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Stability of Thrombin in the Presence of Fibrinolysin.*

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Under certain conditions blood shows marked proteolytic activity, and digests such substrates as casein, gelatine and fibrin.[1] It has recently been shown that the proteolytic activity of serum is due principally to an endopeptidase which is homospecific with an enzymatic component of papain.[2] Earlier work has demonstrated the existence of an enzyme precursor ("profibrinolysin") in blood, which is spontaneously activated following the destruction of an inhibitor by treatment with chloroform or certain other denaturing agents.[3,4] It is also activated specifically by a substance found in streptococcal cultures which is known as streptokinase.[5] To avoid confusion of terminology, the nomenclature suggested by Loomis, George and Ryder[6] has been used throughout this paper. The effect of trypsin on the clotting activity of thrombin was studied by Glazko and Ferguson[7,8] prior to the recognition of fibrinolysin as an important blood protease. The work presented here demonstrates clearly that fibrinolysin has no appreciable effect on thrombin, confirming statements made by others.[6,9] In addition it was observed that the thrombin preparation used in these experiments had some stabilizing effect on fibrinolysin. These results are of interest because of the close association of thrombin with fibrinolysin in globulin fraction III-3,[10] and of prothrombin with profibrinolysin in globulin fraction III-2.[11]

Materials and Methods. In studying thrombin stability, serial dilutions of thrombin were made in 5 ml volumes of buffered saline, containing M/100 phosphate at pH 7.4 and a small amount of phenyl mercuric borate to prevent bacterial growth. The thrombin preparation was obtained commercially as Lederle's "Hemostatic Clotting Globulin." After warming to 38°C in a water bath, 1 ml portions of enzyme solution were added to each thrombin dilution, and the mixtures were maintained at 38°C. Clotting tests were performed at regular intervals by removing 0.5 ml of each mixture and transferring to 1.0 ml portions of 0.15% fibrinogen solution at 25°C. Clotting times were measured from the moment of addition of thrombin to the first appearance of a clot.

The stability of fibrinolysin in the presence of thrombin was estimated by measurement of the time required to lyse a standard fibrin clot. Equal volumes of thrombin and fibrinolysin solutions were warmed to 38°C, mixed and tested at intervals for fibrinolytic activity by transferring 0.5 ml amounts to 0.2 ml of fresh 1:10 thrombin, and then adding 1.0 ml of saline and 0.5 ml of 0.6% fibrinogen solution. Lytic times were measured from the time of addition of fibrinogen to the time at which the gel structure of the

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TABLE I.
Stability of Thrombin in the Presence of Globulin Fraction III-3.

Thrombin dilution	Incubation time (hr)							
	No protease added				With protease added			
	0	1	5	20	0	1	5	20
1:2	4	4	4	4	5	5	5	5
1:4	5	5	5	5	6	7	7	7
1:8	7	7	7	7	9	8	9	8
1:16	9	10	11	13	12	13	13	14
1:32	13	14	16	19	17	16	17	20
1:64	21	23	25	33	20	19	23	28
1:128	34	37	45	67	24	25	28	43
1:256	75	70	85	170	25	25	30	65
No thrombin added	—	—	—	—	30	32	48	40

Body of table gives clotting times in seconds.
Incubation mixture: 5 ml thrombin + 1 ml (= 10 mg) globulin III-3.
Clotting test: 0.5 ml incubation mixture + 1.0 ml 0.15% fibrinogen.

TABLE II.
Stability of Thrombin in the Presence of Fibrinolysin.

Thrombin dilution	Incubation time (hr)									
	No enzyme added					Fibrinolysin added				
	0	1	3.5	21	46	0	1	3.5	21	46
1:2	5	5	5	5	5	5	5	5	5	5
1:4	6	6	6	6	6	6	6	6	6	6
1:8	8	8	8	9	9	8	8	8	9	8
1:16	13	13	13	13	14	12	13	13	13	13
1:32	20	20	21	22	22	20	22	22	24	25
1:64	30	35	39	42	46	30	34	38	42	44
1:128	55	57	70	77	77	50	52	55	65	72
1:256	85	90	120	140	180	85	100	100	125	150
No thrombin added	—	—	—	—	—	—	—	—	—	—

Body of table gives clotting times in seconds.
Incubation mixture: 5 ml thrombin + 1 ml (= 1 mg) fibrinolysin.
Clotting test: 0.5 ml incubation mixture + 1.0 ml 0.15% fibrinogen.

clot broke down on gently tilting the tube.

The fibrinogen, prepared from beef blood, was obtained through the courtesy of Dr. J. D. Porsche of Armour and Company. Negative tests were obtained for the presence of prothrombin, and no lytic activity appeared when the fibrinogen was treated with an active streptokinase preparation.

Two different sources of fibrinolysin were used in these experiments. Globulin fraction III-3‡ was first used as a convenient source of the protease, although it was later found to contain appreciable amounts of free thrombin.[10] A second source of enzyme was the more highly purified fibrinolysin described by Loomis *et al.*,[6] which gave negative tests for thrombic activity. This was used in a buffered saline solution containing 1 mg of the dry preparation per ml.

Results. Table I presents the results of an experiment in which the stability of thrombin was studied in the presence of globulin fraction III-3. The clotting times were found to remain fairly constant with the more concentrated thrombin solutions; but dilute solutions showed some increase in clotting time. However, there were no significant differences in the rate of increase in the clotting times between the controls and the samples containing added protease.[8] The samples to which globulin fraction III-3 had been added showed some additional

‡ Obtained through the courtesy of Dr. John T. Edsall, and prepared under a contract between the OSRD and Harvard University from human blood collected by the American Red Cross.

TABLE III.

Fibrinolytic Activity of Globulin Fraction III-3 After Incubation with Thrombin at 38°C.

Thrombin dilution	Incubation time (hr) 0	1	2
1:1	7′	17′	53′
1:2	6′	13′	46′
1:4	6′	12′	46′
1:8	5′	23′	>4°
No thrombin added	5′	4°38′	>4°

Body of table gives lytic times in hours (°) and minutes (′).

Incubation mixture: 2 ml thrombin + 2 ml (= 2 mg) globulin III-3.

Fibrinolysis test: 0.5 ml incubation mixture + 1 ml saline + 0.2 ml 1:10 thrombin + 1 ml 0.6% fibrinogen.

thrombic activity due to the presence of a small amount of thrombin in this fraction.[10]

Table II shows the results of a similar experiment carried out with the fibrinolysin preparation. Here again it is evident that the stability of thrombin was not affected by the presence of fibrinolysin. All of the clots obtained at the 21-hour testing period were completely lysed within 24 hours; and those obtained at the 46-hour testing period were completely lysed within 54 hours, showing that active fibrinolysin was still present. However, the mixtures containing the highest concentrations of thrombin retained the greatest amount of fibrinolytic activity. The shortest lytic time (after the 46-hour incubation period) was 4 hours for the 1:2 dilution of thrombin; 9 hours for the next higher dilution; and progressively longer lytic times were observed up to a maximum of 54 hours for the sample which contained no thrombin. These observations were checked using globulin fraction III-3 in place of the fibrinolysin preparation, and the results of these lytic time measurements are given in Table III. It can be seen that the solutions containing the higher concentration of thrombin retained their fibrinolytic activity better than samples with little or no thrombin present.

Summary. It has been shown that fibrinolysin does not affect the clotting activity of thrombin, and that the thrombin preparation increased the stability of fibrinolysin under the conditions of these experiments.

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Effect of Iodine and Adrenalin on Thyrotropin in Graves' Disease and in Normal and Thyroidectomized Dogs.

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This study deals with (1) the evaluation of the amount of circulating thyrotropic factor in clinical hyperthyroidism, (2) the effect of iodine on such circulating thyrotropic factor, and (3) the influence of the parenteral injection of adrenalin on thyrotropic discharge from the anterior pituitary in the intact and the totally thyroidectomized experimental animal.

Method. Biological assay was used for the determination of thyrotropic factor. This technic involves histological examination of the thyroids of young guinea pigs not exceeding 200 g in weight, following subcutaneous injection of 5 cc of serum from the patient or experimental animal. The guinea pigs were injected on 2 successive days and were killed with ether 24 hours after the last injection. The thyroid lobes were then removed, fixed in 10% formalin, and stained with hematoxylin-eosin. The thyroids were then examined for hyperplastic changes. Following the injection of varying amounts of pure thyrotropic factor there occur changes in the thyroid characterized by a decrease in the amount of colloid in the follicles, an in-