
Adsorption of plasma by Al(OH)₃ is a requirement for the two stage assay of Factor VIII. It is generally accepted that factors IX, VII, IX and X are removed by this procedure while factors V and VIII are unaffected. Following gel filtration of a Factor VIII concentrate on Sepharose 4B, Factor VIII 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 amino acid fractions with Al(OH)₃ to eliminate the non-F VIII procoagulant activity Factor VIIIc disappeared from the void volume fractions and was much reduced in the low molecular weight region. Factor VIIIc:Ag was also removed from these fractions by Al(OH)₃.

The presence of hemophilia plasma eluting activity remained in both regions suggesting the presence of true Factor VIII activity. Upon addition of 1 IU of Factor VIIIc per ml, a low purity preparation was unaffected by Al(OH)₃ while adsorption of fractions in the high purity concentrate were considerably reduced. Addition of 5% albumin to the high purity preparation prevented this adsorption. It is concluded that under conditions of high purification Factor VIIIc can be adsorbed preferentially on Al(OH)₃ and this appears to be due to removal of Factor VIII:Ag.

A RAPID AND SIMPLE METHOD FOR SEPARATION AND ANALYSIS OF PURE ANTHEMOPHILIC FACTOR FROM PLASMA. H.B. Rutten and C.H. Zhou. Clinical Hematology Branch, WH, Bethesda, Maryland, USA. Current methods of purifying anantemophilic 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 they are separate. The method utilizes a controlled-pore glass column that is assembled in the procedure. AHF separates as an isolated peak in the void volume while all other proteins including inorganic calcium 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°C or 22°C gives the same result. Yields are 40–60% of plasma f VIII activity.

Increasing ionic strength (p) of plasma to >0.3 progressively dissociates vWF and Factor VIII up to μ=0.75, vWF remaining at MW=10⁵ while Factor VIII is retarded by pores as small as 330 A, indicating a MW of approximately 1.5 × 10⁶. Human anti-AHF reacts exclusively with separated Factor VIII, not with vWF; and rabbit anti-AHF reacts primarily with vWF. Findings on normal and hemophilic plasma support the concept that Factor VIII and vWF are distinct components circulating as a weakly associated complex, and that hemophiliacs lack Factor 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. E.H. Knebel, F. E. Martin and R. Kramen. Haematology Department, Royal Prince Alfred Hospital, Sydney, Australia. Gel filtration of human plasma cryoprecipitate on Sepharose 2B indicated the molecular weight of Factor VIII conjugate activity (VIIIc) to be significantly greater than that found in antihemophilic concentrate. Polyethylene glycol at 34% concentration precipitated approximately half of the VIIIc from cryoprecipitate. This activity eluted at high molecular weight material on gel filtration. The addition of more polyethylene glycol to a concentration of 84% 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 antihemophilic 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.