ADSORPTION OF F VIII ON ALUMINIUM HYDROXIDE. M.J. Seghatchian. T. Barrowcliffe. M. Miller-Andersson . NLBTC, Edgeware, U.K. NIBSC, London U.K. AB Kabi Stockholm, Sweden.

Adsorption of plasma by $Al(OH)_3$ is a requirement for the two stage assay of F VIII. It is generally accepted that factors II, VII, IX and X are removed by the procedure, while factors V and VIII are unaffected. Following gel filtration of a F VIII concentrat on Sepharose 4 B F VIII:c 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 adsorption of the fractions with $Al(OH)_3$ to eliminate the non F VIII procoagulant activity F VIII:c 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 $Al(OH)_3$ adsorption. After adsorption of fractions in 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 VIII:c per ml, a low purity preparation was unaffected by $Al(OH)_3$ adsorption whereas both antigen and clotting activity of a high purity concentrate were conciderably reduced. Addition of 5 % albumin to the high purity preparation prevented this adsorption. It is concluded that under conditions of high purification F VIII:c can be adsorbed preferentially on $Al(OH)_3$ and this appears to be due to removal of F VIII:R Ag.

A RAPID AND SIMPLE METHOD FOR SEPARATION AND ANALYSIS OF PURE ANTIHEMOPHILIC FACTOR FROM PLASMA. N.R. Shulman and K.M. Tack. Clinical Hematology Branch, NIH, Bethesda, Maryland USA

Current methods of purifying antihemophilic 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 suggesting the two activities exist on one molecule, others that two molecules are complexed.

Our method utilizes a controlled-pore glass column of pore-size approximately 1000Å. AHF separates as an isolated peak in the void volume while all other proteins including cold insoluble globulin and fibrinogen are retained. We obtain pure AHF, 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 22° gives the same results. Yields are 40-60% of plasma f VIII activity.

Increasing ionic strength ($\mu$) of plasma to >0.3 progressively dissociates vWf and f VIII up to $\mu$=0.75, vWf remaining at MW $>10^6$ while f VIII is retained by pores as small as 330Å, indicating a MW of approximately $1.5 \times 10^5$. Human anti-AHF reacts exclusively with separated f VIII, not with vWf; and rabbit anti-AHF reacts primarily with vWf. Findings on normal and hemophilic plasma support the concept that f VIII and vWf are distinct components circulating as a weakly associated complex, and that hemophiliacs 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 COAGULANT ACTIVITY IN CRYOPRECIPITATE AND POLYETHYLENE GLYCOL PRECIPITATES. K. A. Rickard, T. Exner and H. Kronenberg. Haematology Department, Royal Prince Alfred Hospital, Sydney, Australia.

Gel filtration of human plasma cryoprecipitate on Sepharose 2B indicated the molecular weight of factor VIII coagulant activity (VIIIc) to be significantly greater than that found in antihaemophilic concentrate. Polyethylene glycol at 3% concentration precipitated approximately half of the VIIIc from cryoprecipitate. This activity eluted as high molecular weight material on gel filtration. The addition of more 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 in elution volume to that observed for antihaemophilic 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 either in stability or in reactivity with human antibody to factor VIII were found.