*. Pronounced decrease in fibrinogen (control level, 350 mg.) was observed after the administration of 200,000 units of trypsin. Extreme depression in fibrinogen was observed with 300,000 trypsin units. Quantities too low to

assay appeared when more than 750,000 units of trypsin were administered.

4) *Antithrombin*. The titer of antithrombin varied inversely with the quantity of injected trypsin. This inverse relationship became most apparent after 500,000 units had been administered. "Absolute" depression in antithrombin titer occurred beyond 750,000 trypsin units.

5) *Antifibrinolysin*. Following the administration of trypsin in doses greater than 350,000 trypsin units, the phenomenon of antifibrinolysis could not be demonstrated.

6) *Effect on thrombi*. Lytic changes in thrombi were demonstrable following administration of 100,000 trypsin units.

7) *Blood volume*. Significant changes in blood volume did not occur until trypsin was adminis-

tered in quantities exceeding 500,000 units. At 750,000 units of trypsin, the blood volume dropped to 68 per cent of control; at 1,000,000 units the blood volume was 44 per cent of control. Between 300,000 and 500,000 units, the blood volume varied from 91 to 96 per cent respectively.

8) *Coagulation time*. The coagulation time showed only moderate prolongation prior to the administration of 300,000 units of trypsin. Inordinately prolonged blood coagulation time followed doses exceeding 500,000 units of trypsin. Blood samples obtained one half-hour after 300,000 unit infusions of trypsin showed a mean coagulation time of 42 minutes. Samples obtained one hour after the administration of 300,000 units of trypsin, however, showed normal coagulation time. Following the administration of trypsin in a

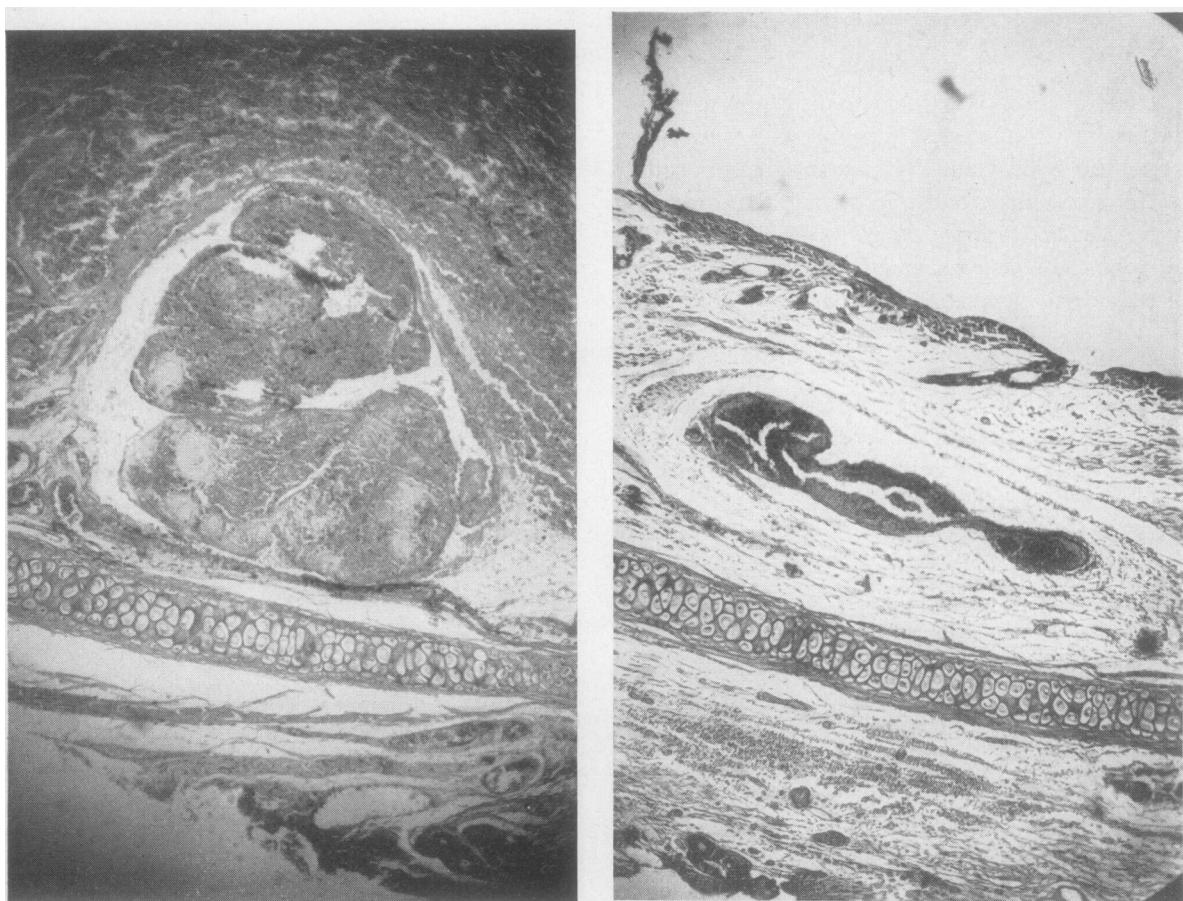


FIG. 1. HISTOLOGIC SECTIONS OF EXPERIMENTALLY PRODUCED EAR VEIN THROMBUS IN RABBIT TAKEN

(A) Immediately following completion of injection of sodium morrhuate, and

(B) Twenty-four hours after intravenous administration of 200,000 Armour Units of trypsin in 250 ml. of isotonic saline, given at the rate of six drops per minute into the marginal vein of opposite ear.

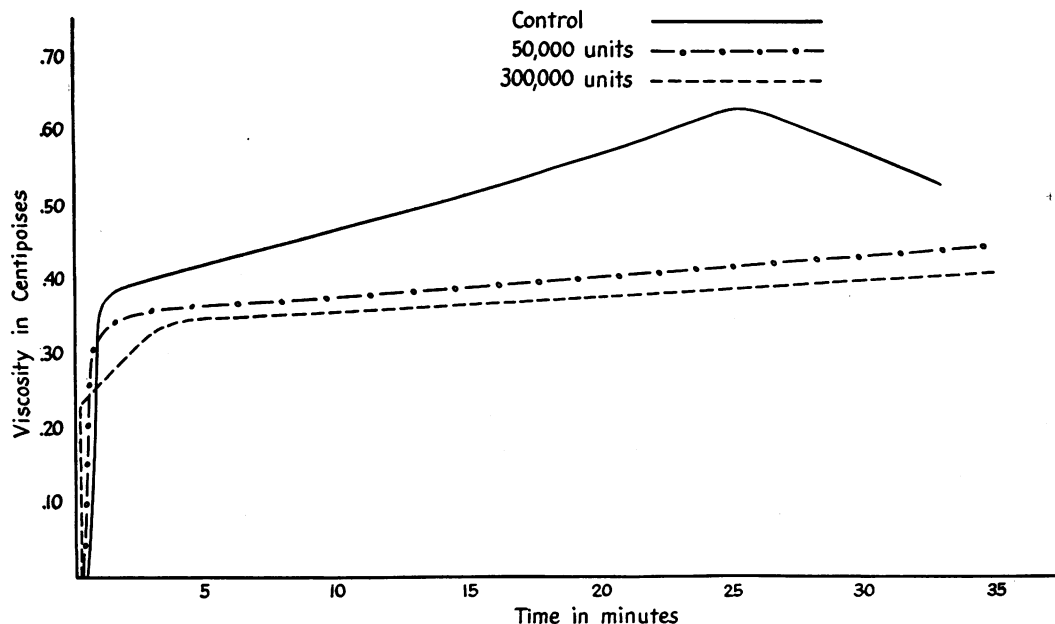


FIG. 2. CHANGES IN BLOOD CLOTTING VISCOSITY FOLLOWING INTRAVENOUS ADMINISTRATION OF TRYPsin TO A DOG

750,000 to 1,000,000 unit range, the coagulation time was greatly prolonged in random blood samples obtained during a 15 hour period of observation. At the end of 24 hours, the clotting time showed only slight prolongation.

9) *Fibrinolysis*. There was no evidence of fibrinolysis prior to the administration of 50,000 units of trypsin. Samples obtained from five of 15 dogs showed evidence of fibrinolysis at the 100,000 units level. Samples obtained from eight of 15 dogs showed fibrinolysis at the 200,000 trypsin unit level, and 14 of the 15 dogs showed fibrinolysis after 350,000 trypsin units. Each sample tested showed fibrinolysis when 1,000,000 units was administered.

10) *Blood clotting viscosity*. A sharp decrease in blood clotting viscosity occurred following the administration of 50,000 units of trypsin to one dog. More marked diminution in viscosity was evident after the administration of 300,000 units to the same animal (2).

DISCUSSION

Activation of proteolytic enzyme systems (fibrinolysis) has been produced by diversified methods in the past two decades. During the course of *in vitro* experiments this phenomenon followed: (a) the conversion of plasminogen to

plasmin by culture filtrates of *Streptococci hemolyticus* (streptokinase) (10), (b) the removal of plasmin inhibitor by certain organic solvents, such as chloroform and ether (11), (c) the activation of proplasmin into plasmin by a "Factor" from mammalian tissue (12), (d) the conversion of an inactive precursor enzyme present in culture filtrates of group A streptococci into a proteinase by autocatalysis or by the action of trypsin (13), (e) the action of pancreatic trypsin upon plasminogen causing the release of plasmin (14, 14a) and (f) the action of trypsin upon fibrin (15).

Although there is convincing evidence that trypsin produces fibrinolysis *in vitro*, our observations on the fibrinolytic effects of intravenous trypsin do not necessarily prove a direct trypsin-fibrin substrate interaction. Trypsin may resemble streptokinase in its capacity to activate a fibrinolytic system by transforming naturally occurring plasminogen into plasmin (14, 14a). Whether plasmin then acts synergistically with trypsin, or the fibrinolytic effects following intravenous trypsin are predominantly due to the liberation of plasmin requires further evaluation.

There have been reports that intravenous trypsin caused shock (16), intravascular coagulation (17, 19) local necrosis (18), sloughing of tissue (18), and was generally "too toxic for intravenous use"

(19). The appearance of shock in our experiments was conditioned by such critical factors as infusion rate, enzyme concentration, and total dose. In a previous study, shock developed within 15 seconds following a rapid intravenous injection of trypsin in a dose of only 2,000 units/kg. (1). In the present investigation, administration of the enzyme solution at 6 to 8 drops per minute in rabbits, and 30 to 40 drops per minute in dogs did not produce shock for several hours during the course of a single infusion, despite massive enzyme concentrations and dose, *i.e.*, 125,000 units/kg. in rabbits and 100,000 units/kg. in dogs. Occasionally, shock did appear when doses exceeding 150,000 units of trypsin were given to rabbits, or greater than 750,000 units were given to dogs.

We could not confirm the observation that a small dose of intravenous trypsin hastens blood coagulation or predisposes toward intravascular thrombosis (19). Following administration of trypsin in the same small dose range previously associated with intravascular clotting (100 to 200 units/kg.) (20), the coagulation time, prothrombin time, factor V, antithrombin and fibrinogen values were normal (Table I). It is conceivable that circulating antitryptic substances adequately inactivate small quantities of injected trypsin. In order to rule out the possibility that multiple thrombi followed small trypsin doses despite normal findings of the circulating constituents measured, seven rabbits and three dogs were sacrificed following small intravenous trypsin doses. After extensive dissection, no evidence of intravascular thrombosis was noted either grossly or microscopically.

Marked anticoagulant effects followed large intravenous doses of trypsin. When the dose of trypsin was steadily increased (continuous intravenous infusion) a point was reached beyond which all clotting proteins were proteolyzed (Tables II and III). In such extreme instances the blood became incoagulable presumably because the protein constituents of the coagulation mechanism had been depolymerized to amino acids.

The lytic effect of intravenous trypsin upon experimentally produced thrombi was most impressive. Gross changes in the rabbit ear vein thrombi following intravenous trypsin included

1) diminution and in several instances disappearance of the thrombus *in situ*, 2) restoration of local circulation, and 3) restoration of vessel wall compressibility. The microscopic features of trypsin treated thrombi included 1) diminution of fibrin, 2) diminution of cellular and platelet masses, and 3) no untoward effect upon vessel walls. Fibrinolytic effects extended into the laminations and interstices of the thrombus.

Gross changes were not observed in control animals with artificially induced clots who were given intravenous saline. Furthermore, it was noted microscopically, in these saline-treated control animals, that there was a persistence of the dense fibrin meshwork, plus homogenous masses of platelets and cells.

It appears probable, therefore, that the intravenous route effectively provides surface contact between proteolytic enzymes and the fibrin meshwork in thrombi, and that lysis of artificially formed thrombi in rabbits and dogs was produced by virtue of fibrinolytic mechanisms activated by intravenous trypsin. On the basis of these experimental data, the enzymatic action of *in vivo* trypsin upon the coagulation mechanism, and its effect upon fibrin and clots *in situ*, merits further study and elucidation.

SUMMARY

1. Under controlled conditions of infusion rate, enzyme concentration, and dosage, the intravenous administration of trypsin is safe and feasible in rabbits and dogs.

2. Small trypsin doses *in vitro* produce clots. The effects of small trypsin doses *in vivo* is apparently nullified by circulating antitryptic substances.

3. Large trypsin doses both *in vitro* and *in vivo* have powerful anticoagulant effects.

4. Intravenously administered trypsin induces lytic effects upon artificially formed intravascular thrombi in rabbits and dogs.

REFERENCES

1. Innerfield, I., Angrist, A., Benjamin, J. W., and Schwarz, A. W., Studies on trypsin: I. The anticoagulant action of trypsin. *Gastroenterology*, 1952, **20**, 630.
2. Quick, A. J., On the quantitative estimation of prothrombin. *Am. J. Clin. Path.*, 1945, **15**, 560.

3. Greenberg, D. M., The colorimetric determination of serum proteins. *J. Biol. Chem.*, 1929, **82**, 545.
4. Innerfield, I., Angrist, A., and Benjamin, J. W., Antithrombin titer in acute pancreatitis. *Am. J. Med.*, 1952, **12**, 24.
5. Stefanini, M., New one-stage procedures for the quantitative determination of prothrombin and labile factor. *Am. J. Clin. Path.*, 1950, **20**, 233.
6. Conley, C. L., Ratnoff, O. D., and Hartmann, R. C., Studies on afibrinogenemia. I. Afibrinogenemia in a patient with septic abortion, acute yellow atrophy of the liver and bacteremia due to *E. coli*. *Bull. Johns Hopkins Hosp.*, 1951, **88**, 402.
7. Lee, R. I., and White, P. D., A clinical study of the coagulation time of blood. *Am. J. M. Sc.*, 1913, **145**, 495.
8. Aust, J. B., Chou, S. N., Marvin, J. F., Brackney, E. L., and Moore, G. E., A rapid method for clinical total blood volume determination using radioactive iodinated human serum albumin. *Proc. Soc. Exper. Biol. & Med.*, 1951, **77**, 514.
9. Yesner, R., Hurwitz, A., Rich, S. R., Roth, W., and Gordon, M. E., Preliminary observations on blood coagulation utilizing ultrasonics for continuous measurement of viscosity. *Yale J. Biol. & Med.*, 1951, **24**, 231.
10. Tillett, W. S., and Garner, R. L., The fibrinolytic activity of hemolytic streptococci. *J. Exper. Med.*, 1933, **58**, 485.
11. Christensen, L. R., The activation of plasminogen by chloroform. *J. Gen. Physiol.*, 1946, **30**, 149.
12. Tagnon, H. J., and Palade, G. E., The activation of proplasmin by a factor from mammalian tissue. *J. Clin. Invest.*, 1950, **29**, 317.
13. Elliott, S. D., A streptococcal proteinase and its precursor. *Tr. New York Acad. Sc.*, 1952, **14**, 137.
14. Innerfield, I., Unpublished data.
- 14a. Kocholaty, W., Ellis, W. W., and Jansen, H. F., Activation of Plasminogen by Trypsin. *Blood*. To be published.
15. Northrup, J. H., *Crystalline Enzymes; the Chemistry of Pepsin, Trypsin and Bacteriophage*. Columbia University Press, New York, 1939.
16. Roche e Silva, M., The histamine theory of anaphylactic shock. *Arch. Path.*, 1942, **33**, 387.
17. Ferguson, J. H., and Erickson, B. N., The coagulant action of crystalline trypsin, cephalin and lung extracts. *Am. J. Physiol.*, 1939, **126**, 661.
18. Dragstedt, C. A., The digestion of living tissues by proteolytic enzymes. *Science*, 1943, **98**, 131.
19. Tagnon, H. J., Effect of intravenous injection of trypsin on the blood coagulation time in hemophilia. *Proc. Soc. Exper. Biol. & Med.*, 1944, **57**, 45.
20. Tagnon, H. J., The nature of the mechanism of the shock produced by the injection of trypsin and thrombin. *J. Clin. Invest.*, 1945, **24**, 1.