Fibrinogen as a Co-factor in the Reaction of Platelets with Kaolin

WE have shown[1] that the clotting time of platelet-rich plasma (PRP) is progressively shortened by incubation at 37° C with a suspension of kaolin, and that this reflects a progressive increase in availability of platelet factor 3 (PF3) as well as activation of the plasma clotting system. Since maximal acceleration of clotting by kaolin was only achieved when platelets and plasma were activated to-togther, it seemed likely that a plasma component was required for the reaction of platelets with kaolin. In order to determine whether a plasma coagulation factor was concerned in this reaction, investigations were carried out on the PRP of patients with a series of congenital coagulation defects. Equal volumes (0·1 ml.) of PRP and kaolin (5 mg per ml. of buffered saline, *p*H 7·3) were incubated at 37° C for 20 min and then recalcified with 0·1 ml. of 0·025 M calcium chloride, 0·1 ml. of a partially purified preparation of the deficient factor (or of buffered saline) being added in each case immediately before or after incubation with the kaolin. In the cases of factor-XII and factor-XI deficiency, activation product (prepared by the method of Nossel[2]) was used in place of the respective purified factors. Factors V and VIII were prepared by ammonium sulphate precipitation from barium sulphate-adsorbed platelet-poor oxalated normal plasma and factors VII, IX and X by adsorption of normal serum on to barium sulphate and subsequent elution into 5 per cent trisodium citrate. The fibrinogen reagent used (kindly provided by the Blood Products Laboratory of the Lister Institute) was prepared from fresh plasma by the method of Kekwick and Mackay[3] and was 66 per cent clottable; it also contained plasminogen and some factor VIII and anti-von Willebrand activity.

The results of this series of experiments are shown in Table 1, from which it can be seen that in every case, except those of fibrinogen deficiency, there was no significant difference between the degree of correction of the clotting time of PRP, whether the deficient factor was added before or after incubation with kaolin. It thus appears that the activation of PF3 by kaolin does not depend on prior activation of factors XII and XI, and is independent of all the clotting factors tested except fibrinogen. Horowitz[4], using a Stypven time system, has also found that factors V, VIII, X, XI and XII are not required for PF3 activation by celite.

In order to determine the quantitative relationship between fibrinogen and PF3 activation, PRP from the patient with a plasma fibrinogen concentration of 15 mg per 100 ml. was incubated for 20 min with an equal volume of kaolin suspension in the presence of a series of dilutions of fibrinogen; a mixture of equal parts of fibrinogen (310 mg per 100 ml.) and 0·025 M calcium chloride was then added, so that the clotting time of the mixture was not significantly influenced by the final fibrinogen concentration, but only by that present during incubation with kaolin. The results (Fig. 1) show that PF3 availability is significantly reduced only when the fibrinogen concentration falls below about 30 mg per 100 ml. This is in close agreement with the fibrinogen concentration of 20 mg per 100 ml. which Caen *et al.*[6], in investigations

Table 1. KAOLIN CLOTTING TIME OF DEFICIENT PLATELET-RICH PLASMA

Deficient factor (Per cent)	Clotting time (sec) Deficient factor added before incubation with kaolin	Deficient factor added after incubation with kaolin	Deficient factor not added
XII (<1)	39	36	110
XI (<1)	38	39	97
X (10)	39	37	65
IX (2)	32	32	70
VIII (<1)	39	44	107
VII (<1)	36	33	29
V (<1)	41	47	120
Fibrinogen (1 mg/100 ml.*)	30	62	>600
Fibrinogen (15 mg/100 ml.†)	30	77	166

* Reference 5.
† Reference 6.

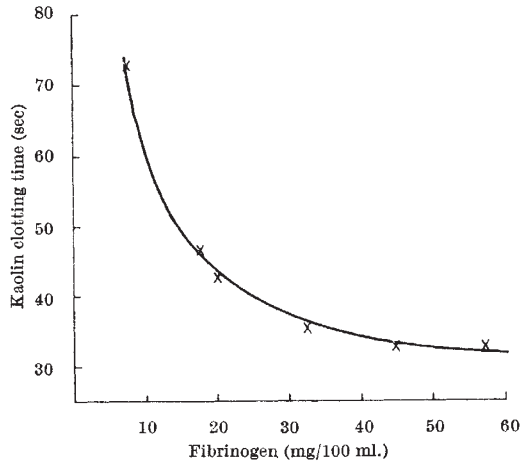

Fig. 1. Relationship between kaolin clotting time of platelet-rich plasma and fibrinogen concentration during incubation with kaolin

on the same patient, found necessary for spontaneous platelet aggregation and adhesion to glass.

Evidence has also been presented[7–9] that fibrinogen is an essential co-factor for the aggregation of platelets by adenosine diphosphate (ADP). We have found that the activation of PF3 by incubation of PRP with kaolin is always associated with the formation of mixed aggregates of platelets and kaolin particles, and that both are inhibited by ADP antagonists and by EDTA, and fail to occur in thrombasthenia[1,10,11]. Platelet/kaolin aggregation also failed to occur during the investigations on afibrinogenaemic PRP reported here, unless fibrinogen was added. These findings thus suggest that PF3 availability in this system results from the adhesion of platelets to kaolin and from their aggregation, and that these reactions are dependent on the action of ADP, which here, as in other systems which have been investigated, requires fibrinogen as a co-factor.

These experiments do not, of course, resolve the question whether other plasma components besides the coagulation factors are also required for this reaction. Similar investigations on patients with von Willebrand's disease have given inconclusive results, chiefly for the reason that the kaolin clotting time of their PRP has usually differed too slightly from that of normal controls to allow the effect of plasma fractions to be properly evaluated.

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