## Supplementary Abstracts

# Factor VIII

## 1015

THE EFFECT OF PURIFICATION ON THE QUARTERNARY STRUCTURE OF FACTOR VIII-VWF. J.A. van Mourik, J. Over, J.A. Hellings. Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

As an index of denaturation, the ratio of Factor VIII coagulant antigen (VIII:CAg) to Factor VIII coagulant activity (VIII:C) as measured in plasma, cryoprecipitate and cryosupernatant plasma from fresh and stored blood and in several concentrates. The ratio of VIII:CAg to VIII:C was close to unity for both cryoprecipitate and factor VIII concentrates, suggesting that VIII:CAg is lost concurrently with VIII:C during cryoprecipitation and further fractionation. Together with VIII:C, a substantial amount of VIII:CAg was recovered in the cryosupernatant. Storage of blood for 24 h before processing resulted in a 30% loss of VIII:C from the separated plasma, but the level of VIII:CAg was not affected. In cryoprecipitate, prepared from this plasma, VIII:C and VIII:CAg were both 30% lower than in that prepared from fresh plasma. A corresponding rise of VIII:CAg, but not of VIII:C, in the cryosupernatant was found.

Gelchromatographic analysis revealed that the rise of VIII:CAg in cryosupernatant, prepared from stored blood, was due to an increased amount of VIII:CAg that eluted after the void volume which was no longer associated with VIIIR:Ag. Such an increase in dissociated VIII:CAg was not detected in the plasma derived from stored blood prior to cryoprecipitation.

It is concluded that during storage of blood molecular changes are induced in the factor VIII complex, which makes the complex more liable to dissociation during the subsequent cryoprecipitation procedure. Similarly, losses of VIII:C during further purification of the Factor VIII complex should be interpreted in terms of dissociation of the VIII:C constituent unit and not merely considered as a result of denaturation of VIII:C present in the native complex.

## 1016

THE EFFECT OF THROMBIN ON THE ELECTROPHORETIC MOBILITY AND FUNCTIONAL ACTIVITY OF VARIOUS FACTOR VIII MOLECULAR FORMS. M.J. Seghatchian and I.J. Mackie. North London Blood Transfusion Centre, Edgware, Middlesex, U.K.

Several distinct populations of factor VIII procoagulant activity (VIIIC) were observed in some clinical concentrates after agarose gel electrophoresis. It is not yet known whether these different forms are the products of proteolytic digestion, or subtle conformational changes of FVIII structure, which may occur during concentrate preparation. Low thrombin concentrations activate FVIII in a time/concentration dependent manner, and the 2 proteins have high affinities for one another. We have studied the properties of the various FVIII forms before and after incubation of concentrate with highly purified α-thrombin. Agarose gel electrophoresis was used to monitor: a) quantitative changes in functional activity (by 2 stage clotting (VIII:C) and amidolytic (VIII:CAm) assays); b) the binding of $^{125}$I-anti VIII:C (*IgG) to FVIII:C; c) the crossed immunoelectrophoretic (CIE) pattern of FVIII: RAg.

In the absence of thrombin, FVIII concentrate showed 2 main populations of FVIII:C and VIII:CAm. The radioactivity (VIII:CAg) pattern correlated with the slower moving of these populations. Low thrombin concentrations (0.0001-0.01 u/ml) caused no detectable change in FVIII:C level in the slow peak but additional peaks of activity appeared at the sample well, and between the well and slow peak. This was accompanied by a decrease in the fast moving peak, and the appearance of a third new peak with slightly less mobility. Higher thrombin concentrations (0.5u/ml) caused a marked decrease in the slow moving peak, with a concomitant increase in the well peak, by all methods. Increasing thrombin concentration progressively reduced *IgG binding, and a pattern similar to serum was obtained. Thrombin caused a slightly faster migration of FVIII:RAg, a well peak was sometimes seen. Thrombin therefore appeared to act preferentially on the lower molecular weight FVIII activity, and the high molecular weight peaks that occurred may represent complexes of FVIII with thrombin and/or other contaminant proteins present in concentrate such as fibrinogen or fibronectin.

## 1017

HYDRODYNAMIC STUDIES OF VON WILLEBRAND PROTEIN AND FACTOR VIII$_C$ USING THE PARTITION CELLS IN THE ANALYTICAL ULTRACENTRIFUGE. Grant H. Barlow, S. Eric Martin and Victor J. Marder. Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, USA.

In an attempt to determine the functional hydrodynamic properties of von Willebrand and Factor VIII$_C$ proteins studies were made using the moving and fixed partition cells in the analytical ultracentrifuge. Experiments using a formalin fixed platelet ristocetin assay as the functional assay showed a non parallel response when the data was plotted as the rate of agglutination vs. the dilution after partition sedimentation. The slope of this plot diminished as a function of the centrifuge time. These observations negate calculations of the sedimentation parameters. It is suggested that this non parallel response in activity is attributable to the different specific activities of the different polymers. It is postulated that hydrodynamic calculations can be made based on the slope of the rate of agglutination vs. dilution curve. On the other hand when functional assays involve the determination of Factor VIII$_C$ activity a normal parallel response is obtained following partitioning. This allows for the calculation of sedimentation coefficients from the activity data. Values ranging from about 12S to 25S are obtained. These differences show that the non parallel response by von Willebrand protein is not anomalous and further indicates that current techniques for quantitating von Willebrand protein should be revised.