**Urea Denaturation of Antithrombophilic (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 1.5 M resulted in 100% loss of vWF, 70% loss of AHF, and 35% loss of factor VIII related antigen (VIII Ag) in two hours, while 3 M 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. Lysolecithin and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) are also important. These 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 (Antithrombophilic Factor).** H. Purlan, T. Jakob and E.A. Beug. Central Haematology Laboratory, Inselspital, Bern, 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 triacylglyceride lipase from Rhizopus arrhizus. They retained full functional activity (procoagulant and fibrinogen cofactor). The subunits reaggregated spontaneously at 37°C into a reconstituted complex which remained functionally fully active. The resulting aggregate could be repeatedly dissociated by addition of fresh lipase. The reaggregation process was enhanced by phenylmethylsulphonyl fluoride or ethylenediamine tetraacetae, but was inhibited at 4°C. The treatment with lipase renders factor VIII more susceptible towards plasmin which destroys its functional properties.


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 Wa) was retained for two hours, and then declined slowly during the subsequent incubation. Analysis of the 72-hour breakdown products by immuno-electrophoresis, Sepharose 4B chromatography and SDS-polyacrylamide electrophoresis revealed three distinct groups of fragments recognized 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 Wa activity, which was found only in Group II, appeared to be associated with glycopeptide(s) of up to 150,000 daltons.