THE DETECTION OF A CIRCULATING ANTICOAGULANT ACTIVE AGAINST BLOOD THROMBOPLASTIN FORMATION

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A circulating anticoagulant which interferes with the formation of blood thromboplastin is well recognized as one cause of a haemorrhagic disorder. Several techniques for their demonstration have been described, depending on the ability of the patient's blood to prolong the clotting time of normal whole blood (Singer, Mond, Hyman, and Levy, 1950) or plasma (Biggs and Macfarlane, 1953), or to interfere with the prothrombin consumption of normal blood (Stefanini and Damashek, 1955), or on the results of thromboplastin generation tests (Biggs and Douglas, 1953; Hougie, 1953; Evans, 1955). Hougie (1955a) considers these last to be the most sensitive and reliable for the detection of clotting inhibitors of this type.

In a case recently available for investigation (Nussey and Dawson, 1957) recalcification methods on plasma mixtures and the thromboplastin generation test failed to reveal an anticoagulant, but by a modification of Hougie and Fearnley's (1954) procedure for determining the action of antithromboplastinogens its presence, as well as its action, was established.

Modified Hougie and Fearnley Test

Normal plasma, serum, and platelets are prepared as for the standard thromboplastin generation test of Biggs and Douglas (1953). The serum is diluted at least two hours before use (Bergsagel, 1955).

Alumina plasma, 0.2 ml., diluted 1 in 5, and 0.2 ml. serum, diluted 1 in 10, are incubated with 0.2 ml. calcium chloride for 10 minutes at 37° C. The generation mixture is then completed by the addition of 0.2 ml. platelet suspension, and sampling carried out at minute intervals. The substrate may be recalcified 15 seconds before the addition of the thromboplastin sample to permit ease of performance of the test by one individual.

In the first test 0.1 ml. neat control aluminium hydroxide treated serum is added to the plasma-serum-calcium mixture before its incubation, in the second immediately after. The same tests are then carried out, substituting the patient's neat alumina serum for the control.

Results

Fig. 1 shows the results of these tests. Curves 2 to 5 show acceleration of thromboplastin generation, but curve 4 shows that incubation of the plasma-serum-calcium mixture with the patient's serum results in defective generation.

The same results were obtained when the alumina plasma was replaced with a preparation of anti-haemophilic globulin, and factor V added with the platelet suspension. This is the unmodified Hougie and Fearnley test.

A subsequent test in which antihaemophilic globulin was incubated with dilutions of the patient's

FIG. 1.—Hougie and Fearnley test. Curve 1 = normal plasma and normal serum. In curves 2 to 5 these reagents are incubated in the presence of calcium for 10 minutes before the addition of the platelet suspension at 0 minutes. In curve 2 the control serum is added before incubation, in curve 3 after incubation. Curves 4 and 5 are similar procedures to curves 2 and 3 but replacing the control serum by patient's serum.

alumina serum showed a quantitative destruction of this globulin by the serum to a titre of 1 in 5.

Comments and Summary

Methods involving the use of whole blood for the detection of anticoagulants are inconvenient because they have to be carried out at the patient's bedside. Lewis, Ferguson, and Arends (1956) record unsatisfactory findings with whole blood mixtures probably because, as Biggs and Macfarlane (1953) have noted, anticoagulants may require time for their full effect to be seen. It is for this reason that plasma mixtures are often incubated for varying periods before testing. Hougie (1955b), however, has pointed out that the calcium clotting time of normal platelet-free plasma lengthens on incubation and in practice this makes the results of tests on plasma mixtures difficult to interpret. Clotting time and prothrombin consumption tests demonstrate the presence of an anticoagulant if it is potent, but do not differentiate an antithromboplastinogen from other inhibitors of coagulation, whereas the thromboplastin generation test does.

For an anticoagulant to be detected by the thromboplastin generation test it must be active

immediately and be potent at a dilution of 1 in 40, if Hougie's (1955a) criteria are applied. By the method described, time is allowed for it to act, and its dilution can be reduced to 1 in 7. The modification of Hougie and Fearnley's test makes the preparation of antihaemophilic globulin and factor V unnecessary and therefore of application in any laboratory that does the standard thromboplastin generation test. It appears that the procedure described is more sensitive than those detailed in the literature for the demonstration of an antithromboplastinogen.

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