Time

13.15 **0363** MECHANISMS OF HUMAN BLOOD VII ACTIVATION IN PLASMA DURING CONTACT ACTIVATION, CLOTTING AND EXPOSURE TO COLD.

U. Seligsohn*, B. Østerud, S.F. Brown, J.H. Griffin and S.I. Rapaport. Department of Medicine, University of California, San Diego and Department of Immunopathology, Scripps Clinic and Research Foundation, San Diego, U.S.A.

Factor VII (VII) is activated, giving shorter clotting times with tissue factor, when plasma is exposed to kaolin, is clotted or exposed to cold. The mechanisms involved were studied. Incubation of plasma with kaolin resulted in: No activation in XII deficiency plasma (dp), partial activation (2.5 fold) in Prekallikrein (PK) dp and High Molecular Weight Kininogen (HMWK) dp, and 4.5-9 fold activation in normal or other dp. Clotting plasma by recalcification resulted in: No activation with XII dp, HMWK dp, XI dp and IX dp, and 4-5 fold activation with VIII dp, X dp and V dp. The mechanism of cold promoted activation of VII in plasma was studied by adding purified ^{125}I-XII or ^{125}I-IX to plasma before storage at 4° and observing the extent of their proteolysis (measure of activation) from their radioactivity profiles on reduced polyacrylamide gel following electrophoresis in the presence of SDS. Significantly greater ^{125}I-IX and ^{125}I-XII proteolysis was observed in plasma from 4 subjects whose VII activated in the cold, than in plasma from 5 subjects whose VII was not activated in the cold. Addition of anti-IX antiserum inhibited 50% of the observed cold activation of VII. Thus, with kaolin XII_a was the principal activator of VII; after clotting IX_a was the principal activator and in cold activation both XII_a and IX_a played roles.

13.30 **0364** SOME EFFECTS OF FACTOR VII ON RATES OF THROMBIN PRODUCTION AND ONE STAGE PT's

D. J. Baughman* and A. Lytwyn, Ortho Diagnostics Inc., Raritan, NJ.

Attempts to automate a chromogenic one stage PT assay required hastening the onset of thrombin production without altering the rate of production. Assays were performed by combining 0.25ml chromogenic substrate S-2238 (2mM), 0.40ml tissue thromboplastin,0.05 plasma, and 0.050ml diluted serum (40%v/v) in 1.35ml TRIS buffer (pH=8.5,I=0.15). Sera were prepared by using the supernatant of a)whole blood, b)recalcified plasma, c)recalcified plasma and tissue thromboplastin, d)recalcified plasma and partial thromboplastin (cephalín and ellagic acid) and e)thrombin clotted plasma. The results indicated that S-2238, a potent thrombin inhibitor, delayed the onset and altered the rate of thrombin production. Small amounts of thrombin shortened the initial generation of thrombin without altering its rate. Sera from a),c) and d) but not b) and e) resulted in immediate thrombin production at most plasma concentrations without affecting the rate, had no residual prothrombin, and had similar residual S-2238 activity not reduced by hirudin At III-heparin. In particular, serum b) had ~80% plasma Factor X which could be adsorbed without affecting thrombin production, had ~40% Factor V and ~300% Factor VII. These results suggest that when Factor VII activity is greater than ~7% there is an effect on the time but not on the rate of thrombin production via positive thrombin feedback scheme. Thus, the standard PT can measure the time of thrombin appearance, and/or the rate of thrombin production, and/or fibrin polymerization.

13.45 **0365** PREPARATION OF A FACTOR VII CONCENTRATE

A. J. MacLeod* and I. Dickson, Scottish National Blood Transfusion Service, Protein Fractionation Centre, Edinburgh. U.K.

A factor VII concentrate has been prepared from pooled citrated fresh frozen plasma following removal of cryoprecipitate and factors II, IX and X. The method involved batch adsorption on DEAE-Sephadex A-50, fractionation of the subsequent batch eluate by PEG precipitation and passage through a column of DEAE-Sepharose CL-6B. A phosphate-citrate buffer pH 6.9 was used throughout, this was made 0.2M with NaCl for the batch elution and a 0 - 0.2M NaCl linear gradient was used to elute the components from the column. Factor VII activity was clearly resolved from the bulk of the protein, including caeruloplasmin, and could be recovered as a concentrate at about 20 U FVII/ml with a specific activity of in excess of 1 U FVII/mg of protein and an overall rcovery of 40% to 50%

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.