ADSORPTION OF F VIII ON ALUMINIUM HYDROXIDE. M.J. Segrath, E. T. Barrowcliffe, M. Miller-Anderson, W.E. Ehrlich, E.F. Stow. Clinical Hematology Branch, NIH, Bethesda, Maryland USA

Adsorption of plasma by Al(OH)₃ is a requirement for the two stage assay of F VIII. It is generally accepted that factors V, VIII, IX and X are removed at the procedure, while factors V and VIII are unaffected. Following gel filtration of a F VIII concentrate on Sepharose 4 B F VIIIic was found in the low molecular weight area, as well as in the void volume as expected. This activity was found with both one and two stage techniques. After one adsorption with Al(OH)₃ to eliminate the non F VIII procoagulant activity F VIIIic disappeared from the void volume fractions and was much reduced in the low molecular region. F VIII: R Ag was also removed from these fractions by Al(OH)₃.

The presence of hemophilia plasma clotting activity remained in both regions suggesting the presence of true F VIII activity. Thus at concentration of 1 IU of F VIIIic per ml, a low purity preparation was unaffected by Al(OH)₃, adsorption. After adsorption of fractions in the presence of hemophilic plasma clotting activity remained in both regions suggesting the presence of a large amount of non-F VIII activity.

A RAPID AND SIMPLIFIED METHOD FOR SEPARATION AND ANALYSIS OF PURE ANTHEMOPHILIC FACTOR FROM PLASMA. L.B. Shulman and K.H. Tuck. Clinical Hematology Branch, NIH, Bethesda, Maryland USA

Current methods of purifying anhemophilic factor (AHF) are complicated and lengthy, leading to marked loss of factor VIII (F VIII) activity despite low temperature processing. Uncertainties concerning denaturation or cryo-aggregation hinder clear interpretation of relationships between the F VIII and von Willebrand factor (VWF) activities of AHF, some utilizing the two activities exist on one molecule, others that two molecules are complexed.

Our method utilizes a controlled-pore glass column of Bio-Gel P-300. AFH separates as an isolated peak in the void volume while all other proteins including cold insoluble globulin and fibrinogen are retained. We obtain pure AFH, judged by SDS acrylamide gel and immunologic criteria, from large volumes of citrated plasma by a single filtration within one hour of obtaining blood. Processing entirely at 37° or 21° gives the same results. Yields are 40-60% of plasma F VIII activity.

Increasing ionic strength (pH) of plasma to >0.3 progressively dissociates VWF and F VIII up to 50%, VWF remaining at Nf 10⁶ while F VIII is retained by pores as small as 3300, indicating a MW of approximately 1.5 x 10⁵. Human anti-AHF reacts exclusively with separated F VIII, not with VWF, and rabbit anti-AHF reacts primarily with VWF. Findings on normal and hemophiliac plasma support the concept that F VIII and VWF are distinct components circulating as a weakly associated complex, and that hemophiliac lack F VIII by functional and immunologic criteria.

Preparation of pure AHF by this technique on a scale appropriate for clinical use is feasible.

HIGH MOLECULAR WEIGHT FACTOR VIII CONJUGATE ACTIVITY IN CRYOPRECIPITATE AND POLYETHYLENE GLYCOL PRECIPITATES. K. J. Richart, T. K. Murray and S. R. Robinson. Hematology Department, Royal Prince Alfred Hospital, Sydney, Australia

Gel filtration of human plasma cryoprecipitate on Sepharose 2B indicated the molecular weight of factors VIII with conjugated activity (VIIIc) to be significantly greater than that found in anhemophilic concentrate. Polyethylene glycol at 34% concentration precipitated approximately half of the VIIIc from cryoprecipitate. This activity eluted as high molecular weight fraction on gel filtration. The addition of pure polyethylene glycol to a concentration of 8% precipitated most of the remaining VIIIc from cryoprecipitate. This activity appeared to be of significantly lower molecular weight, approximately corresponding to clotting volume to that observed for anhemophilic concentrate. The possibility that an antibody to VIIIc generated in a patient treated with cryoprecipitate might be directed against the higher molecular weight form of factor VIII was investigated. However, no significant differences between the higher and lower molecular weight forms of factor VIII were found.