HAEMORRHAGIC DIATHESIS DUE TO A CIRCULATING ANTICOAGULANT INVESTIGATED BY THE THROMBOPLASTIN GENERATION TEST

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A circulating anticoagulant which inhibits or delays the formation of thromboplastin is now a recognized cause of acquired haemorrhagic diathesis (Biggs and Macfarlane, 1953). Reported cases of this condition may be divided into three groups. The first group consists of true haemophiliacs in whom the appearance of the anticoagulant follows blood transfusion. In the second group the anticoagulant develops shortly after a pregnancy in women who previously had shown no haemorrhagic tendency. The third group is a rather heterogeneous collection in which the anticoagulant is unassociated with haemophilia or recent pregnancy. It may develop in the absence of any other recognized disease (Joules and Macfarlane, 1938; Harrington, Desforges, Stohlman, Crow, and Moloney, 1950; Pons and Torregrosa, 1952; Hougie, 1953), but more often it is associated with some other condition, for example, syphilis and tuberculous lymphadenitis (Lozner, Jolliffe, and Taylor, 1940), non-specific lymphadenopathy (Conley, Rathbun, Morse, and Robinson, 1948), rheumatoid arthritis with amyloid deposits in lymph nodes (Collins and Ferriman, 1952), chronic nephritis and syphilis (Conley *et al.*, 1948), pemphigus treated by arsenicals (Quick and Stefanini, 1948; Dieter, Spooner, and Pohle, 1949), dermatitis (Tzanck, Soulier, and Blatrix, 1949), and myocardial infarction (Singer, Mond, Hyman, and Levy, 1950). The cases to be described belong to the third group.

Methods of Investigation

Thromboplastin Generation Test.—This test was performed as described by Biggs and Douglas (1953). Some of these tests were kindly undertaken by Dr. R. Biggs at the Radcliffe Infirmary. The remainder were performed at the Derbyshire Royal Infirmary. A number of tests were carried out in parallel at both laboratories.

The clotting times were measured by the method of Lee and White (normal 4–10 minutes), bleeding times by Duke's method (normal 2–5 minutes), and platelets by Lempert's method (normal 150,000–350,000/c.mm.), and prothrombin activity by Quick's one-stage method.

Case Summaries

Case 1.—Mrs. D., aged 45 years, a forewoman in hosiery, was well until May, 1952, when she developed severe bruising of the limbs following trivial injuries. There was no history of transfusion or recent pregnancy. She had two healthy children aged 19 and 14 years. The bruises gradually subsided on symptomatic treatment. In October, 1952, further bruising occurred, with haemorrhages into the neck and mouth causing dysphagia and trismus. Remissions and exacerbations followed. During 1953 she developed haemarthroses of both knees. Retrobulbar haemorrhage of the right eye, following a blow, resulted in blindness of this eye. In April, 1954, severe exsanguination followed massive perirenal haemorrhage. She recovered, but the bruising tendency still persists.

Investigations.—Clotting times were 4 to 50 min., usually over 30 min., and bleeding times $2\frac{1}{2}$ to 7 min. Prothrombin activity was 100%. Platelets numbered 260,000–470,000/c.mm. Haemoglobin was 3.7 g.% and red cells 1,770,000/c.mm. (after haemorrhage). The marrow showed a normoblastic reaction. Liver function tests were normal. A thromboplastin generation test showed no antihæmophilic globulin and a circulating anticoagulant.

Treatment.—Transfusions of fresh blood controlled the anaemia, but did not significantly reduce the clotting time. Cortisone, 50–100 mg. daily, reduced neither the clotting time nor the titre of the anticoagulant, but appeared to check the bruising tendency.

Case 2.—Mrs. B., aged 61 years, a bus cleaner, in October, 1951, suddenly developed severe spontaneous bruising, beginning in the left arm and becoming generalized. She had no history of bruising or transfusions before. At 59 years she suffered symmetrical arthritis of the wrists, elbows, shoulders, and knees. In March, 1952, she developed severe haematuria and melaena lasting two months. In July, 1952, a spontaneous haematoma of the right eye spread to involve the face (Fig. 1). With rest and fresh blood transfusion this subsided (Fig. 2).

Investigations.—Bleeding times were 2 to 3 min. Prothrombin activity was 100%. Platelets numbered 300,000–440,000/c.mm. Haemoglobin was 6.4 g.% and red cells 2,500,000/c.mm. (during haematuria). Liver

FIG. 1.—Case 2, 11 months after the onset of disease.

FIG. 2.—Case 2, 14 months after the onset of disease.

function tests were normal. Clotting times were 15–40 min. until April, 1953. The clotting time was reduced from 33 min. to 18 min. by the addition of 1 part normal blood to 9 parts patient's blood. (A corresponding addition of normal to known haemophilic blood reduced the clotting time from 31 to 6½ min.) A thromboplastin generation test (January, 1953) showed no antihæmophilic globulin but a circulating anticoagulant.

Treatment.—Fresh blood transfusions controlled hæmorrhages but never reduced clotting times below 15 min. During cortisone therapy clotting times fell to within normal limits (Fig. 3). No relapse followed withdrawal of cortisone. In May, 1953, the anticoagulant was barely detectable. By April, 1954, it had disappeared and antihæmophilic globulin activity had returned. The patient is now well.

Experimental Results

Thromboplastin Generation Test.—The presence of a circulating anticoagulant in these patients was demonstrated using the thromboplastin generation test. In this test a mixture is made of $\text{Al}(\text{OH})_3$ -treated normal plasma, normal serum, normal platelets, and CaCl_2 . In this mixture a very powerful thromboplastin develops. An anticoagulant which inhibits thromboplastin formation can be demonstrated by its action when added to this normal system. The approximate potency of the inhibitor can be measured by finding the highest dilution at which inhibition can be demonstrated. The method can of course also be used to demonstrate anti-

haemophilic globulin deficiency by replacing the normal $\text{Al}(\text{OH})_3$ plasma by that of the patient.

Demonstration of the Inhibitor in Case 1.—Throughout the patient's illness her plasma was found to inhibit thromboplastin formation. Initially the inhibitor could only just be detected. Later it increased in potency, reaching a fluctuating maximum after about 18 months. On occasions it was detected at dilutions of the patient's plasma of over 1 in 1,000. The inhibitor's effect is clearly demonstrated in Fig. 4.

Absence of Antihæmophilic Globulin in Case 1.—At no time was any antihæmophilic globulin activity detected. Even in the early stages, when the anticoagulant was very weak, replacement of the $\text{Al}(\text{OH})_3$ -treated plasma in the "normal system" by that of the patient resulted in negligible thromboplastin formation (Fig. 5). The possibility of Factor V deficiency was excluded by the normal prothrombin time.

Ability to Correct the Clotting Defect in Haemophilia.—Initially when the anticoagulant was very weak the ability of the patient's plasma to correct the clotting defect in hæmophilia was tested (Fig. 6). The patient's plasma did not correct the hæmophilic abnormality.

Results with Same Tests in Case 2.—This patient differed from Case 1 in that the anticoagulant finally disappeared. During the active phase of the disease the anticoagulant was clearly demonstrated (Fig. 7). After 18 months the patient was clinically improved and little anticoagulant activity could be

FIG. 3.—Clotting times during treatment with A.C.T.H. and cortisone in Case 2.

FIG. 4.—Thromboplastin generation test demonstrating inhibitor in Case 1 18 months after the onset of disease. "Normal system" = normal serum, normal platelets, and normal $\text{Al}(\text{OH})_3$ plasma, with CaCl_2 which initiates incubation; 1 vol. 0.85% NaCl added to the control "normal system"; 1 vol. diluted patient's plasma added to test the "normal systems." Dilutions are final dilutions in incubation mixture. Thromboplastin formation inhibited by high dilutions of patient's plasma.

FIG. 5.—Thromboplastin generation test showing absence of anti-haemophilic globulin activity in Case 1 five months after the onset of disease when the anticoagulant was very weak. "Patient's serum" = "normal system" with serum replaced by patient's serum. "Patient's plasma" = "normal system" with $\text{Al}(\text{OH})_3$ plasma replaced by patient's $\text{Al}(\text{OH})_3$ plasma. Thromboplastin formation was negligible with the latter.

FIG. 6.—Test showing failure of plasma from Case 1 to correct haemophilic clotting defect five months after the onset of disease. N = normal $\text{Al}(\text{OH})_3$ plasma. P = patient's $\text{Al}(\text{OH})_3$ plasma. H = haemophilic $\text{Al}(\text{OH})_3$ plasma. Mixtures as indicated replaced $\text{Al}(\text{OH})_3$ plasma in "normal system." The haemophilic defect was not corrected by patient's plasma.

FIG. 7.—Test demonstrating inhibitor in Case 2 14 months after the onset of disease. Dilutions of patient's plasma are final dilutions in incubation mixture.

FIG. 8.—Test during recovery of Case 2 showing deficiency of anti-haemophilic globulin but little inhibitory effect 18 months after the onset of disease. "Patient's serum" = "normal system" with serum replaced by patient's serum. "Patient's plasma" = "normal system" with $\text{Al}(\text{OH})_3$ plasma replaced by patient's $\text{Al}(\text{OH})_3$ plasma, thromboplastin formation being negligible. "Normal system" + patient's plasma = patient's $\text{Al}(\text{OH})_3$ plasma added to normal system, thromboplastin formation being practically normal.

detected. At this time it is of note that, although little inhibitory effect could be detected, the patient's plasma still showed a deficiency of antihaemophilic globulin (Fig. 8). A year later the antihaemophilic globulin had returned to normal.

Discussion

Both cases present a clinical picture which resembles haemophilia starting in middle life. Haemarthroses occurred, but haemorrhage into the soft tissues was a more prominent feature. As in haemophilia the clotting time was prolonged and the bleeding time was normal. This resemblance to haemophilia is in keeping with the absence of antihaemophilic globulin activity shown by the thromboplastin generation test, further confirmed by the failure of the patient's blood to correct the clotting defect of a known haemophiliac. In addition a circulating anticoagulant was clearly demonstrated in both cases.

There was an approximate correlation between the patient's clinical condition and the titre of the anticoagulant. In both cases acute haemorrhagic episodes were associated with high titres of anticoagulant. The recovery phase of Case 2 was

associated with diminution and finally the disappearance of the anticoagulant. It must, however, be mentioned that Case 1 appeared clinically well for several weeks when the circulating anticoagulant was demonstrated at titres of over 1 in 400. It is not clear whether this apparent temporary immunity to the circulating anticoagulant was related to the coincident cortisone therapy. Nor is it certain that the disappearance of the anticoagulant in Case 2 was due to the cortisone given at that time.

The thromboplastin generation test findings indicate that the anticoagulant acts by inhibiting thromboplastin formation. The absence of antihaemophilic globulin activity in both cases suggests that the site of action of the anticoagulant is on the antihaemophilic globulin itself.

In Case 2 the antihaemophilic globulin activity returned following the disappearance of the circulating anticoagulant. This is what would be expected if the anticoagulant specifically inactivated antihaemophilic globulin.

Craddock and Lawrence (1947) presented evidence that the anticoagulant which develops in some haemophiliacs is an immune antibody whose corresponding antigen is the antihaemophilic globulin of transfused blood. This has been confirmed by Frommeyer, Epstein, and Taylor (1950). It has also been suggested that the anticoagulant in both haemophiliacs and non-haemophiliacs may be of a similar nature (Quick and Stefanini, 1948; Collins and Ferriman, 1952). This would imply that the anticoagulant developing apparently spontaneously in non-haemophiliacs may be auto-antibody. By analogy with the auto-antibody of acquired haemolytic anaemia a favourable response of at least some cases to cortisone and A.C.T.H. is not a surprising finding.

Summary

Two cases are presented of severe acquired haemorrhagic diathesis occurring in women unassociated with haemophilia or recent pregnancy.

They resemble haemophilia clinically and show a prolonged clotting time with a normal bleeding time.

The thromboplastin generation test revealed an anticoagulant which inhibited thromboplastin formation. It also demonstrated the absence of antihaemophilic globulin activity.

The absence of antihaemophilic globulin activity was related to the presence of the anticoagulant. In the second case disappearance of the anticoagulant coincided with cortisone therapy, after which antihaemophilic globulin activity returned.

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