

CONVENIENT MICROTITER ASSAY FOR VON WILLEBRAND'S FACTOR. B. B. Weksler, T. Cheng and C. Ley.  
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A simple method for measurement of von Willebrand's factor (VWF) suitable for the clinical coagulation laboratory would substantially aid in the differential diagnosis of bleeding disorders. We have developed a simple macroscopic titration assay of plasma VWF using the Ristocetin induced aggregation of washed, fixed normal platelets. The assay employs arithmetic dilutions of test plasma in buffer, Ristocetin and washed, fixed platelets in a final volume of 0.4 ml per well. After 20 minutes' agitation on a rotary shaker at room temperature, an endpoint of visible aggregation can be determined. Fifty normal plasmas tested had a mean titer of 1/500 (range 1/240 to 1/853) whereas 8 plasmas from patients with known von Willebrand's disease (VWD) had a mean titer of 1/21 (range 1/10 to 1/186). Deviation from the normal range correlated with the severity of the VWD. Reproducibility of results was within  $\pm 10\%$ .

Platelets washed before fixation with paraformaldehyde (method modified from Allain et al, J. Lab. Clin. Med. 85:318, 1975) appear almost twice as sensitive to VWF in this assay as those fixed in platelet rich plasma and washed subsequently. Fresh and frozen plasmas give equivalent results, permitting storage of specimens. This microtiter assay thus offers a sensitive measure of VWF Ristocetin cofactor activity, utilizes stable and standardizable reagents, requires only small amounts of patient plasma (or other biological materials) and permits rapid testing of different specimens simultaneously.

THE ASPIRIN BLEEDING TIME - A SCREENING TEST FOR EVALUATION OF von WILLEBRAND'S DISEASE. Marie J. Stuart, Merrill Miller, Joel Wolk and Fredrick Davey. Depts. of Peds. and Path., SUNY, Upstate Med. Ctr., Syracuse, N.Y.

In an attempt to elucidate the usefulness of the Bleeding Time (BT) post Aspirin (ASA) ingestion this test was done with other tests of coagulation in 30 controls and 77 patients evaluated for a possible bleeding diathesis. Coagulation studies included PT, PTT, TT, VIII AHF & AGN, IX, XI, XIII platelet retention and aggregation, and modified Ivy BTs pre and 2 hrs. post 600 mgms ASA. The mean control BT in 25 normals was  $3.6 \pm 3.2'$  (3SD). Following ASA the BT was  $6.4 \pm 4.1'$  (3SD). 5/30 "controls" without bleeding histories had abnormal BTs post ASA. In this group, 4 were proven to have unrecognized von Willebrand's disease (VWD) and one a platelet defect. Of the 74 patients studied, 28 had initial BTs that were abnormal (23 with VWD and 5 with platelet defects). Of the remaining 46 with initially normal BTs, 26 had abnormal BTs post ASA. 13/26 had VWD at their first evaluation. In 9/26, however, the abnormal BT post ASA was the only abnormality at initial evaluation. On repeated testing, these patients were also shown to have VWD. 4/26 with abnormal BTs post ASA were found to have platelet abnormalities. The remaining 20 patients had normal BTs pre and post ASA. 16/20 revealed no hemostatic abnormality. In 4 eventually proven to have VWD, the BT post ASA was normal. The use of the BT post ASA raised the sensitivity of the BT as a screening test from 48% to 93% in the abnormal patient population. VWD appears to be the most common symptomatic/asymptomatic bleeding disorder. The BT post ASA is a valuable screen in the evaluation of hemostatic defects; i.e., mainly VWD, as also the occasional patient with a thrombocytopathy.

COMPARISON OF FACTOR VIII LEVELS AFTER ADRENALIN IN HEMOPHILIA A AND VON WILLEBRAND'S DISEASE (VWD) M. E. Eyster, J. O. Ballard and D. Prager - Pennsylvania State University School of Medicine, Hershey, Pa. and The Allentown General Hospital, Allentown, Pa., U. S. A.

Two-fold or greater increases of Factor VIII procoagulant activity (VIII AHF) have been described following the administration of adrenalin in normal individuals and in some patients with VWD. VIII AHF and Factor VIII-like antigen (VIII AGN) levels were measured pre and one hour post the s. q. injection of adrenalin 0.35 cc. in 4 adult VWD patients and in 10 adult hemophiliacs with VIII AHF levels ranging from 1-29%, normal VIII AGN levels and normal VIII VWF levels as measured by the washed platelet ristocetin assay. Eighteen normal adults served as controls. The four patients with VWD showed two-fold or greater increases of VIII AHF with variable VIII AGN responses. All 10 hemophiliacs showed no change in VIII AHF with significant increases in VIII AGN when analyzed by the paired t-test.

The fixed rate of VIII AHF production, release or activation observed in hemophiliacs may help to explain the constancy of VIII AHF levels in individual hemophiliacs and their affected family members. Furthermore, this simple provocative test may be helpful in distinguishing certain VWD patients from those with hemophilia.