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0145 EVIDENCE FOR THE PRESENCE OF FVIII SUBUNITS IN PLASMA AND CLINICAL FVIII CONCENTRATES USING QUANTITATIVE ELECTROPHORESIS

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X-Electrophoresis of normal plasma and low purity FVIII concentrates indicated two major peaks of cross reacting FVIIIR:Ag, extending from the front of fibrinogen to the α_2 region. In high purity concentrates (Hemofil and Koate) smaller aggregates, with faster mobility predominated, confirming the heterogeneous nature of FVIII concentrate preparations.

Analysis of the gel content for other FVIII related activities revealed that FVIIIR:RCF was associated only with larger aggregates, while FVIII:C as determined by both chromogenic and clotting techniques were mainly associated with the smaller forms. The coexistence of FVIII subunits, with an immunological identity line, is brought about by using high concentrations of FVIII. The presence of such an entity in plasma was confirmed by in vitro addition of high purity concentrate to plasma, FVIII:C neutralization activity and reduction with Dithiothereitol (D.T.T.). The subunits found after D.T.T. treatment gave a symetrical peak with similar molecular distribution of the fast moving peak, present in high purity concentrate.

It is concluded that each population of FVIII aggregates would consist of the subunits cross-linked by disulphide bonds. The relative concentration of these aggregates, present in differing amounts in various preparations, could be determined quantitatively: Thus an extremely useful method for the identification of the native forms of FVIII and the quality control of concentrate preparation is provided.

P6-104 0146 SPECIFIC FACTOR VIII FRAGMENTATION IN COMMERCIAL FACTOR VIII CONCENTRATES

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Factor VIII antigen (VIIIag) is felt to consist of a series of oligomers that demonstrate immunologic reactions of identity with each other in normal plasma. This study demonstrates a fragment of VIIIag (VIIIag FRAG) which is present in commercial Factor VIII concentrate (VIII CONC) but not detected in cryoprecipitate (CRYO).

Crossed immunoelectrophoresis of 10 bags of CRYO and 10 different VIII CONC (5 different brands) was carried out against specific antibody to VIIIag. As expected, the mobility of VIIIag was less anodic in CRYO than in VIII CONC, but a specific fragment of VIIIag (VIIIag FRAG) was detected in the VIII CONC. VIIIag FRAG is more anodic and demonstrates a reaction of partial identity with normal VIIIag. VIIIag FRAG is immunologically distinct from our previously reported von Willebrand's disease antigen II. None of the CRYO samples demonstrate VIIIag FRAG.

	N	R_f† (VIIIag)	R_f (VIIIag FRAG)
NORMAL PLASMA		0.27	not detected
CRYO	10	0.24 ± .03	not detected
VIII CONC	10	0.34 ± .04	0.54 ± .04

†(R_f = ratio of distance migrated of unknown vs. albumin.)

Thus proteolysis in the preparation of commercial VIII CONC has produced VIII antigen that is deficient in some of the normal Factor VIII antigenic determinants. VIIIag in CRYO does not demonstrate this alteration. This proteolysis of VIIIag may contribute to the failure of VIII CONC to correct the bleeding time in von Willebrand's disease.

P6-105 0147 COMPARATIVE IN VITRO AND IN VIVO STUDY OF VARIOUS FACTOR VIII PREPARATIONS

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A comparison of nine commercial and non-commercial Factor VIII preparations was made. They consisted of 1 lyophilized cryoprecipitate, 4 intermediate and 4 high purity concentrates. Protein, Fibrinogen, Factor VIII complex, IgG, anti-A and anti-B antibody levels were measured. Factor VIII:C content varied from 4-7 u/ml in cryoprecipitate, 12-31 u/ml in intermediate and 21-40 u/ml in high purity concentrates. These three categories of Factor VIII preparations can be better defined by 2 ratios : u F VIII/mg proteins and u F VIII/mg fibrinogen. They were respectively < 0.5 and < 1 in cryo, 0.5-1 and 1-3 in intermediate purity concentrates, > 1 and > 3 in high purity concentrates. The F VIII:C/F VIII:AG ratio ranged from 0.3 to 0.6 in any preparation. The F VIII:C/F VIII:VWF ratio was always lower than 1.

Each preparation was injected to several classic hemophilia A patients for treatment of minor hemorrhages. The peak of activity was always found 1 hour post-injection and the recovery ranged from 80 to 105%. The Factor VIII half-life ranged from 10 to 12.5 hours. No significant differences in half-life or recovery was found, and the clinical efficacy was similar. With the exception of fibrinogen load, all products carry similar risk for hepatitis, anti-IgG immunization and hemolysis. The differences lie in the ease of injection, the price and the yield of Factor VIII from starting plasma. Nevertheless, high purity concentrates should be used when high doses are required for surgery or treatment of patients with inhibitor.