

ASSESSMENT OF FVIII FUNCTIONAL ACTIVITIES WITH QUANTITATIVE ELECTROPHORESIS. M.J. SEGATCHIAN, North London Blood Transfusion Centre, Edgware, Middlesex. U.K.

Electrophoretically separated fractions of FVIII concentrates were quantitatively assayed to assess heterogeneity in FVIII related proteins. The method was successfully applied to the characterisation of multiple forms of FVIII in normal, pathological, haemophilia A, and v.WD. post-infused samples. Similarly, FVIII:C inactivation properties of several FVIII antibodies, which differed from one another on the basis on their complex formations, were investigated by: i) identifying the molecular forms of the Residual FVIII and ii) by monitoring the electrophoretic distribution of the complexes using a radiolabelled VIII:C IgG. 200 ul samples were applied to rectangular wells (22 x 3mm) cut in a 2.5mm thick agarosegel (1-2%). Electrophoresis in the first dimension was performed in 0.025M barbital buffer (pH8.2). The gels were then cut into 20-30 equal slices, eluted with 0.025M Tris-saline buffer (pH7.4) and assayed by currently used methods.

The results indicate that FVIII concentrates are highly heterogeneous, consisting of several subpopulations with differing functional and inhibitor neutralization activities. Most classical assays (including IRMA) were sensitive to the larger forms but methods based on the optimal Fxa generations (VIII:C 2 stage & VIII:C amidolytic) measured smaller forms of FVIII more sensitively. In the post-infused samples a transient complex was observed which reflected the rate of clearance of FVIII from the circulation. Furthermore the novo synthesis of FVIII in v.WD patients appeared to be quantitatively indistinguishable from circulating FVIII present in these patients. Some populations of FVIII were totally and specifically inactivated by some FVIII:C inhibitors but partially by the others. The inhibitor which inactivate totally FVIII:Cam also failed to produce high molecular weight radiolabelled complexes. On the basis of these results, inhibitor patients could be treated specifically with an appropriate subpopulation of FVIII.

THE USE OF FACTOR VIII:C DEPLETED NORMAL PLASMA IN THE FACTOR VIII:C ASSAY. N. Pancham, D. Bogdanoff, C. Mintz, S. Wada and M. Coan. Cutter Laboratories, Inc., Berkeley, California.

Normal plasma was made deficient in functionally active FVIII:C. This plasma supplemented with bovine FV was used as a substrate and compared to hemophilic plasma in the FVIII:C assays. The assays were performed on mechanical as well as optical clot detecting systems using the one-stage procedure.

Using a statistically valid experimental design, the plasma artificially-deficient in FVIII:C was shown to be comparable to hemophilic plasma, when both FVIII concentrates and pooled normal plasma samples were assayed. The plasma artificially deficient in FVIII:C was stable upon freezing and thawing and upon reconstitution after freeze-drying. Batch-to-batch variation was not significant. The introduction of this plasma as a reagent eliminates the need for collecting hemophilic plasma which may contain inhibitors to FVIII.

The main conclusions of the analyses are that artificially deficient FVIII substrate is fully comparable to naturally deficient human substrate in both accuracy and precision.

HEMAGGLUTINATION ASSAY FOR DETERMINATION OF HUMAN FACTOR VIII:C. E.P. Kang, D.I. DeSouza, M.J. Fitzpatrick and S.P. O'Neill. Electro-Nucleonics Laboratories, Inc., Bethesda, Md., U.S.A.

New methods for the determination of human factor VIII clotting protein (F. VIII:C) have been developed utilizing hemagglutination technique without using any other coagulation proteins in the assay. A 50% ammonium sulphate precipitate of the plasma from a hemophilic patient containing high titer of F. VIII:C inhibitor was purified on an affinity column of sepharose 4B covalently linked to F.VIII:C by CNBr. The 2.8 M MgCl₂ eluted protein, after dialysis against 0.1 M phosphate buffered saline (pH 7.4), was coupled on either fresh or stabilized human erythrocytes by using either chromic acid or glutaraldehyde as the coupling agent. Serial dilutions of human plasma or F. VIII concentrate were added to the anti-F.VIII:C coated red cell suspension on a microtiter plate. For the stabilized cells, the plate was incubated on a vibration-free surface at room temperature. The results were read after two hours. For the fresh cells, the plate was incubated at room temperature for 30 minutes and was centrifuged at 300 Xg for 1/2 minute. It was then placed on a tilted platform at a 60° angle. The results were read after 10 minutes. By these methods, it is possible to determine the detectable level of F.VIII:C in plasma and the F.VIII:C concentration in cryoprecipitates and F.VIII concentrates.

CHARACTERISATION OF ACTIVE COMPONENTS OF FIX CONCENTRATES USED IN FVIII INHIBITOR PATIENTS. M.J. Seghatchian and I.J. Mackie. N.L.B.T.C. Edgware, Middlesex, U.K.

It is well recognised that haemostasis may be achieved in patients with FVIII inhibitors using FIX concentrates (PTX). However, it has not been possible to correlate the clinical response with enzymicity, FVIII levels and other *in vitro* results. This is partially due to the presence of variable amounts of inhibitors and stabilizers such as: citrate, heparin, and anti proteases in different preparations. Agarose gel electrophoresis was performed in an attempt to separate these inhibitors so that the various activities may be measured relative to one another. PTX from 4 sources were used in this study, (Kabi Vitrum, Immuno, Oxford and Edinburgh fractionation centres); FVIII was measured by two stage clotting (FVIII:C) and amidolytic (CAM) assays, and its electrophoretic distribution was followed by preincubation with ¹²⁵I-anti VIII:C (VIII:Cag). Enzymicity was measured with various substrates (S2160, S2222, S2302, S2238, S2288, S2251). All concentrates showed a fast moving peak of FVIII:C and VIII:CAM activity, in amounts in excess of that detected before electrophoresis. This peak correlated well with the distribution of bound radiolabel (VIII:Cag). Preincubation of PTX with FVIII concentrate caused the VIII:Cag peak to migrate more slowly, in its normal α 2 position. FVIII:Rag was present in much lower amounts than FVIII:C. When a twin-peak of VIII:CAM activity was sometimes seen, the slower peak was associated with S2288 activity, which may represent the action of activated factors VII or IX: other substrates showed variable patterns of enzymicity but the strongest activity was with S2160 and S2302 from the well to the β region, and this could be completely inhibited by addition of anti Kallikrein. The presence of an abnormal molecular form of FVIII, and the discrepancy between pre and post electrophoresis FVIII levels, indicate that FVIII could play a major role in the action of PTX. This may involve 1) a complex with phospholipid, FIX and FX making it potentially more effective; 2) enhancement of VIII:C activity *in vivo* by binding to FVIII:Rag; 3) altered antigenic sites on the FVIII, making it unrecognisable to inhibitor.