POSTER SYMPOSIUM III

Factor VIII: Purification and Assay.

SEPARATION OF HUMAN BLOOD PLATELETS FROM FACTOR VIII (VON WILLEBRAND'S FACTOR) BY COMBINED ALBUMIN GRADIENT-GEL FILTRATION METHOD. S. Timmons and J. Hawiger. Vanderbilt University and VA Hospital, Nashville, Tennessee, USA.

Currently used gel filtration methods were investigated in order to obtain a system which would effectively separate platelets from plasma proteins, such as Factor VIII/von Willebrand's Factor (FVIII/vWF), in relatively small samples of human blood which are not amenable to repeated centrifugation. BioGel A150, which has a higher exclusion limit than the presently used Sepharose 2B, was found to be equally effective for preparing gel-filtered platelets. However, it did not completely separate the FVIII/vWF as judged by ristocetin-induced platelet aggregation. Therefore, a stepwise albumin gradient was employed as a preceeding step to gel filtration on BioGel A150. Platelets obtained by this modification were not responsive to ristocetin unless human plasma, as a source of FVIII/vWF, was added. Tyrode buffer, which shows variation in pH due to changes in pCO_2 , was replaced by the more stable zwitterionic buffer containing N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) pH 7.4. The need for control of CO_2 was thereby obviated, reactivity of platelets in vitro was maintained, and their functional life span was extended from 1 to 2 hours. This modification of existing methods allows efficient separation of human platelets from FVIII/vWF and from other plasma macromolecules in relatively small samples of blood with preservation of the functional integrity of obtained platelet preparations.

DIFFERENTIATION AND STABILIZATION OF AHF SEPARATED FROM vWF BY POLYELECTROLYTES. V.E. Macdonald, M. Semar, A.J. Johnson and J. Brind. New York University Medical Center, New York, N.Y. U.S.A.

Low-charge density, solid-phase ethylene maleic anhydride polyelectrolytes (PEs) have been used to quantitatively separate factor VIII-antihemophilic factor (AHF) from factor-VIII von Willebrand factor (vWF). Under these circumstances, the AHF is adsorbed on the resin while the vWF remains in the supernatant plasma. Addition of large amounts of proteolytic inhibitors does not influence the separation. The vWF can be purified further by subsequent adsorption on high-charge density PEs. The PE-adsorbed AHF and vWF show marked differences in elution patterns and stability. The AHF is eluted in high salt while the vWF is eluted at lower salt concentration. Addition of certain amino acids to the eluting buffers results in extremely stable AHF preparations, whereas the same amino acids have the opposite effect on the vWF eluates. Further purification by selective PEG precipitation results in 100-fold purification of both materials. These intermediate-purity concentrates, prior to gel chromatography, were found to contain complement components not previously reported. Comparison of several commercial factor VIII preparations demonstrates that this is a common contaminant in all preparations examined thus far. The differences in properties of the separated AHF and vWF seen during fractionation and purification by these methods strongly suggest that they are two independent molecules.