PLATELET FACTOR XI. P.A. Castaldi, J. Connellan, I. Smith and H. Michel. Haematology Department Austin Hospital, Melbourne, Australia and Laboratoire d'Hémostase et de Thrombose Expérimentale, Hôpital Saint-Louis, Paris, France.

Contact factors are known to be associated with platelets. There is evidence to suggest that F XI may mediate an enhancement of coagulant activity in response to collagen stimulation of platelets. In this study we further explore this relationship using fresh platelets and extracts prepared from normals and a F XI deficient subject. Antisera against plasma F XII and F XI were used in tests of collagen induced coagulant activity and the latter antibody in immunoprecipitation studies with platelet extracts. Comparisons were made with antisera to isolated platelet membranes.

Collagen induced coagulant activity was inhibited by anti F XI and to a slight degree by anti F XII. F XI-like coagulant activity could be detected in extracts of washed platelets and this activity adsorbed to heparin-sepharose as does plasma F XI. Similar activity could be detected in platelet extracts from the F XI deficient patient. Normal platelets contained an antigen that precipitated with anti F XI.

F XI activity and antigen are closely associated with platelets. This may provide a mechanism for enhancement of coagulation.

PROTEASES IN BLOOD PLATELETS : COLLAGEN INDUCED ACTIVATION OF PROELASTASE BY A TRYPSIN-LIKE ENZYMES. Y.J. Legrand and J.P. Caen, I.N.S.E.R.M. U. 150, Hôpital Lariboisière, Paris, (France)

Arterial elastolysis is an important feature of atherosclerosis and the comprehension of the activation mechanism of platelet elastase (proelastase) is essential to the knowledge of the role of platelet proteases as an agent of vascular alterations. Trypsin, chymotrypsin and elastase-like proteases were purified from a platelet protein fraction by a three step affinity chromatography procedure. Three electrophoretically homogenous proteins of molecular weights equal to 40×10^3 (trypsin), 32×10^3 (chymotrypsin) and 26×10^3 (elastase) daltons were obtained. Proelastase is strongly activated by the purified trypsin-like enzyme, whereas the chymotrypsin-like enzyme is ineffective. After the separation of platelet organelles on a discontinuous sucrose gradient proelastase, elastase and trypsin were found associated with the light granules and membranes. Fibrillar collagen incubated with platelets produces a rapid activation of proelastase; elastase is then released in its active form. This effect of collagen is likely indirect and may involve an intermediary step during which proelastase becomes available to the trypsin-like enzyme.

RADIOIMMUNOASSAY OF HUMAN PLATELET FACTOR 4. C.N. Chesterman, J.R. McGready and F.J. Morgan. University of Melbourne Department of Medicine and St. Vincent's School of Medical Research, St. Vincent's Hospital, Melbourne, Australia.

Plasma levels of the platelet specific protein, platelet factor 4 (PF4), may reflect alteration in platelet turnover or activation. Measurements more sensitive and specific than bioassay are required to test this hypothesis.

Highly purified human PF4 prepared by affinity chromatography on heparin/agarose and cation exchange chromatography has been used to develop a sensitive radioimmunoassay. A standard double antibody technique with goat antirabbit serum as second antibody has been used. Anti-PF4 antibody was raised in rabbits with the multiple subcutaneous injection technique. Following a single booster an antibody was obtained which, at a final dilution of 1:75,000, binds 37% of tracer PF4. Iodinated tracer PF4 was produced by the lactoperoxidase labelling technique and purified by gel chromatography in heparin containing buffer to prevent absorptive losses. The assay buffer also contains heparin 1 U/ml.

The tracer shows good stability (4-8 weeks) and adequate immunoreactivity. Non-specific binding was $2.4 \pm 0.8\%$ in 11 consecutive assays. Sensitivity of the assay, expressed as 50% displacement of the tracer, is normally 0.3-0.4 ng per tube with a working range of 0.06-1.25 ng (20-80% displacement). Twelve replicate determinations of a single plasma sample yielded a within-assay standard deviation of 0.6 ng/ml and coefficient of variation of 0.1%. The assay is highly specific, the closest cross reactivity being obtained with β -thromboglobulin which produces 50% displacement of tracer with 5 μ g/tube. Plasma preparation may not yet be optimal, but normal values in carefully prepared samples lie below 10 ng/ml.