INTERACTION OF THE FIRST COMPONENT OF COMPLEMENT (C1) IN PLATELET ADHESION AND AGGREGATION INDUCED BY COLLAGEN. J.L. Watier, H. Souchon, A.P. Feltier and J.P. Caen, Hôpital Lariboisière, Paris, France.

The attachment of C1 to human platelets through C1q and the inhibition of platelet aggregation induced by C1r and C1s prompted us to investigate a possible interaction between these activated C1 and collagen. C1q is a molecule composed of two moieties: a collagen-like region and a globular region composed of polypeptides which may be isolated for each of it. The globular region and specific antigens (anti-C1q, anti-C1r, anti-C1s) were tested for their effect on collagen-induced aggregation and adhesion of gel filtered platelets. The results suggest that C1q containing the collagen-like region inhibited platelet aggregation and adhesion to collagen while collagenase treated C1q (globular moiety) did not modify the reaction. Preincubation of collagen with C1s inhibited platelet adhesion and aggregation. In other experiments platelets modified by anti-C1s did not react with collagen while anti-C1q, anti-C1r or anti-fibrinogen did not influence the reaction with collagen. These results suggest that the collagenlike region of C1q binds to C1s with a possible reaction between C1s associated with the platelet or a platelet structure similar to C1s and collagen.

THE INVOLVEMENT OF FACTORS XI, XII IN THE PLATELET COAGULANT ACTIVITY. J.H. Connellan, G. Bowden, and P.A. Castledi. Austin Hospital and University of Melbourne, Melbourne, 3084, Australia.

Platelets provide coagulant activities, other than the phospholipid Platelet factor 3, which can be released from the platelet. In Platelet Poor Plasma, the initiation of the intrinsic pathway is proposed to proceed via Factors XII & XI as well as 2 other factors, Prekallikrein and Kininogen. The role of these factors in the initiation of coagulation in Platelet Rich Plasma is under investigation. Walsh, Spr.J.Haematol.22:795,1972) and Hume (Proc.R.Soc.34:941,1974) have shown that platelets devoid of Factor XII will still produce a coagulant activity when Platelet Rich Plasma is incubated with collagen. However, we have recently shown that this reaction requires the presence of factor XI. Factor XII has been purified of XIIa by collagenase and pepstatin (34:941,1974), and Factor XI has been purified by the method of Connellan, Casteldi and Hume. (Haemostasis, in press 1977).

Specific antibodies were developed in rabbits and the IgG from the antisera was coupled to Sepharose. These antibodies could be adsorbed from plasma by the immobilized antibodies and could then be eluted by the use of alkaline or guanidine HCl, thus resulting in a rapid purification of these factors.

Platelets were washed free of loosely bound clotting factors and provided significant amounts of coagulant activity when ruptured by either freezing and thawing or by glycerol treatment. Platelets from a factor XI deficient patient also provided this activity. The platelet activity was extracted by passage through both Sepharose-heparin columns and Sepharose-anti-factor XI column: the activity appeared to be identical to plasma factor XI.

DIFFERENT REQUIREMENTS FOR INTRINSIC FACTOR-XA FORMING ACTIVITY AND PLATELET FACTOR 3 ACTIVITY AND THEIR RELATIONSHIP TO PLATELET AGGREGATION AND RELEASE. P.H. Walsh and M.R. Lipscomb, Temple University School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Platelets provide coagulant activity in part by making available membrane phospholipids which promote the reactions of: (1) Factors X, VIII, IX and calcium to activate factor IX (intrinsinc factor-Xa forming activity or XaPA) and, (2) Factors Xa, V and calcium to activate platelet factor 3 activity (PF3A). We have studied the availability of XaPA and PF3A, platelet aggregation and [14C]-SNAP release in washed platelet suspensions, incubated with collagen. XaPA developed rapidly (100% activity at 15-30 sec) well before maximum [14C]-SNAP release (2-4 min) and decayed to 10% at 5-10 min. PF3A developed slowly (100% activity after 20 min stirring with collagen well after maximal aggregation and release. Maximal XaPA availability occurred in unstimulated platelet suspensions incubated with collagen and not undergoing aggregation or release, and was not enhanced by stirring, whereas PF3A did not become available unless platelet suspensions were stirred with resultant aggregation and release. PF3A, XaPA, aggregation and release were inhibited by pretreating platelet suspensions with p-chloromercuribenzenesulfonate, heparin, and adenosine A, whereas pretreatment with indomethacin inhibited aggregation, release and PF3A availability but not XaPA availability. These results indicate that the biochemical determinants of XaPA and PF3A are different. PF3A developed only when aggregation and release occurred whereas XaPA was independent of aggregation and release.