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Observations on the Conversion of Prothrombin to Thrombin.

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Evidence from several sources has indicated that the rate of formation of thrombin in plasma upon the addition of a given thromboplastin is affected by some factor besides the concentration of the prothrombin itself. Seegers, Loomis and Vandenbelt[1] found that thrombin was formed more slowly from very highly purified prothrombin than from prothrombin naturally contained in plasma. In studies of patients who had cirrhosis[2] or pernicious anemia,[3] of newborn infants[4] and of normal dogs and rabbits,[5] the concentration of prothrombin in the plasma has been reported to be less when determined by the 2-stage procedure of Warner, Brinkhous and Smith[5] than by the one-stage method of Quick.[6] In Quick's method the time required for conversion of prothrombin to thrombin constitutes part of the observed clotting time. These differences are attributed by the originators of the 2-stage method to an increased rate of conversion of prothrombin in the one-stage technic. On the other hand Quick[6] thought the differences were due to incomplete conversion of prothrombin in the high dilution required by the 2-stage method. However, when the plasma of patients treated with dicumarol[7] or normal plasma after storage[8] are studied by both methods the 2-stage procedure gives the higher values. Quick[9] has presented evidence that the increase in prothrombin time of plasma after storage is due to the disappearance of a substance termed "prothrombin A" which remains in fresh plasma depleted of prothrombin by treatment of patients or animals with dicumarol or addition of aluminum hydroxide to the plasma. Fantl and Nance[10] also found that plasma freed of prothrombin by treatment with barium carbonate still contained a substance which accelerated the conversion of prothrombin to thrombin. Because in blood coagulation the rate of thrombin formation is more important than the total amount of available prothrombin, such a factor or factors affecting conversion appear to deserve further study.

Method. The prothrombin time of fresh or stored (one to 2 months) human plasma was determined by Quick's method as previously described,[11] except that just before addition of thromboplastin, 0.1 ml of a 0.9% solution of sodium chloride, or whatever material was being tested for action on the conversion rate, was added. Thus, the volume of the clotting system was increased from 0.3 to 0.4 ml.

Standard thromboplastin was prepared by Quick's original method. With this preparation, normal plasma has a prothrombin time of from 17 to 19 seconds. Fresh tissue thromboplastin was made by macerating fresh or frozen rabbit brain with twice its weight of ice-cold normal saline solution. A commercial preparation of acetone-dehydrated rabbit brain* was used. Thromboplastin was prepared from human placenta by the method

1 Seegers, W., Loomis, E., and Vandenbelt, J., *Arch. Biochem.*, 1945, **6**, 85.

2 Ziffren, S., Owen, C., Warner, E., and Peterson, F., *Surg., Gynec. and Obst.*, 1942, **74**, 463.

3 Warner, E., and Owen, C., *Am. J. M. Sc.*, 1942, **203**, 187.

4 Owen, C., Hoffman, G., Ziffren, S., and Smith, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 181.

5 Warner, E., Brinkhous, K., and Smith, H., *Am. J. Physiol.*, 1939, **125**, 296.

6 Quick, A., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, Springfield, Ill., Charles C Thomas, 1942, pp. 36 and 40.

7 Hurn, M., Barker, N., and Mann, F., *Am. J. Clin. Path.*, 1947, **17**, 712.

8 Lord, J., and Pastore, J., *J. A. M. A.*, 1939, **113**, 2231.

9 Quick, A., *Am. J. Physiol.*, 1943, **140**, 212.

10 Fantl, P., and Nance, M., *Nature*, 1946, **158**, 708.

11 Hurn, M., Barker, N., and Magath, T., *J. Lab. and Clin. Med.*, 1945, **30**, 432.

of Zondek and Finkelstein.[12]

The materials tested for prothrombin-converting activity were plasma and serum from human beings and crude platelet extract. The platelet extract was prepared as follows. Oxalated plasma was separated from the cells by gentle centrifugation; then the suspension of platelets thus obtained was centrifuged for 30 minutes at 3,000 revolutions per minute. The plasma was carefully drained from the tubes, after which the sticky pellet of sediment was removed with a stirring rod. The sediment, which thus contained a minimal amount of plasma, was triturated in a mortar with 0.9% solution of sodium chloride containing 0.001 molar sodium oxalate. One milliliter of this fluid was used for the amount of sediment obtained from 16 ml of plasma. Serum was used only after it had stood 3 to 4 hours at room temperature to allow for destruction of thrombin. Neither the platelet extracts nor the serum used caused clotting when mixed with oxalated plasma. The plasma tested was drawn and oxalated as for a prothrombin determination or in some experiments the silicone technic of Jaques and his associates[13] was used. When the silicone technic was used all pipets and tubes used in handling the plasma throughout the procedure were coated with silicone.

Results. Fresh plasma, serum and platelet extracts were all found to reduce the prothrombin time of stored plasma markedly (Tables I, II and III). The fresh plasma was so highly diluted that little of the effect could be attributed to its content of prothrombin; the same was true of platelet extract. Platelet extract showed considerably more prothrombin-converting activity than did serum or plasma. When the thromboplastin of rabbit brain was replaced with normal saline solution the clotting time was greatly prolonged, showing that the platelet extract potentiated the action of the tissue thromboplastin. Platelet extract was observed to potentiate the action of thromboplastin from dried rabbit brain, fresh rabbit brain, acetone-dehydrated rabbit brain and human placenta on stored

TABLE I.
Effect on Stored Plasma with Standard Thromboplastin of Plasma and Serum Drawn from Same Subject at the Same Time.

Material added to plasma	Dilution	Clotting time, sec
Saline		82
Plasma	1:10	36
	1:20	41
	1:40	49
	1:80	57
Serum*	1:10	31
	1:20	37
	1:40	43
	1:80	57

* Undiluted serum usually restored the clotting time to the normal value of 18 seconds.

TABLE II.
Potentiation of Standard Thromboplastin with Platelet Extract.

Plasma	Material added	Thrombo-plastin	Clotting time, sec
Stored	Saline	Standard	90
	Platelet extract	"	13
	" "	None	86
	Plasma from which extr. made dil. 1:5	Standard	28
Fresh	Saline	"	18
	Platelet extr.	"	13
	" "	None	47
	Saline	Standard dil. 1:50	39
	Platelet extr.	Standard dil. 1:50	23

TABLE III.
Potentiation of Fresh Tissue Thromboplastin with Platelet Extract.

Plasma	Material added	Thrombo-plastin	Clotting time, sec
Stored	Saline	Fresh tissue	52
	Platelet extr.	" "	15
	" "	None	45
Fresh	Saline	Fresh tissue	20
	Platelet extr.	" "	16
	" "	None	56
	Saline	Fresh tissue, dil. 1:50	32
	Platelet extr.	Fresh tissue, dil. 1:50	23

* Obtained from the Difco Laboratories, Inc., Detroit, Mich.

12 Zondek, Bernhard, and Finkelstein, Michael, PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 374.

13 Jaques, L., Fidlar, E., Feldsted, E., and Macdonald, A., *Canad. M. A. J.*, 1946, **55**, 26.

TABLE IV.
Effect of Plasma Drawn from Same Subject by Various Technics. Standard Thromboplastin. Stored Plasma.

Material added	Dilution	Clotting time, sec
Saline		145
Oxalated plasma	1:10	30
	1:40	49
Oxalated plasma (silicone technic)	1:10	31
	1:40	46
Native plasma* (silicone technic)	1:10	23
	1:40	36

* Tested within 5 minutes after blood drawn; blood clotted in original tube after 30 minutes.

plasma. A similar, but less striking, potentiation between platelet extract and tissue thromboplastin was observed when fresh plasma was used. Thus although the prothrombin-converting activity of fresh plasma is rather great it is still not maximal, especially if dilute thromboplastin is used. However, since the extracts represent 16 times their volume of plasma, it would seem reasonable that the activity of the plasma might not be less than that of its platelets. Plasma had the same converting activity when drawn and handled with silicone technic as when exposed to glass (Table IV). Thus it seems unlikely that this activity is a product of processes incident to the drawing of blood. Native plasma drawn with silicone technic appears to have more prothrombin-converting activity than the same plasma after decalcification. As a rule, all materials tested lost about 90% of their prothrombin-converting activity on standing 24 hours in the icebox. By heating at 56°C for 30 minutes, most of this activity was destroyed.

Comment. No specific term will be applied at present to the substance which potentiates the conversion of prothrombin to thrombin by tissue thromboplastin. This material appears to be the same as Quick's prothrombin A but it is not converted into thrombin since it remains in the serum. By definition, the conversion-favoring substance might reasonably be regarded as part of the thromboplastin complex. Quick[14] has justly emphasized the fact that if any appreciable amount of active thromboplastin were present in the circulation the blood would not be fluid. This material does appear to exist in the circulation and presumably cannot alone bring about formation of thrombin. The prothrombin-converting activity of the plasma, however, may well be a determining factor when the blood is exposed to a minimal amount of tissue thromboplastin, as it probably often is in cases of thrombosis.

Summary. Fresh plasma, serum and platelet extracts contain a material which potentiates the action of tissue thromboplastin on stored plasma. Platelet extract apparently contains a greater degree of this activity than plasma or serum and potentiates the action of tissue thromboplastin on fresh plasma. This factor favoring prothrombin conversion apparently is present in the circulating blood.

14 Quick, A., *The Hemorrhagic Diseases and the Physiology of Hemostasis,* Springfield, Ill., Charles C Thomas, 1942, p. 70.