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SYMPOSIA SESSION

I Von Willebrand's Factor and the Vessel Wall

MULTIPLE MOLECULAR FORMS OF FACTOR VIII ANTIGEN IN NORMAL INDIVIDUALS AND VON WILLEBRAND'S DISEASE PATIENTS. T. Zimmerman, J. Kimball, T. Edgington and C. Abildgaard. Scripps Clinic & Res. Fndn., La Jolla, California and University of California, Davis, California, U.S.A.

Factor VIII antigen is present in normal individuals in multiple molecular forms which can be separated according to size. The smaller forms have little or no ristocetin co-factor activity. In order to evaluate the possibility that Factor VIII antigen forms of large size may be an artifact of *in vitro* aggregation, we have ultracentrifuged plasma on a 20% sucrose cushion at 37°C for 10 min at 254,000 xg (peak). The rate of clearing of Factor VIII antigen was compared to that of other plasma proteins. The results indicate that Factor VIII-related antigen forms of high S exist even when plasma is maintained at physiological temperature and analyzed with minimal delay, suggesting that these larger molecular forms also exist as such *in vivo*.

In von Willebrand's disease (vWd) all forms of Factor VIII antigen may be present but decreased in quantity (Type I) or large forms may be missing with smaller forms present in normal or increased quantities (Type II). Factor VIII antigen was isolated from plasma of three patients with Type I and three patients with Type II vWd by counter immunoelectrophoresis. The Factor VIII antigen was then reduced and electrophoresed on SDS-containing polyacrylamide gels. The presence of carbohydrate was evaluated by staining with perchloric acid-Schiff's reagent (PAS). The 210,000 MW Factor VIII antigen subunit from each patient was PAS-positive. Though subtle changes in carbohydrate content or composition could not be evaluated by this technique, a total defect of glycosylation is unlikely in this sample of vWd patients.

THE INTERACTION OF THE PLATELET AND VON WILLEBRAND FACTOR. D. N. Fass, F. Booyse, J. C. Lewis, and E. J. W. Bowie. Mayo Clinic, Rochester, Minnesota and Rush University, Chicago, Illinois.

A culture of pig aortic endothelial cells was used for experiments to investigate the interaction between the platelet and von Willebrand factor. An antibody was raised in rabbits to purified porcine von Willebrand factor. A semi-confluent culture of pig endothelial cells was stained immunofluorescently by the sandwich technique using anti-Willebrand factor IgG. An extensive extracellular meshwork of microfilaments was revealed. In endothelial cell cultures from von Willebrand pigs, no immunoreactive microfilaments were found. Immunoelectronmicroscopy with peroxidase linked antibody has been used to identify similar filaments in normal pig endothelial cells. Washed platelets were shown to adhere to semiconfluent or damaged normal endothelial cell cultures. If the cultures had been previously incubated with anti-Willebrand factor IgG, the washed platelets did not adhere. There was no adherence of platelets when they were added to semiconfluent or damaged von Willebrand endothelial cells.