

THE RECOVERY OF THROMBIN FROM PLASMA

Sternberger and coworkers (1951, 1952, 1956) reported that small amounts of thrombin could be recovered from normal plasma. Larger amounts were recovered from the plasma of patients with postoperative complications and of rabbits with experimental thrombosis. They suggested that thrombin is continually formed by *in vivo* coagulation in normal individuals, and that the formation is increased in patients with thrombosis. Some of the thrombin is adsorbed to fibrin, and some is neutralized through the formation of an inactive complex with anti-thrombin. They apparently recovered the thrombin from this complex.

These findings, if confirmed, are extremely important. First, the presence of thrombin in normal plasma would indicate that a slow continuous coagulation takes place in the circulation of normal individuals. Still far from proved, such a hypothesis is frequently suggested, and several authors list Sternberger's findings as evidence (Copley 1954, Roos 1957, Lasch, Mechelke & Nusser 1957). Second, the presence of increased amounts of thrombin in conditions which predispose to thrombosis may suggest that thrombin plays an important role in the early phase of thrombus formation, and also that Sternberger's test might disclose candidates for thrombosis.

In reviewing the arguments in favor of continuous *in vivo* coagulation, we were forced to take a stand for, or against Sternberger's claims. We are not aware of any attempts to confirm the findings. Therefore, we repeated the key experiments.

Sternberger (1952) used the following technique. Citrated plasma is diluted with an equal of saline, and the pH is adjusted to 11.3 with sodium hydroxide, all at a tempe-

rature of 1° C. This supposedly dissociates the thrombin-antithrombin complex. After two minutes, an equal volume of 50 per cent ethanol is added to prevent reassociation of the components, and the pH is returned to 7.2 with hydrochloric acid. The resulting suspension of precipitated proteins, still in 25 per cent ethanol, is tested for thrombin activity on a substrate of oxalated plasma or fibrinogen. Fibrinogen is unable to form an ordinary clot at this concentration of ethanol (final concentration 12.5 per cent), but the appearance of granules was taken to indicate clotting. The "clotting time" or the "granule appearance time" was therefore used to measure the recovered thrombin.

Following this technique, we prepared suspensions from normal plasma which produced a granular precipitate when mixed with human plasma or bovine fibrinogen at 18° C. However, the granulation did not form in plasma at 37° C; in fact, the granulation which had formed at 18° C disappeared at 37° C. Therefore, the endpoint, *i. e.* the "clotting time", could have been fibrinogen precipitated by ethanol in the test material, and not fibrin clotted by thrombin. To settle this problem, we centrifuged the last mixture after the granulation had formed. The sediment dissolved rapidly in saline at 18° C, and clotted solidly after addition of thrombin. The supernatant was nearly free of fibrinogen. We also found that a mixture of equal parts of 50 per cent ethanol and citrated saline produced a granular precipitate when added to the substrate, although it took somewhat longer to form and was not as coarse.

The suspension prepared from plasma contains fibrinogen which has been completely precipitated by the ethanol. Fibrin clots

or clumps should nevertheless occur if the suspension also contained free of recovered thrombin. However, clots were not observed, a fact which suggests that it may not contain thrombin. As a further test, we added an equal volume of commercial bovine thrombin (Topostasine, Hoffmann-La Roche, Switzerland) diluted in saline. This resulted in the formation of coarse fibrin clumps which were not soluble at 37° C and differed markedly from the fine granular precipitate described above. With as little as 0.3 NIH units per ml thrombin solution, this change, which we believe is due to coagulation, occurred in 60 seconds. Similar results were obtained with bovine thrombin in 25 per cent ethanol and with human thrombin prepared by Sternberger's method (1948). This observation confirms that the suspension did not contain thrombin.

Sternberger & Maltaner (1951) added thrombin prepared by Sternberger's method (1948) to serum, waited for complete inactivation, and then recovered the thrombin quantitatively by the method describer. The thrombin was measured by the "granule appearance time". We have repeated this experiment many times, and have always failed to recover any thrombin which could produce the coarse, irreversible fibrin clumps of ordinary thrombin.

From these observations we must reject Sternberger's claim that normal plasma contains thrombin which can be recovered by this method. We believe that his assay is unspecific, and that granulation of the substrate is caused by ethanol precipitation of fibrinogen. It therefore does not measure thrombin.

Reich & Sternberger (1956) found that more thrombin could be recovered from the

plasma of patients with postoperative complications than from normal individuals. Since we have failed to demonstrate any thrombin in normal plasma, we have not repeated this work. Their patients also had high concentrations of fibrinogen which may have influenced the assay. Thus, we found that granulation occurred more rapidly if we added "thrombin" recovered from plasma than that from the same patient's serum. We therefore believe that the assay is influenced by unspecific effects of slightly denatured proteins, especially fibrinogen.

Our experiments do not rule the possibility that plasma may contain thrombin, nor that continuous *in vivo* coagulation may be a normal phenomenon. However, if our observations and conclusions are correct, Sternberger's observations should no longer be used as evidence in favour of the occurrence of thrombin in normal plasma.

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