ing or by removal of the salt by dialysis prior to centrifuging. Recrystallization was readily affected by the procedure just described.

Summary. A simple method for recrystallizing catalase has been described. Some of the cow liver catalase crystals present in the mixture have exactly the same shape as those of beef erythrocyte catalase. This fact points to a multi-molecular nature of cow liver catalase. Since it is difficult, however, to remove all of the blood present in liver, it is quite possible that the described prisms originate from erythrocytes and that the higher active component described by other investigators may have a similar origin.

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Initiation of the Clotting of Blood.* (19515)

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It was recently observed that when hemophilic blood was put in a glass tube which previously had contained a solution of thrombin, but which had been rinsed repeatedly with saline solution, clotting was considerably faster than in a control test tube that had contained only saline(1). Since this simple experiment serves as a useful means for obtaining salient information concerning various factors that play a role in the clotting of blood, it was repeated with certain modifications as recorded in Table I.

From the recorded results, it can be seen that hemophilic blood clotted almost completely in 10 minutes when placed in a test tube which had contained thrombin but which had been repeatedly rinsed with saline. The same blood in a control test tube rinsed only with saline clotted in 40 minutes. Platelet-rich plasma obtained from the same hemophilic subject likewise formed a semi-solid clot in 10 minutes in a test tube that had contained thrombin, whereas the same plasma depleted of platelets by high centrifugation required 90 minutes before a clot of similar density was formed.

Obviously the primary cause of the marked shortening of the clotting time in the test tube that had contained thrombin was a minute amount that remained. Since the tube had been rinsed repeatedly, it seems likely that the thrombin was adsorbed to the glass surface probably as a thin film. Since the platelet-poor plasma clotted very slowly, although the tube contained the same amount of residual thrombin, it is clear that the minute quantity of adsorbed thrombin was not enough by itself to account for the accelerated clotting. Since the platelet-rich plasma, like the whole blood, clotted rapidly, one may conclude that more thrombin was formed in this plasma, which accounts for the decreased clotting time. Since this did not occur in the platelet-poor plasma, it seems evident that the platelets participated in increasing the forma-

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TABLE I. Adsorption of Thrombin on Glass and Its Influence on the Clotting Time of Hemophilic Blood and Plasma.

	Surface of test tube exposed to			
	Saline only	Thrombin*		
	Blood†	Blood†	Plasma†	
			Platelet-rich	Platelet-poor
Semi-solid clot	30 min	10 min	10 min	90 min
Solid clot	40	20	20	120
Prothrombin consumption test‡	8 sec	8 sec	8 sec	8 sec

* One cc of a concentrated solution of thrombin was transferred into small test tubes (13 × 100 mm). After 1 min the solution was poured out, the test tube drained, then filled with saline solution, and again drained. The rinsing and draining was repeated 4 times.

† Blood was collected with a silicone-coated syringe and needle. The platelet-rich plasma was prepared by centrifuging blood at 800 r.p.m. 5 min, while the platelet-poor plasma was obtained by centrifuging the blood 10 min at 8000 r.p.m. The latter plasma contained approximately 3000 platelets per mm^3. The centrifuge tubes were coated with silicone, and the blood chilled in an ice bath. For determination of the clotting time, 1 cc of blood or plasma was transferred to the test tube which was placed in a water bath at 37°C.

‡ The reagents and tests employed were carried out as described recently (1). These studies were repeated on the bloods of several hemophiliacs.

tion of thrombin. It may be postulated that an interaction of thrombin and platelets caused the generation of more thrombin. Actually, the additional amount of thrombin formed, which was sufficient to bring the clotting time nearly to normal, must have been extremely small since no utilization of prothrombin was demonstrable with the prothrombin consumption test.

These findings clearly show that an extremely small quantity of thrombin is sufficient to inaugurate *in vitro* clotting even of hemophilic blood. The results further indicate that thrombin in some manner interacts with platelets to bring about the formation of additional thrombin. One may assume that this is presumably an autocatalytic reaction which in hemophilia is limited by the lack of a plasma factor which has been designated as thromboplastinogen. Because of the marked effect of an extremely minute amount of thrombin on the clotting time, it is easy to understand why a faulty veni-puncture may introduce enough tissue juice which is rich in thromboplastin to reduce the clotting time to such an extent that a false normal value may be obtained. Furthermore, in the clotting of hemophilic blood a solid gel may form before less than one-third of the fibrinogen has been changed to fibrin. Thus, the clotting time is not necessarily a true measure of complete coagulation, and a normal value is no assurance that the clotting reaction is quantitatively normal. To have any significance, the clotting time must be meticulously standardized since seemingly small deviations in technic such as using improperly cleaned test tubes may induce serious errors. It is to be emphasized that in studying the clotting time in test tubes, one introduces artificial factors such as foreign surfaces and interphases that are absent in physiological *in vivo* clotting.

Summary. A minute amount of thrombin such as remains adherent to the wall of a test tube after repeated rinsing is enough to shorten markedly the clotting time of blood from a severe hemophiliac. Since the same effect is observed in platelet-rich but not in platelet-poor hemophilic plasma, it appears that although an extremely small quantity of thrombin can initiate clotting, platelets participate in accelerating the reaction.

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