SIGNIFICANT ALTERATION IN THE STOICHIOMETRY OF FACTOR VIII INHIBITOR INTERACTIONS IN THE PRESENCE OF 0.25 M $CaCl_2$. C.B. Harbury, P.L. Perry, M.E. Janszen. 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 undissociated VIII. Conditions were chosen so that multiplicity of neutralization would occur with low frequency, favoring one Ab molecule per VIII molecule. Remaining VIII (R8) was kept at 65% to 85% of original VIII activity. Ab units were calculated by units of VIII neutralized x 1/dilution of antisera. As R8 fell below 65%, the apparent potency of Ab units decreased. A 25-donor pool of lyophilized plasma was made up with H_2O or 0.25 M $CaCl_2$. 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 plasmas were used. Samples were incubated 2 hours @ 22° C. Controls remained stable for the duration. The two-stage factor VIII assay of Pool et al allowed sufficient dilution of $CaCl_2$ for accurate assay.

For 15 independent experiments, $\bar{X}_{Ca}$ = 53.4 ± 12 Ab u/ml and $\bar{X}_{H_2O}$ = 25.8 ± 10 Ab u/ml

For 31 independent experiments, $\bar{X}_{Ca}$ = 437 ± 78 Ab u/ml and $\bar{X}_{H_2O}$ = 174 ± 50 Ab u/ml

For 12 independent experiments, $\bar{X}_{Ca}$ = 80 ± 9 Ab u/ml and $\bar{X}_{H_2O}$ = 32 ± 9 Ab u/ml

Thus, there was 2.07, 2.51, and 2.50 times more VIII neutralized in the 0.25 M $CaCl_2$ vs. H_2O incubation. Experiments were also run at 37° C, slightly increasing Ab u/ml for H_2O; but the H_2O control often lost significant activity. Possible explanations include: 1) the antibody is bivalent 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.

FACTOR VIII-LIKE ACTIVITY OF BASIC AMPHOTERIC POLYELECTROLYTES. T. Exner, K. A. Rickard and H. Kronenberg. Haematology Department, Royal Prince Alfred Hospital, Sydney, Australia.

The broad-range Ampholine (LKB) 3.5-10 containing a mixture of materials with isoelectric points between pH 3.5 and 10 was found to correct the prolonged PTTK of factor VIII-deficient plasma, and this effect was investigated. Ampholines which are used as carrier ampholytes or pH gradient stabilizers in isoelectric focussing are condensation products of propionic acid and polyethylene polyamines.

The most effective of the commercial Ampholine mixtures were found to be those with the most alkaline isoelectric points. Ampholine 9-11 at 0.2% concentration was found to have factor VIII and factor IX activity equal to that of normal plasma as well as somewhat less activity as factor XI. It had no significant correcting effect on plasmas deficient in factors V, VII, X, II or XII. In contrast to polyglutamic and polyaspartic acids which have also been reported to correct for factor VIII deficiency in the PTTK, Ampholine 9-11 was effective whether added before or after kaolin contact activation of the test plasma. However, Ampholine 9-11 inhibited contact activation of plasma in the absence of kaolin and showed less correcting activity in partial thromboplastin time tests utilizing activators other than kaolin.

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

Human antihaemophilic factor has been purified by hydroxylapatite chromatography following precipitation from plasma and gel filtration on Sepharose 6B.

Application to hydroxylapatite was in 0.02 M tris HCl (pH 7.35) - 0.14 M NaCl and after washing with 5mM phosphate (pH 6.8) - 0.1M NaCl the antihaemophilic factor was eluted with 0.1M phosphate (pH 6.8) - 0.1M NaCl. Factor VIII coagulant 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-relationship 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 remains to be elucidated.