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