SIGNIFICANT ALTERATION IN THE STOICHIOMETRY OF FACTOR VIII INHIBITOR INTERACTIONS IN THE PRESENCE OF 0.25 M CaCl₂. G.E. Harkavy, P.L. Perry, M.R. Jameson, Stanford Univ. School of Medicine.

The major aim of this study was to assess whether the hemophilic VIII antibody (Ab) interacted more readily with "monomeric" factor VIII than with unidissociated VIII. Conditions were chosen so that dissociation would occur with low frequency, favoring the Ab molecule per VIII molecule. Remaining VIII (B) was kept at 65% to 85% of original VIII activity. Ab units were calculated by units of VIII neutralized x L/dilution of antisera. As PB fell below 65%, the apparent potency of Ab units decreased. A 25-donor pool of lyophilized plasma was made up with Na₂O or 0.25 M CaCl₂. Thus, within and between experiments, unknown but constant concentrations of VIII molecules and VIII antigens were present. Unknown but constant concentrations and types of Ab molecules from each of three inhibitor plasma were used. Samples were incubated 2 hours at 22°C. Controls remained stable for the duration. The two-stage factor VIII assay of Pool et al. allowed sufficient dilution of CaCl₂ for accurate assay.

For 21 independent experiments, R₀ = 417 ± 78 Ab u/ml and R₁Ca = 25.8 ± 10 Ab u/ml.

Thus, there was 2.07, 2.54, and 2.50 times more VIII neutralized in the 0.25 M CaCl₂ vs. Na₂O incubation. Experiments were also run at 37°C, slightly increasing Ab u/ml for Na₂O, but the Na₂O control often lost significant activity. Possible explanations include: 1) the antibody is bi-variate when VIII is monomeric; 2) Previously-hidden VIII-neutralizing Antigen sites are revealed in the VIII monomeric form; or 3) antigen/antibody affinity is altered.

STUDIES ON THE STRUUCTURE AND SUBUNIT COMPOSITION OF HUMAN ANTIHAEMOPHILIC FACTOR. J.J. Gorman, Department of Clinical Hematology and Oncology, Royal Children's Hospital, Parkville, Victoria 3052, Australia.

Human antihaemophilic factor has been purified by hydroxyapatite chromatography following precipitation from plasma and gel filtration on Sephacryl SE.

Application to hydroxyapatite was in 0.02 M tris-HCl (pH 7.5) - 0.14 M NaCl and after washing with 50mL phosphate (pH 6.8) - 0.1 M NaCl the antihaemophilic factor was eluted with 0.1M phosphate (pH 6.8) - 0.1 M NaCl. Factor VIII conqueulent activity, factor VIII related antigen and von Willebrand factor activity eluted simultaneously.

The protein(s) had a molecular weight in excess of 500,000 and multiple subunits as shown by electrophoresis in 5% acrylamide gels containing sodium dodecyl sulphate; without reduction the protein failed to enter these gels but following reduction multiple bands were observed, the major band had a molecular weight around 200,000.

Thin layer peptide mapping demonstrated structural inter-relationships between the 200,000 dalton protein and three of the smaller species, however, two other unrelated smaller species were evident.

It is apparent from these findings that human factor VIII may exist as multiple molecular forms due to heterogeneity of one subunit (MW around 200,000) and the molecular structure may include other smaller non-identical subunits. The structure-function relationships of these subunits remain to be elucidated.