C. S. Grace and P. Wolf (Prince Henry Hospital, Matraville 2036 Australia): **A High Titre Circulating Inhibitor of Human Factor V: Clinical, Biochemical and Immunological Features and its Treatment by Plasmapheresis. (20)**

A 44 year old male with a two month history of epistaxis and haematuria was admitted because of melaena. He had not received antibiotics or blood transfusions. Haemostatic studies showed a complete absence of Factor V activity and this was not corrected by the addition of an equal part of normal plasma. Incubation studies showed that the inhibitor was specific for human Factor V. Factor VIII and IX activities were low by one stage assay methods but were normal after the addition of bovine Factor V.

The titre of the antibody was very high; a 900 fold dilution causing a 50% reduction in the Factor V level of an equal volume of normal plasma on incubation at 37° C for 30 mins. The inhibitor appeared in the second protein peak on Sephadex G200 fractionation and was neutralized by antihuman IgG antiserum. Kinetic studies showed the inhibition to be first order with respect to Factor V and inhibitor.

The patient was treated with plasmapheresis on a IBM Celltrifuge. Four litres of plasma were removed on each of four occasions over a period of 14 days. The antibody titre dropped from 900 to about 100. Despite the addition of immunosuppresive therapy the antibody has persisted at a titre of about 200 and has now been present over 5 months. No anamnestic response occurred following multiple transfusions.

H. R. Roberts, C. R. Fuller, H. Worden, J. Stuart, H. Reisner, K. Koehler and W. J. Yount (University of North Carolina School of Medicine, Chapel Hill, North Carolina, U.S.A.): **Immunochemical Characterization of a Factor IX Inhibitor Following Anamnestic Response. (21)**

We previously characterized a human inhibitor for Factor IX in patient P.W.B. with Hemophilia B as an IgG4, λ immunoglobulin of restricted electrophoretic mobility. This restriction to a minor IgG subclass led us to characterize a second Factor IX inhibitor occurring in patient R. J. after an anamnestic response to Factor IX. On preparative zone electrophoresis the inhibitor migrated with a broad zone of mobility in the anodal portion of the γ peak and was restricted to the anodal portion of the IgG containing fractions. Gel filtration on calibrated 1.5 M sepharose columns revealed inhibitor activity in fractions corresponding to a molecular weight of 150,000. The inhibitor was further characterized by the technique of antibody neutralization using monospecific antisera to immunoglobulin classes, subclasses and light chain types in the zone of antibody excess. The inhibitor was completely neutralized by antibody to IgG whereas antisera to IgA, IgM, IgD and IgE had no effect. Neutralization was abolished by absorption of the IgG antiserum with purified IgG. Neutralization with antisera specific for light chains indicated a mixture of light chain types with an estimated κ/λ ratio of 6/1. Neutralization with antisera specific for IgG subclasses revealed a mixture of IgG subclasses. The Factor IX inhibitor was thus characterized as a polyclonal IgG immunoglobulin. Sepharose conjugates of R. J. globulin effect complete removal of Factor IX from normal plasma on an immunoabsorbent column and biologically active Factor IX may be eluted with 1600-fold purification.

K. Ørstavik and B. Østerud (Institute of Medical Genetics, University of Oslo, Norway, and Institute of Medical Biology, University of Tromsø, Norway): **Electroimmunoassay of Human Coagulation Factor IX in the Detection of Variants of Haemophilia B. (22)**

Factor IX was quantified by an electroimmunoassay (EIA, rocket immunoelectrophoresis), using a rabbit antiserum against human factor IX. The EIA was performed on plasma samples from 38 healthy persons. The range was 65–150% relative to a standard plasma pool, and a positive correlation was found between determinations of factor IX antigen and factor IX activity.

Seven patients with severe and six patients with moderate haemophilia B were tested for plasma cross-reacting material (CRM) by the EIA and by an antibody-neutralization test. Five of the seven patients with severe haemophilia had no detectable CRM by any

of the two techniques whereas the remaining eight patients had CRM varying from low to normal values. The coefficient of correlation was 0.82 between the determinations of CRM by the two techniques.

One of the patients with severe haemophilia and normal amounts of plasma CRM showed a prolonged prothrombin time using ox brain thromboplastin (haemophilia B_M). As a group, the other patients had a significantly reduced prothrombin time when compared to 20 healthy males.

Our results confirm that haemophilia B is a heterogeneous disorder and show that the EIA may be used to detect genetic variants of this disease.

M. Matsuoka, M. Ito, N. Sakuragawa and K. Takahashi (Niigata University School of Medicine, 951 Niigata, Japan): **Immunological Method for the Detection of the Carrier of Haemophilia B.** **(23)**

Both the immunoassay and bioassay were performed on factor IX activity of haemophilia B patient. Their values were compared with each other.

The immunoassay by neutralization was performed as follows: antibody to factor IX was obtained by immunization of purified factor IX to rabbit which was isolated by the technique of DEAE-Sephadex column chromatography using eluate from $BaSO_4$ which absorbed factor IX of normal human plasma.

In approximately 90% of the cases of definite carriers of haemophilia B, the activity of factor IX by bioassay was observed to be lower than that of factor VIII of haemophilia A carrier. The factor IX activity was observed to be at the same level as factor IX antigen by immunoassay in almost all of the cases, but in the cases of the mother of haemophilia B, and the North Carolina type of haemophilia B the factor IX antigen was much greater than that of activity by bioassay. The same results were obtained by the above mentioned methods using inhibitor substance arising from severe haemophilia B patient.

It was suggested that the immunoassay method is useful in detecting the carrier of haemophilia B and North Carolina type of haemophilia B.

P. A. Castaldi and K. M. McGrath (Austin Hospital and University of Melbourne, Melbourne, 3084, Australia): **Cyclical Variation in Factors VII and VIII Associated with Oral Contraception.** **(24)**

Sequential studies of the levels of coagulation factors II, VII, VIII (activity and antigen), IX, X and fibrinogen, were carried out in three females using oral contraceptive agents, and two normal controls. A striking increase in the levels of factor VII activity was seen in the second half of the menstrual cycle in all test subjects. In two test subjects, a marked cyclic increase in factor VIII activity occurred and this did not have any fixed relationship to VIII antigen levels.

The increase in factor VII activity was associated with shortening of the thrombotest in plasma tested after storage at –20 C. Activation of factor VII can be induced in vitro in 60% of oral contraceptive plasmas by incubation at 4 C for 16 hours. This cold promoted activity (CPA) also varies throughout a menstrual cycle, is independent of the prothrombin complex, being present in Al_2OH_3 adsorbed plasma, and is contained in the supernatant of the euglobulin fraction in CPA positive plasmas. It can induce activation of factor VII in normal plasma, but not in contact factor deficient plasma. The nature of this cold sensitive activity and its relationship to the cyclical increase seen in factor VII activity and to oestrogen induced clotting in vivo, remains to be determined.

K. Miloszewski and M. S. Losowsky (St. James's Hospital, Leeds LS9 7TF, England): **Factor XIII Concentrate in the Long Term Management of Congenital Factor XIII Deficiency.** **(25)**

Congenital Factor XIII deficiency causes a serious and disabling bleeding diathesis with a high risk of fatal intracranial haemorrhage. Blood levels of Factor XIII of a few percent of normal are sufficient to control bleeding and the in vivo half-life of Factor XIII is long, making permanent prophylaxis practicable.