Deficiency of the Hageman Factor Demonstrated by the Thrombin Generation Test

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Ratnoff and Margolius jr. (1955) and Ratnoff and Colopy (1955) have described a coagulation defect in the plasma thromboplastin system caused by lack of a thermostable serum factor, which they named the Hageman factor. The Hageman factor remained in absorbed normal serum heated to 56° C. for half an hour. It could be precipitated from this serum by 25—40% saturation with $(NH_4)_2SO_4$.

Ratnoff et al. described 3 persons with the Hageman trait, a 37 year old man and unrelated to him 2 sisters, 46 and 50 years old respectively. The patients had never had symptoms of haemorrhagic diatheses. All the patients had been operated upon without bleeding accidents. The two sisters had had tooth extractions without haemorrhages. Both of them had given birth to children. The only demonstrable defect in the coagulation system was a considerably prolonged coagulation time. In addition two of the patients had decreased capillary resistance. Frick and Hagen (1956) reported two men with the same trait. Both of them had been operated upon without haemorrhagic tendency. One of them had been wounded during the second world war without bleeding complications.

Experimental

Case report: The patient is a 37 year old man, who has never suffered from severe diseases. Especially there has never been observed signs of liver diseases. There has never been bleeding attacks, not even after dental extraction. In the family no haemorrhagic disorders have been observed.

Methods: The thrombin generation test was performed as described before (Macfarlane and Biggs [1953], Pitney and Dacie [1953], Sjølin [1956a]). During this test the

recalcification time on diluted plasma is also determined. Quicks prothrombin time was determined according to Biggs and Macfarlane (1953). The capillary resistance was measured with the Bexelius method. Barium sulfate absorbed serum was prepared from normal blood after spontaneous coagulation as follows: The blood sample with the clot was left for 4 hours in a waterbath at 37° C. and stored overnight in an ice-box (4° C). The sample was then centrifuged at 2500 r. p. m. for 15 minutes and the clot removed. The serum was absorbed twice with barium sulfate (Brodthagen [1953]). Part of the serum was placed in a waterbath for 30 minutes at 56° C. The samples of absorbed and heated serum were stored at — 20° C.

Platelet suspensions were prepared from the patient's citrated blood. The blood sample was centrifuged at 1000 r. p. m. for 2 minutes. The plasma was again centrifuged at 2500 r. p. m. for 20 min. and the plasma discarded. The platelets were washed 4 times in saline. The sedimented platelets were resuspended in saline. One sample contained 32 000 platelets per mm^3, the other 100 000 platelets per mm^3.

Results. Quicks prothrombin time was 18, 16 and 16 sec. (control: 17). Platelet count: 324 000/mm^3 plasma. The recalcification time on diluted plasma was 5 min. (control: 1½ to 4 min). Bleeding time: 3 minutes. Bexelius: 3 Petechiae.

The thrombin generation test showed that the maximum of thrombin concentration was obtained 10 min. after recalcification (Fig. 1A). The lag period was 4 minutes and the increase in thrombin concentration was rather slow. It should be mentioned, that the clotting times in the first 4 tubes with fibrinogen solutions exceeded 23 minutes, which is an extremely long coagulation time for the thrombin estimation.

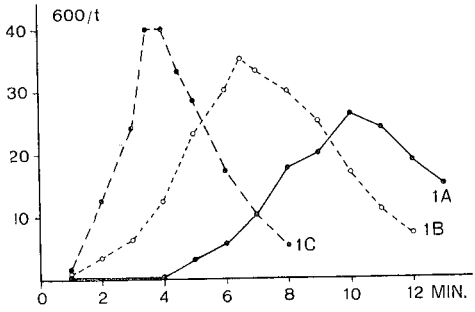

Fig. 1: *Thrombin generation in the patient's plasma.* Curve A is obtained with no addition. Curve B is obtained after addition of fresh normal plasma, and curve C after addition of reabsorbed heated serum. *Abscissa:* Reaction time in minutes. *Ordinate:* Reciprocal clotting time expressed as 600/t (t in seconds).

After the addition of 0.2 ml fresh normal plasma to 1 ml of the patient's plasma, the lag period disappeared and the thrombin generation became normal (Fig. 1B). The recalcification time on diluted plasma fell to 2 min. 30 sec. After the addition of 0.2 ml reabsorbed heated serum to 1 ml plasma the thrombin generation became even better (Fig. 1C). The recalcification time was now 1 min. 50 sec. The addition of 0.2 ml of the patient's plasma to 1 ml plasma from patients with Christmas disease (Fig. 2A) and classical haemophilia (AHF deficiency) (Fig. 2B) gave normal thrombin generation curves in both cases. Fig. 2C

and D show the spontaneous thrombin generation in the patient with Christmas disease and AHF deficiency respectively before addition of the Hageman plasma. In Fig. 3 is shown the influence of freezing of the patient's plasma. Curve A shows the thrombin generation in the patient's platelet poor plasma, which had been stored for 24 hours at — 20° C., and contained less than 1000 platelets per mm^3 plasma. The lag period is almost normal, about 3 minutes, but the velocity of the thrombin formation and the amount of thrombin formed are decreased. Addition to this plasma of 1 ml platelet suspensions prepared as described and containing 32 000 and 100 000 platelets per mm^3 respectively instead of 1 ml saline, gave completely normal thrombin generation curves (Fig. 3B and C). The platelet suspensions had not been frozen. Fig. 3D shows the thrombin generation in the patient's plasma with its own normal platelet content after it had been stored at — 20° C for 24 hours. The thrombin formation was now completely normal.

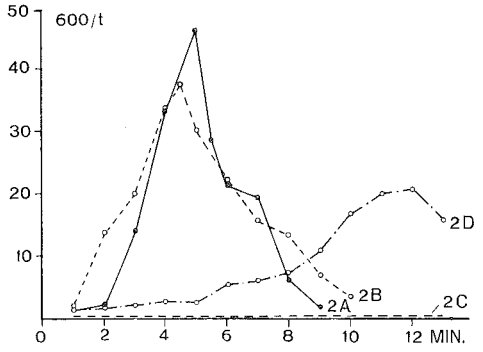

Fig. 2: Thrombin generation in patients with Christmas and AHF deficiencies. Curves C and A show the thrombin generation in the plasma from a Christmas patient before and after the addition of Hageman plasma respectively. Curve D and B the corresponding experiments with plasma lacking AHF (antihaemophilic factor). Values recorded as in Fig. 1.

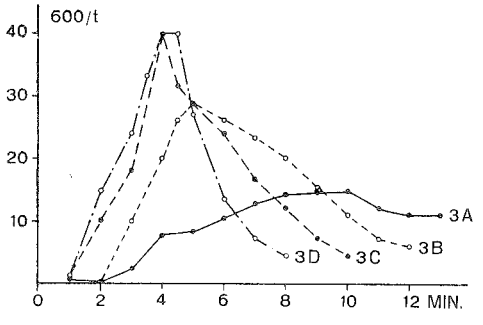

Fig. 3: *Effect of platelets and of freezing on the patient's plasma.* For details see text. Values recorded as in Fig. 1.

Discussion

The experiments reported here indicate, that the patient has a deficiency in the plasma thromboplastin system corresponding to the Hageman trait. There was no bleeding tendency. The recalcification time was slightly prolonged and the thrombin generation was delayed. There was no sign of an inhibitor, as the thrombin generation became normal after addition of 20% normal plasma. The plasma was deficient neither in Christmas factor nor in antihemophilic factor, as it made the coagulation system in plasma samples from these patients normal. We have not had the opportunity to test the plasma against plasma from patients with PTA defiency nor from patients lacking the fourth thromboplastin Factor of S p a e t et al., but both these factors should be missing in a sample of stored, heated and absorbed serum.

The results obtained by freezing the plasma are significant. The fact that the addition of the patients own fresh platelets to his previously frozen platelet poor plasma could make the thrombin generation normal cannot be explained at the present time. This phenomenon has also been demonstrated in plasma samples from a group of patients with manifest haemophiloid disease belonging to the Christmas group (S j ø l i n , in preparation). The finding that the thrombin generation in the patient's plasma with its normal platelet content became normal after storage at — 20° C. is also found in cases of a haemophiloid disease belonging to the Christmas group (S j ø l i n [1956 b]).

The defect in the patient's clotting system is in severity comparable to the defects demonstrated by the thrombin generation test in plasma from patients with a manifest haemophiloid disease. The fact that it does not give clinical symptoms indicate, that the experimentally demonstrated coagulation defect in haemophiloid diseases is not the sole cause of the haemorrhagic tendency.

Summary

A coagulation defect in the plasma from a 37 year old man with no bleeding symptoms is described. The defect was revealed by the thrombin generation test and seemed to be caused by lack of the Hageman factor. The coagulation defect could be corrected by freezing of the plasma.

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Résumé

Description d'une anomalie de coagulation chez un homme de 37 ans en bonne santé et sans hémorragies. L'anomalie a été détectée grâce au test de la

formation de la thrombine et semble être causée par une déficience du facteur Hageman. La congélation du plasma corrige l'anomalie de la coagulation plasmatique.

Zusammenfassung

Es wird ein Koagulationsdefekt im Plasma eines 37 Jahre alten Mannes ohne Blutersymptom beschrieben. Der Defekt konnte im Thrombingenerationstest nachgewiesen werden und schien auf dem Fehlen des Hagemanfaktors zu beruhen. Durch Gefrierenlassen des Plasmas konnte der Defekt behoben werden.

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