CROSS-LINKING OF FACTOR VIII BY DIMETHYL SUBERIMIDATE: STRUCTURAL IMPLICATIONS. C.G.Cockburn. Department of Haematology, Institute of Child Health, London, England.

A dilute solution of highly purified human FVIII was cross-linked by dimethyl suberimidate (negligible intermolecular cross-linking), incubated in 0.2M mercaptoethanol for 35 min. at 37°C, and eluted through a Sepharose 4B column. Unreduced SDS-polyacrylamide gel electrophoresis (3.75%:1%) of the material applied to the column showed 6 clear bands believed to represent cross-linked FVIII oligomers (on the grounds that electrophoretic mobility was directly proportional to log oligomer number). The staining intensity of the bands was roughly as follows:

$$(FVIII)_1 \backsimeq (FVIII)_2 > (FVIII)_3 \backsimeq (FVIII)_4 > (FVIII)_5 \backsimeq (FVIII)_6$$

This indicates that a single FVIII subunit is in intimate proximity with up to 5 other subunits and suggests large areas of close contact between subunits.

After cross-linking virtually all the FVIII RAg was lost, but a little FVIIIC (4% yield) eluted at the void volume (Vo) of the Sepharose column. No detectable protein in the Vo fraction penetrated very large pore size SDS-polyacrylamide gels, indicating a very high molecular weight cross-linked structure.

IMMUNOLOGICAL EVIDENCE THAT HUMAN FACTOR VIII IS COMPOSED OF TWO LINKED MOIETIES. J. Koutts, J.-M. Lavergne and D. Meyer, Institut de Pathologie Cellulaire, Hôpital de Bicêtre, Paris, France.

Whether the three measurable parameters of factor VIII (procoagulant activity, VIII:C; ristocetin cofactor activity, VIIIR:WF; and factor VIII related antigen, VIIIR:AG) reside on a single protein remains disputed. A solid phase immunoadsorption system, in which homologous antibodies to VIII:C arising in haemophiliacs were insolubilized onto Sepharose, was used to examine the action of such antibodies and the inter-relationship between VIII:C, VIIIR:WF and VIIIR:AG. Homologous antibodies were shown to bind specifically VIII:C and to induce a spontaneous separation of VIII:C from VIIIR:WF/VIIIR:AG. The bond between VIII:C and the homologous antibodies bound to Sepharose appeared to be very stable and could not be broken with the usual antigen-antibody dissociating agents. Following prolonged incubation with antibody-sepharose, concentrated VIIIR:WF/VIIIR:AG (20 u/ml), completely devoid of VIII:C and inhibitor-neutralizing activity, was obtained. The loss of VIII:C had no detectable effect on the molecular size, antigenicity or electrophoretic mobility of the original molecule. The concentrated VIIIR:WF/VIIIR:AG was used to absorb heterologous antisera raised against factor VIII. Specific heterologous antisera to VIII:C, no longer neutralizing VIIIR:WF nor precipitating with VIIIR:AG, were obtained. Immunization of rabbits with VIIIR:WF/VIIIR:AG resulted in antisera which potently neutralized VIIIR:WF and precipitated with VIIIR:AG but also weakly neutralized VIII:C. These antibodies, like 4 other heterologous antibodies to Factor VIII studied, did not neutralize VIII:C which had been dissociated from VIIIR:WF/VIIIR:AG.

The results indicate that VIII:C and VIIIR:WF/VIIIR:AG are two different, but linked entities.

EVALUATION OF HIGHLY PURIFIED BOVINE F. VIII AND BOVINE PLASMA AS REAGENTS TO INDUCE PLATELET AGGREGATION. M. A. Lazzari, C. Simonetti, G. Casillas and M. Pavlovsky. Instituto de Invest. Hematológicas. Buenos Aires. Argentina.

Purified bovine F. VIII is a known reagent for human platelets aggregation used for the study of thrombopaties. As bovine plasma (BP)is easier to prepare and standardize it was studied as an alternative reagent, comparing it with purified bovine F. VIII. Platelet aggregation was studied by a turbidimetric technique(Aggregometer Bryston AG 1). The substrates were platelet rich plasma (PRP)and gel filtered platelets using between 150,000 and 200,000 platelets/mm^3 to standardize the platelets surface. 1/10 dilution of bovine plasma was found to be the best for PRP and 1/1 for filtered platelets. Incubation of the substrates with bovine plasma at 37°C for 5 min seems to enhance PAF activity of the platelet aggregation factor (PAF). Primary aggregation was obtained in PRP treated with 2% tetrasodic EDTA, 3. 4% sodium citrate and in plasma with aspirin. No secondary aggregation was observed in EDTA or aspirin plasma. Unlike platelets treated with ADP, their shape did not change since the optical density was not modified. No synergism or competition between PAF and ADP or adrenalin were found. High concentrations of NaCl or urea interfere with PAF, and esposure to 56°C inactivates it. In 50 normal PRP the percentage of total aggregation were: BP 1/10 42% $\pm$ 12 ; BP 1/1 79% $\pm$ 13 ;purified bovine F. VIII 1/10 86% $\pm$ 6 and in 10 batches of filtered platelets BP 1/1 82% $\pm$ 7. We consider an advantage to use bovine plasma for platelets aggregation studies.