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# AN ACCELERATING PROPERTY OF PLASMA FOR THE COAGULATION OF FIBRINOGEN BY THROMBIN<sup>1, 2</sup>

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The last step in the clotting process is the conversion of a soluble protein, fibrinogen, into an insoluble protein, fibrin. This conversion takes place as the result of the action of an enzyme, thrombin. Among the many factors determining the rate of this reaction are the concentrations of fibrinogen and thrombin, the ionic strength of the medium, and the presence or absence of certain bivalent cations.

Biggs and Macfarlane (1) observed that the clotting time of mixtures of oxalated plasma and thrombin varied in different conditions. They suggested the possibility that there are factors in plasma which accelerate the thrombin-fibrinogen reaction. In the present report, such a factor, present in normal plasma, and capable of accelerating the conversion of fibrinogen to fibrin is described. Some of the characteristics of this accelerating property of plasma will be discussed.

## MATERIALS

*Oxalated human plasma* was obtained by mixing venous blood with one-ninth its volume of 0.1M sodium oxalate solution and centrifuging the mixture at room temperature for 15 minutes at 2,500 rpm in a Size 1, Type SB International centrifuge. The plasma was stored between 0° and 4° C. until used. In some experiments, the plasma was shaken for 10 minutes with a carboxylic acid ion exchange resin, Amberlite IRC-50 (Rohm and Haas) in the sodium phase (pH 7.7), in order to remove bivalent cations.

Commercial bovine *thrombin* (Parke, Davis and Co.) was dissolved in barbital-saline *buffer* in a concentration of 1,000 National Institutes of Health (N.I.H.) units per ml. (2) and stored at -25° C. until used. Amberlite-treated thrombin was prepared in the same manner as amberlite-treated plasma. The clotting time, using this calcium-deficient thrombin, was somewhat longer than with untreated thrombin.

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<sup>2</sup> Presented at the 26th Annual Session of the Central Society for Clinical Research, October 31, 1953.

The *buffer* used was 0.025M barbital in 0.125M sodium chloride solution, at pH 7.5, prepared by dissolving 2.76 Gm. of barbital, 2.06 Gm. of sodium barbital and 7.30 Gm. of sodium chloride in one liter of water.

*Defibrinated plasma* was prepared by mixing 100 parts of oxalated plasma with 3 parts of a solution of bovine thrombin containing 1,000 N.I.H. units per ml. The clot which formed was removed either by wrapping it around a glass rod and expressing the defibrinated plasma, or by shaking the contents of the tube with about one-tenth volume of crushed pyrex glass. The fibrin adhered to the glass so that the defibrinated plasma could be decanted. The defibrinated plasma was then incubated at 37° C. for 30 minutes to inactivate the thrombin. The adequacy of defibrination was tested by the addition of fresh thrombin to an aliquot. To test whether the thrombin had been inactivated, the defibrinated plasma was mixed with a solution of bovine fibrinogen; an absence of clotting was taken to mean that the thrombin was no longer active.

*Barium sulfate-adsorbed plasma* was prepared by mixing oxalated plasma or defibrinated plasma with approximately one-tenth its volume of powdered barium sulfate (Baker). The mixture was incubated at 37° C. for 10 minutes with frequent stirring, and the plasma was then separated by centrifugation for 15 minutes at 2,500 rpm in the International centrifuge.

*Dialysed plasma* or its fractions was prepared by dialysis for 16 to 24 hours in cellophane sacs (Visking No-Jax sausage casings, one inch in diameter) at 4° C. against 0.15M sodium chloride solution. When the solutions contained ammonium sulfate, they were first dialyzed against running cold tap water for several hours before dialysis against sodium chloride. The term *dialysate* will be used to define the solution of sodium chloride against which the plasma or its fraction had been dialyzed.

Crude *fractions of plasma* were prepared by the addition of solid ammonium sulfate to plasma which had first been defibrinated and adsorbed with barium sulfate. Each mixture of ammonium sulfate and plasma was kept at 0° C. for 30 minutes. The precipitate which formed was separated by centrifugation at 4,500 rpm. for 5 minutes at -5° C. in a Servall Type SS-1 angle centrifuge. In this manner, the plasma was separated into a fraction precipitated at 33 per cent saturation with ammonium sulfate, a fraction precipitated between 33 and 60 per cent saturation, and a fraction not precipitated with 60 per cent saturation. The two precipitates were each dissolved in a volume of water equal to that of the original

volume of plasma, and the ammonium sulfate was then removed by dialysis.

The fraction of plasma precipitated between 33 and 60 per cent ammonium sulfate was refractionated by acidification to pH 4.8 with sodium acetate buffer of pH 4.0 and ionic strength 0.15. The acidified fraction was diluted to ionic strength 0.10, cooled to  $-5^{\circ}\text{C}$ ., and ethyl alcohol added drop-wise, with stirring, to a concentration of 18 per cent. The mixture was allowed to stand for 30 minutes at  $-5^{\circ}\text{C}$ . and then separated by centrifugation at  $-5^{\circ}\text{C}$ . in the angle centrifuge in the manner described. The concentration of alcohol was then increased to 45 per cent, and the precipitate collected in the same manner. The precipitates were dissolved in a volume of 0.15M sodium chloride solution equal to that of the original plasma. When the activity of such fractions was to be compared with the activity of the ammonium sulfate fractions, the alcohol precipitates were dissolved in the sodium chloride solution against which the ammonium sulfate-precipitated fractions had been dialyzed.

Fractions of human plasma prepared by the low-temperature-ethanol method of Cohn<sup>3</sup> were dissolved in buffer in a concentration of 5 or 10 per cent and serially diluted with buffer. The insoluble material in fractions II-III and IV-1 was removed by centrifugation.

*Bovine fibrinogen*<sup>4</sup> prepared by the method of Ware, Guest, and Seegers (3) was dissolved in buffer and undissolved residue was separated by filtration through Whatman No. 1 filter paper. In all experiments, a portion of dried fibrinogen weighing 100 mg. was dissolved in 20 ml. of buffer. This preparation contained 0.9 Gm. of sodium chloride for every 3.1 Gm. of protein. Assay of the filtrate demonstrated approximately 300 mg. of coagulable protein per 100 ml. of solution. There was a variation of as much as 50 mg. of coagulable protein per 100 ml. from preparation to preparation, but this did not influence the results obtained. In any given experiment, the same solution of fibrinogen was used throughout.

*Heated plasma* was prepared by incubating defibrinated plasma in a pyrex glass tube in a water bath. The temperature was measured with a thermometer immersed in the plasma.

#### METHODS

The clotting time of mixtures of oxalated plasma and bovine thrombin, to be designated as the *thrombin time*, was measured in a  $37^{\circ}\text{C}$ . water bath by adding 0.2 ml. of thrombin to 0.2 ml. of plasma in a pyrex test tube with an internal diameter of 8 mm. The tubes were tilted until clotting occurred. When the thrombin time was 30 seconds or less, duplicate determinations usually checked within two seconds. When the thrombin time was 50 seconds or less, duplicate determinations usually checked within 3 or 4 seconds. When the thrombin time was

<sup>3</sup> Human plasma fractions were obtained through the courtesy of the late Dr. Edwin Cohn.

<sup>4</sup> Bovine fibrinogen was obtained through the courtesy of Dr. Walter Seegers.

greater than 60 seconds, the results of duplicate determinations were more erratic.

The following procedure was used to compare normal and pathologic plasma. The activity of solutions of thrombin varied from preparation to preparation. To obviate this difficulty, the thrombin time of each plasma was determined at several concentrations of thrombin. A graph was then drawn of the thrombin time of the normal plasma against the concentration of thrombin, using a logarithmic scale for both the ordinate and abscissa (4). From this graph, the concentration of thrombin which clotted the normal plasma in 30 seconds was determined by interpolation. In the same manner, the thrombin time of the abnormal plasma was plotted as a function of the concentration of thrombin. From this graph, the thrombin time of the abnormal plasma was determined at that concentration of thrombin which clotted normal plasma in 30 seconds. In this manner, the plasma to be tested was compared with a normal plasma, the thrombin time of which was arbitrarily set at 30 seconds. This interpolation of data was used only to render clinical comparison of normal and abnormal plasmas possible. In the majority of the experiments to be described, such interpolation was not necessary to demonstrate the points in question; the actual, rather than the interpolated data are reported.

To determine the effect of different preparations, suitable amounts were added to a sample of plasma or fibrinogen solution and the thrombin time was then determined in the manner described.

#### RESULTS

##### *The thrombin time of fresh and aged normal oxalated plasma.*

In normal subjects, the thrombin time of fresh oxalated plasma was remarkably uniform. In Table I are the relative thrombin times of six normal adults, tested simultaneously. In many other

TABLE I

##### *The thrombin time in normal subjects*

Clotting time (sec.) of a mixture of 0.2 ml. of fresh oxalated plasma and 0.2 ml. of bovine thrombin. The relative thrombin times of the plasmas were then determined by interpolation, assuming first that the value for subject No. 1 and then that of the value for subject No. 6 was 30 seconds.

Subject	Thrombin time	
	No. 1 = 30 seconds	No. 6 = 30 seconds
1. G	30	28
2. Si	30	28
3. W	31	29
4. Sm	32.5	30
5. W	32.5	30
6. T	32.5	30

experiments, two or three normal subjects were used as controls. In no instance has the thrombin time among normal individuals differed by more than 4 seconds, when the data were so interpolated that the thrombin time of any given normal plasma was 30 seconds.

In contrast, the thrombin time of normal plasma which had been stored at 4° C. for four months was prolonged compared with fresh plasma. For example, in one experiment the thrombin time of fresh normal plasma was 19 seconds, and of the same amount of aged normal plasma was 43 seconds. The thrombin time of an equal mixture of the two plasmas was 28 seconds, longer than that of the fresh plasma, and shorter than that of the stored plasma.

Moreover, the thrombin time of aged plasma was shortened by the addition of fresh *defibrinated* plasma. Thus the thrombin time of an equal mixture of aged plasma and 0.15M sodium chloride was 77 seconds, while that of an equal mixture of aged plasma and fresh defibrinated plasma was 30 seconds. This makes it seem likely that the prolonged thrombin time of the aged plasma could not be accounted for entirely by a qualitative or quantitative change in its fibrinogen. The fresh plasma appeared to supply some factor other than fibrinogen which shortened the thrombin time of the aged plasma. In contrast, the addition of an equal volume of fresh defibrinated plasma did not significantly alter the thrombin time of fresh oxalated plasma. Subsequent experiments appear to demonstrate that the clot accelerating property of defibrinated plasma is not removed by dialysis.

#### *The thrombin time in pathologic states.*

The thrombin time of plasma drawn from patients with a variety of diseases was determined. In hepatic disease, multiple myeloma and eclampsia or severe pre-eclampsia the thrombin time was commonly prolonged (Figure 1). In many of these patients, the hematocrit was below normal, and as a result the proportion of oxalate solution to plasma varied. However, the abnormally prolonged thrombin times in the pathologic plasmas could be demonstrated regularly in plasma which had been dialyzed for 24 hours against 0.15M sodium chloride solution, as well as in plasma which had been treated with a cation exchange resin to substitute sodium ions for other cations.

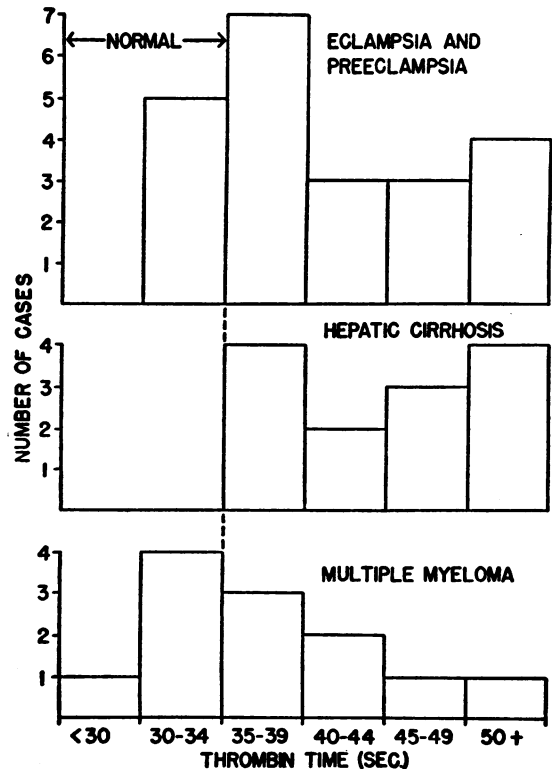


FIG. 1. THE THROMBIN TIME IN MULTIPLE MYELOMA, CIRRHOSIS OF THE LIVER, AND ECLAMPSIA AND SEVERE PRE-ECLAMPSIA

Each plasma was compared with a normal plasma studied simultaneously; the values were interpolated so that the thrombin time of the normal plasma was 30 seconds.

A study of plasmas in which the thrombin time was prolonged supports the view that normal plasma contains an accelerator of the conversion of fibrinogen to fibrin. Normal plasma shortened the thrombin time of abnormal plasma. However, the thrombin time of the mixture was always longer than that of the normal plasma alone. Indeed the thrombin time of an equal mixture of normal and abnormal plasmas was predictable from the thrombin time of each individual plasma. This was determined in the following manner. By interpolation, the amount of thrombin which produced a clot at 30 seconds was measured experimentally for each plasma alone and for the mixture of normal and abnormal plasmas (Table II). At the same time the amount of thrombin which would produce a clot in the equal mixture at 30 seconds was estimated by calculating the average of the

TABLE II

*The effect of mixing normal and abnormal plasmas on the coagulating effect of thrombin\**

Patient	Diagnosis	Amount of thrombin which clots plasma in 30 secs.			
		Control	Patient	Equal mixture (experimental)	Equal mixture (calculated)
1	Eclampsia	1.62	2.75	2.23	2.19
2	Pre-eclampsia	1.80	2.70	2.20	2.25
3	Metastatic carcinoma in liver	1.45	2.15	1.92	1.80
4	Cirrhosis	2.08	3.42	3.02	2.75
5	Cirrhosis	2.12	2.90	2.60	2.51

\* The amount of thrombin (N.I.H. units) required to clot a mixture of 0.2 ml. fresh oxalated plasma and 0.2 ml. bovine thrombin in 30 seconds at 37° C. The amount of thrombin was determined experimentally for each abnormal plasma and a simultaneously studied control. The amount of thrombin required to clot an equal mixture of abnormal and control plasmas in 30 seconds was determined experimentally. This was compared with the calculated average of the amount of thrombin required to clot each plasma separately. Different solutions of thrombin were used to study each patient, and the data for any one patient cannot be compared with that for other patients.

amount of thrombin required to clot the normal and the abnormal plasmas separately. It will be noted that the calculated result and the experimental result closely approximated each other. For this reason, it seems unlikely that the prolonged thrombin time in these abnormal plasmas was the result of a circulating anticoagulant; the abnormal plasmas had no greater effect than one would expect from mere dilution of the normal with the abnormal. Moreover, unlike normal plasma, fresh plasma from patients with myeloma or eclampsia did not shorten the thrombin time of aged normal plasma. These experiments, then, suggest that the plasma of such patients may be deficient in an accelerating property. They do not rule out the possibility that the accelerator is present but for some reason is physiologically inactive.

#### *Some characteristics of the accelerator in human plasma.*

Studies were performed to determine the properties of the substance or substances in normal plasma which shortened the thrombin time of aged or pathologic plasmas. The thrombin time of normal plasma was not altered by adsorption of the plasma with barium sulfate. Prothrombin, serum prothrombin conversion accelerator and Christmas factor are all adsorbed by the barium sulfate. It was therefore possible to study the effect of thrombin on a plasma in which these thrombin-producing factors had been removed. The thrombin time was also not prolonged by dialysis against sodium chloride solution. Indeed, dialyzed plasma had a slightly shorter thrombin time than undialyzed plasma.

The effect of heat on the thrombin time of normal plasma was tested. Fresh oxalated normal plasma was first defibrinated with thrombin to obviate precipitation of fibrinogen by the heating process. This defibrination did not remove the property of plasma which shortened the clotting time of mixtures of plasma and thrombin. The defibrinated plasma was adsorbed with barium sulfate and dialyzed against 0.15M sodium chloride solution for 16 hours at 4° C. A portion of the dialyzed plasma was then heated to 60° C. for 20 minutes, and the effect of unheated and heated dialyzed plasma and of the dialysate upon the thrombin time of normal plasma was measured. In one such experiment, the thrombin time of normal plasma, diluted with an equal volume of the dialysate, was 57 seconds; that of an equal mixture of normal plasma and defibrinated, dialyzed plasma was 42 seconds; and of an equal mixture of normal plasma and defibrinated plasma heated to 60° C. for 20 minutes, was 54 seconds. Thus the accelerating effect of normal defibrinated plasma was diminished by heating it to 60° C. for 20 minutes. Similarly, normal defibrinated plasma heated to 60° C. for 20 minutes lost its ability to shorten the thrombin time of pathologic plasmas.

When plasma was fractionated with ammonium sulfate, the bulk of the accelerating property was present in the fraction precipitated between 33 and 60 per cent saturation (Table III). When this fraction was further subdivided by precipitation with ammonium sulfate, the activity was distributed in each sub-fraction. The fraction precipitated between 33 and 60 per cent saturation was refractionated by precipitation with ethanol at low temperatures. The major portion of the ac-

TABLE III

*The effect of plasma fractions on the thrombin time \**

Fraction	Thrombin time (sec.)
Saline dialysate	45
Normal dialyzed plasma	26
0-33% ammonium sulfate precipitate	41
33-60% ammonium sulfate precipitate	26
Supernate of 33-60% precipitation	37
0-18% ethanol precipitate	36
18-45% ethanol precipitate	28

\* Clotting time (sec.) of a mixture of 0.1 ml. fresh oxalated plasma from a patient with eclampsia, 0.1 ml. of the fraction to be tested, and 0.2 ml. of bovine thrombin (5 units/ml). The fractions were prepared from oxalated normal plasma which had been defibrinated and adsorbed with barium sulfate. The ethanol fractions were reprecipitated from the fraction precipitated between 33 and 60 per cent ammonium sulfate.

tivity was now present in the portion precipitated between 18 and 45 per cent ethanol (Table III). These fractions were effective when mixed with portions of normal or abnormal plasmas. When either defibrinated plasma or the fractions which were prepared were added to bovine fibrinogen solutions, no acceleration of the thrombin time was noted.

The effect of proteins fractionated by the low temperature-ethanol method of Cohn was tested on a solution of human fraction I, on bovine fibrinogen solutions, and on normal and abnormal human oxalated plasma. Fractions III, IV-1, IV-4, IV-7, and V accelerated the thrombin time of oxalated human plasma from a patient with lupus erythematosus (Table IV). They were without appreciable effect on normal oxalated plasma, and were ac-

TABLE IV

*The effect of Cohn's fractions of normal plasma on the thrombin time of abnormal plasma*

Clotting time (sec.) of a mixture of 0.1 ml. fresh oxalated plasma from a patient with lupus erythematosus, 0.1 ml. fraction tested, and 0.2 ml. bovine thrombin (5 N.I.H. units/ml.).

Fraction	Final concentration of fraction (Gm./100 ml.)						
	2.5	1.25	0.625	0.312	0.156	0.078	0
II	22	22	22	22	22	21	24
II-III	—	19	19	22	21	21	
III	14	16	18	20	21	22	
IV-1	—	18	19	21	21	22	
IV-4	14	16	21	19	23	23	
IV-7	—	15	16	20	21	24	
V	14	15	17	19	24	25	

tually slightly inhibitory on bovine fibrinogen solutions. None of the fractions tested had any effect upon the thrombin time of solutions of human Fraction I.

*The effect of calcium upon the thrombin time.*

The reaction between fibrinogen and thrombin is accelerated by the presence of calcium ions (1, 2, 5, 6). Experiments were performed to determine the influence of calcium ions on the thrombin time of abnormal plasma. Oxalated plasma and thrombin solutions were treated with amberlite IRC-50 in the sodium phase to remove bivalent cations. Calcium chloride in varying concentration was then mixed with the thrombin, and the mixture in turn added to the plasma. In some experiments, the plasma was first adsorbed with barium sulfate to remove prothrombin, serum pro-

TABLE V

*The effect of calcium chloride on the thrombin time*

Clotting time (sec.) of a mixture of 0.2 ml. of oxalated plasma and 0.2 ml. of an equal mixture of bovine thrombin (20 N.I.H. units/ml.) and calcium chloride solution. The plasma had been adsorbed with barium sulfate. The plasma and thrombin had been treated with amberlite IRC50 to remove bivalent cations. The calcium chloride solutions were diluted serially with 0.15M sodium chloride to maintain a constant ionic strength.

Plasma	Final concentration of calcium chloride (M/liter)						
	0.0125	0.0062	0.0031	0.0015	0.0007	0.0004	0
Normal	14	26	27	30	39	48	180
Cirrhosis	18	34	44	52	62	86	∞

thrombin conversion accelerator and Christmas factor. In this way, the effect of calcium on the thrombin time was studied independently of any effect that this ion might have on the conversion of prothrombin to thrombin.

These studies demonstrated that calcium shortened the thrombin time of abnormal as well as normal plasmas. In some instances, this effect was sufficiently pronounced that no significant difference between the normal and abnormal plasmas was detected. In others, the thrombin time was still prolonged relative to that of normal plasma even though the concentration of calcium ions was as high as 0.06M or more, an amount far in excess of that present in the circulating blood (Table V).

## DISCUSSION

Normal human plasma appears to have the property of accelerating the coagulation of fibrinogen by thrombin. This accelerating property was not removed by defibrination by thrombin, by dialysis against saline solution, nor by adsorption of the plasma with barium sulfate. The accelerating property was concentrated in that fraction of plasma which is precipitated between 33 and 60 per cent saturation with ammonium sulfate. In turn, the accelerator could be reprecipitated at a concentration of alcohol above 18 per cent at  $-5^{\circ}$  C. and ionic strength 0.1. The accelerating property was diminished in plasma which had been stored at  $4^{\circ}$  C. for several months, and in fresh plasma which had been heated to  $60^{\circ}$  C. for 20 minutes. These characteristics seem to distinguish the accelerator of the conversion of fibrinogen to fibrin from prothrombin, serum prothrombin conversion accelerator, Christmas factor, plasma thromboplastinogen, accelerator globulin, and fibrinogen itself.

Seegers and Smith (2) have shown that solutions of acacia will shorten the clotting time of mixtures of fibrinogen and thrombin. Whether the plasma accelerating property which has been described is due to the presence of a specific nondialyzable substance, or is a non-specific colloidal property of the plasma proteins is not known. It is significant that, unlike acacia, neither the fractions prepared here nor those of Cohn accelerated the coagulation of purified fibrinogen by thrombin. This suggests that the mechanism by which plasma accelerates the clotting of fibrinogen is probably indirect. Perhaps it exerts its action by neutralizing the naturally existing antithrombins which are found in plasma but not in solutions of fibrinogen. Experiments to determine its mode of action are in progress.

Studies with proteins prepared by the low temperature-ethanol method of Cohn indicated that fractions III, IV, and V shortened the abnormally long thrombin time of the oxalated plasma of a patient with lupus erythematosus, but were without effect on normal plasma or on bovine or human fibrinogen solutions. This observation is susceptible to several interpretations. For example, it is possible that the fractions contained a contaminant which shortened the thrombin time.

Another possibility is that the accelerator is coprecipitated with proteins over a broad range of conditions, in the same manner that plasminogen is found in many plasma fractions. One cannot, of course, conclude that the accelerator found in these fractions is necessarily identical with those prepared by ammonium sulfate precipitation.

It is known that bivalent cations will accelerate the conversion of fibrinogen to fibrin (1, 2, 5, 6). However, the data presented seem to preclude the possibility that the accelerating activity described was due to the presence of such cations; defibrinated plasma which had been treated with a cation exchange resin still accelerated the clotting of normal plasma by thrombin. No evidence was obtained concerning the source of the plasma accelerator. Ware, Fahey, and Seegers (7) have described an accelerator of the fibrinogen-thrombin reaction obtained from platelets. However, no differences were observed in the reaction between fibrinogen and thrombin in the plasmas of thrombocytopenic as compared to normal subjects. Moreover, the plasma from which active fractions were obtained had been rendered platelet-deficient by two successive centrifugations, suggesting that the accelerator did not arise from a destruction of platelets *in vitro*.

When bovine thrombin was mixed with oxalated human plasma, the clotting time or thrombin time was strikingly uniform in normal subjects. However, it has been demonstrated (1, 8, 9) that in patients with hepatic disease the thrombin time may be considerably prolonged. In the present study, these observations have been confirmed. Other conditions in which a prolonged thrombin time has been noted include the neonatal state (10), multiple myeloma (11), eclampsia and severe pre-eclampsia (12), amniotic fluid embolism (13), and lupus erythematosus (14). In contrast, the thrombin time was normal in the plasma of normal parturient women (15), patients with uncomplicated thrombocytopenic purpura (14), hemophilia (14) and some patients with obstructive jaundice (8). Unfortunately the thrombin time has not been normal in a sufficient proportion of those patients with obstructive jaundice who have been tested thus far to justify its use as a differential diagnostic test.

Three possible mechanisms for a prolonged thrombin time were considered. Firstly, the reac-

tion between fibrinogen and thrombin might be slowed by an alteration either in the reactivity of fibrinogen or in the polymerization of the fibrin molecule. Secondly, the reaction between fibrinogen and thrombin might be blocked by the presence of an inhibitor. Thirdly, there might be a deficiency of the accelerating property which has been described.

It seems likely that under different conditions, each of these three mechanisms may be responsible for a prolonged thrombin time. For example, Biggs (10) has reported that fibrinogen prepared from the plasma of the newly-born clotted after a longer time than the fibrinogen of adults, when each was mixed with thrombin. Similarly, in a case of cirrhosis, suggestive evidence was obtained that a prolonged thrombin time was due in part to the presence of fibrinogen which reacted more slowly than normal fibrinogen. Definitive studies could not be made because the patient died. Secondly, Conley, Hartmann, and Morse (16) have described patients in whom the thrombin time was prolonged apparently because of the presence of a circulating anticoagulant. No such cases were observed in the present study. In still other patients with a prolonged thrombin time, the plasma behaved as if it were deficient in the accelerator of the coagulation of fibrinogen by thrombin. However, the experiments which have been described do not preclude the possibility that the accelerator is present but in some way rendered ineffective.

When the plasma and thrombin were mixed with calcium ions, the difference between normal and abnormal plasmas was decreased and in some cases disappeared, as had been reported earlier by Biggs and Macfarlane (1). Calcium ions have been shown to increase the tensile strength of fibrin clots (17, 18) and to decrease the solubility of such clots (19). This change in the fibrin molecule apparently requires the presence of a factor in the serum (20, 21). Recently, Shulman (22) has shown that this factor is present in fraction V of bovine plasma. No information is available as yet whether the factor which decreases the solubility of fibrin and the factor which shortens the thrombin time are the same.

The prolongation of the thrombin time observed in pathologic plasma is, of course, a phenomenon demonstrated *in vitro*. Ordinarily, the thrombin

time is measured with oxalated plasma. However, when calcium ions were added in the concentration found in plasma, the thrombin time was still prolonged in many of the patients who were studied. For this reason, it is likely that a defect in the coagulation of fibrinogen by thrombin may be responsible for some instances of the hemorrhagic phenomena which are often observed in patients with liver disease, multiple myeloma, and eclampsia or severe pre-eclampsia.

In a preliminary note, the name "thrombin accelerator" was proposed for the accelerating principle in plasma (23). This name seems inappropriate. No evidence has been obtained that the accelerator affects the interaction between fibrinogen and thrombin; it is entirely possible that its influence is upon the polymerization of fibrin. By analogy with the prothrombin conversion accelerators, it might be called "fibrinogen conversion accelerator," but until more data are available, it might perhaps better go unnamed.

#### SUMMARY

The thrombin time, that is, the clotting time of a mixture of oxalated human and bovine thrombin, was prolonged in patients with liver disease, multiple myeloma, eclampsia and severe pre-eclampsia. The conditions responsible for the prolongation of the thrombin time were investigated. These studies indicate that normal plasma contains a heat-labile, non-dialyzable component which accelerates the coagulation of fibrinogen by thrombin.

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