

LARGE SCALE PURIFICATION AND CHARACTERISATION OF PORCINE FACTOR VIII FOR CLINICAL USE

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Animal factor VIII can be useful in the treatment of haemophilic patients with high titres of human factor VIII antibody when the antibody shows low cross-reactivity towards animal factor VIII. The use of currently available preparations of animal factor VIII is associated with rigor, anaphylaxis and transient thrombocytopenia, which limit their therapeutic value. Analysis of some of these preparations has shown low IgM and high IgG concentrations and haemagglutinating activity against a panel of human red blood cells. It has been demonstrated that purified porcine IgG and IgM agglutinate human red blood cells, while IgM is also complement fixing.

A large scale purification procedure has been developed for producing porcine factor VIII from platelet-poor plasma. It involves various precipitations followed by gel filtration through Sepharose 4B-CL on a KS sectional column. The pooled void-volume fractions are concentrated by using the Pellicon cassette system, giving a final yield of approximately 20% of the starting plasma. Porcine factor VIII prepared by this method is stable. Its greatly reduced fibrinogen content facilitates final filtration and prevents the accumulation of fibrinogen in patients treated with high doses. Non-specific haemagglutinins which may have contributed to some of the post-transfusion reactions have been removed. Specific activities of at least 50 U/mg of protein are obtained, and doses greater than 10,000 units can be given in 10 ml.

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CHEMICAL CROSS-LINKING OF FACTOR VIII SUBUNITS. M. Furlan, T. Jakab and

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Dimethyl suberimide is a bifunctional reagent known to react with amino groups of proteins. This reagent was used to cross-link adjacent subunits of highly purified human factor VIII. Reaction products were reduced with β -mercaptoethanol and examined by polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate. Low concentrations of dimethyl suberimide (< 0.5 mM) produced dimers of the subunit polypeptide chains and virtually no oligomers of larger size. Treatment with higher concentrations of the cross-linking agent resulted in an almost simultaneous appearance of both trimeric and tetrameric products, suggesting the existence of specific intradimer contacts. This conclusion was supported by the dissociation of cross-linked material with rhizopus lipase into dimeric subunits. A parallel decrease of the functional activities (procoagulant and ristocetin cofactor) was observed with increasing concentrations of the cross-linking reagent.

ELEVATIONS IN VIII:C, VIIIIR:Ag AND VIIIIR:vW. Ute Hasiba, Joel A. Spero, and Jessica H. Lewis.

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High levels of factor VIII:C and VIIIIR:Ag have been described following exercise, in pregnant women and in liver diseases. The objective of the present study was to compare levels of VIII:C to VIIIIR:Ag and VIIIIR:vW in patients with a variety of disorders. The normal range in this laboratory for each of the VIII components is 0.50 to 1.50U/ml. Following exercise all three components rose. Hemophiliacs, who have had no recent treatment with VIII concentrate, have low VIII:C and, usually, normal VIIIIR:Ag and vW. A few have high levels which may relate to liver dysfunction. In twelve hemophiliacs with anti-VIII, VIIIIR:Ag was above 2.0 in six and between 1.5 and 1.9U/ml in another three; VIIIIR:vW was elevated in only four of these. In all four patients with acquired anti-VIII, who were tested, VIIIIR:Ag and vW were elevated. In the last six months, all patients' plasma samples in which VIII:C assayed above 2.0U/ml were also assayed for VIIIIR:Ag and VIIIIR:vW. In seventy-four patients all three components were elevated. Many diseases were represented: liver disease, thrombo-embolism, leukemia and cancer, infection, etc. No common factor was ascertained, although, these are all clinical settings in which disseminated intravascular coagulation (DIC) is found. Twelve of these patients had coexistent laboratory evidence of DIC. In these patients VIIIIR:Ag tended to be higher than VIIIIR:C. Those studied without DIC generally showed a 1:1 correspondence between the levels of VIIIIR:Ag and VIIIIR:C. In seven patients not included in the above group of seventy-four the VIII:C was high, but VIIIIR:Ag and vW fell within the normal range, suggesting that some activation may have occurred.