UREA DENATURATION OF ANTIHEMOPHILIC (AHF) AND von WILLEBRAND (vWF) FACTORS IN THE PRESENCE AND ABSENCE OF SELECTED AMINO ACIDS. R.B. Harris, J. Newman and A.J. Johnson. New York University School of Medicine, New York, New York, U.S.A.

AHF and vWF are functionally independent activities in human plasma and a large body of data suggests that they may be different proteins, including a striking difference in the rate and extent of denaturation by urea. Thus, concentrations of urea below 1.5M resulted in 100% loss of vWF, 70% loss of AHF and 35% loss of Factor VIII related antigen (VIII Ag) in two hours, while 3M urea caused a loss of 100% vWF, 90% AHF and 45% VIII Ag in 30 hours. Full activity of urea denatured vWF and VIII Ag could be restored by dialysis of the urea while the AHF activity returned to about 75% of the initial value. Substitution of acetamide for urea resulted in only partial denaturation of the AHF (50% of that obtained with urea) suggesting that urea may interact with these proteins through the formation of bifunctional hydrogen bonds. Lysine and structural analogs of lysine with free amino groups prevented urea denaturation of the AHF but urea denaturation of vWF was not prevented by these amino acids. These data suggest that loci essential for the biological activity of AHF, vWF and VIII Ag are denatured by direct interaction with urea. Since VIII Ag and AHF are more resistant and slower than vWF in their reaction with urea, other bonds or groups are also important. The differences in denaturation, renaturation and protection against urea denaturation of AHF and vWF by selected amino acids could be explained if the reactive sites for each activity were located on different molecules as suggested by other in vitro and in vivo observations or were remote from one another on one molecule.

LIPASES PRODUCE FUNCTIONALLY INTACT SUBUNITS OF FACTOR VIII (ANTIHAEMOPHILIC FACTOR). M. Furlan, T. Jakab and E.A. Beck. Central Haematology Laboratory, Inselspital, Berne, Switzerland.

Cryoprecipitates of fresh human plasma were fractionated by gel filtration on Sepharose CL-2B. Factor VIII was eluted in the void volume together with a non-proteolytic enzyme which was capable of degrading factor VIII into smaller subunits. Similar subunits were observed following treatment of factor VIII with a triglyceride lipase from Rhizopus arrhizus. They retained full functional activity (procoagulant and ristocetin cofactor). The subunits reaggregated spontaneously at 37° into a re-constituted complex which remained functionally fully active. The resulting aggregate could be repeatedly dissociated by addition of fresh lipase. The reaggregation process was enhanced by phenylmethylsulfonyl fluoride or ethylenediamine tetraacetate, but was inhibited at 0°. The treatment with lipase renders factor VIII more susceptible towards plasmin which destroys its functional properties.

VON WILLEBRAND FACTOR ACTIVITY IN PLASMIN DIGESTS OF FACTOR VIII. J.A. Guisasaola, C. Cockburn and R.M. Hardisty. Department of Haematology, Institute of Child Health, London, England.

Purified human factor VIII was incubated for up to 24 hours with plasmin, and the activity of the breakdown products studied at intervals. Factor VIII coagulant activity was lost within the first hour, but von Willebrand factor activity (FVIII:WF) was retained for two hours, and then declined slowly during the subsequent incubation. Analysis of the 24-hour breakdown products by immuno-electrophoresis, sepharose 4B chromatography and SDS polyacrylamide electrophoresis revealed three main groups of fragments recognised by rabbit anti-human factor VIII anti-serum, and having molecular weights in the following ranges: Group I 300,000-500,000; Group II, 150-200,000; Group III, 100,000. FVIII:WF activity, which was found only in Group II, appeared to be associated with glycopeptide(s) of up to 155,000 daltons.