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THE FUNCTIONAL HETEROGENEITY OF CLINICAL FVIII CONCENTRATES
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There is an increasing tendency to use more highly purified FVIII preparations, and more sophisticated fractionation methods. In some cases new populations of FVIII:RAg have been produced that have a greater or lesser physiological activity. The characterisation of clinical concentrates on the basis of their biological activity is therefore extremely important in the search for more effective therapy.

We have investigated the activity of several high purity concentrates (Hemofil, Koate, Actif-VIII) as well as low purity pooled cryoprecipitate, with and without added ^{125}I-anti-VIII:C(*IgG or *FAB), after electrophoresis in 1% agarose gels.

In concentrates with a high FVIII:RAg concentration(25-30Iu/ml), 2 or more FVIII:RAg peaks were seen, with partial or complete identity. The main one migrated in the α 2 region, but a more anodal peak was frequently seen. The relative concentration of fast moving VIII:RAg in cryoprecipitate was too low to allow detection by immunoelectrophoresis. In the presence of *IgG or *FAB, only one main peak of radiolabelled VIII:C was noted, which corresponded with the larger molecular forms of VIII:RAg. In contrast, bioassays based on FXa generation (VIII:C and VIII:CAm), revealed 2 main peaks of activity in concentrates, cryoprecipitate and plasma. The slower moving peak correlated well with the radiolabelled VIII:C pattern. The fast moving peak in concentrate corresponded with the trailing edge of the fast moving VIII:RAg peak. This activity was not neutralised by increasing the *IgG level, or addition of anti-IXc IgG to the sample. No enzymic activity was detected in this region using a wide range of chromogenic substrates. However, $Al(OH)_3$ adsorption did remove the activity. These forms of FVIII may represent a native subpopulation or proteolytic breakdown product, which has lost some antigenic determinants, but retained biological activity. This material, which has a lower affinity for anti VIII:C and RAg, could be present in plasma and be selectively concentrated during fractionation procedures.

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HEATED LYOPHILIZED FACTOR VIII CONCENTRATE - ADDITIONAL PRELIMINARY IN VITRO STUDIES.
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The possibility that heating of lyophilized factor VIII in a water bath at 60°C for 10 hours will not be sufficient for significant inactivation of hepatitis virus in the lyophilized powder led to different heating experiments. Heating of lyophilized factor VIII at 100°C for 30 minutes led to severe deep brown discoloration and this experiment was abandoned. Heating of lyophilized factor VIII powder for 16 hours at temperatures between 62-64°C followed by storage at 6°C for 4 weeks still resulted in greater than 80% recovery of factor VIII as compared to an unheated control, absorbance of the reconstituted factor VIII at 580 nm resulted in .127 for the heated reconstituted factor VIII and .117 for the unheated control; immunoelectropheresis against human serum showed overall increase in anodal migration as compared to an unheated control. Clearly it must be determined with future chimpanzee studies which heating temperature and length of time is necessary for significant inactivation of hepatitis virus.

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DEVELOPMENT OF A RAPID IMMUNOMETRIC ASSAY FOR THE FACTOR VIII ANTIGENS IN PLASMA.
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Immunoradiometric assays (IRMA) for both Factor VIII related antigen (VIII R:Ag) and Factor VIII clotting antigen (VIII C:Ag) have been described, involving the preparation of specific radiolabeled antibodies. The alternative methodology described in this report for F VIII R:Ag involves three steps: 1. the immobilization of a crude preparation of F VIII R:Ag (0.05 units/ml) on polyvinyl plates (96-well); 2. the incubation of known amounts (defined by a freeze-dried plasma standard) of F VIII R:Ag with a predetermined excess (1/2000) of a monospecific rabbit antiserum to F VIII R:Ag (called first antibody, ab'); 3. the binding of the various residual amounts of ab' (from step 2) to the immobilized F VIII R:Ag. The residual ab' bound to the coated polyvinyl plates is measured using a radiolabeled (^{125}I) goat IgG to rabbit IgG (called second antibody, ab"). Thus the radioactivity in each well of the plate is inversely related to the amount of F VIII R:Ag in the incubation mixture. A linear standard curve was established for pooled plasma between 0.04-0.002 units of F VIII R:Ag per ml. Test plasmas gave parallel dose-response curves with this standard curve. Thus the F VIII R:Ag levels in a variety of plasmas and concentrates were established and found to correlate with data obtained by Laurell 'rocket' immunoelectrophoresis. A similar assay to that described above has been attempted for Factor VIII C:Ag in plasma. The antiserum was obtained from a patient with an elevated level of 'inhibitor' (antibody) to F VIII C:Ag. A standard curve has been established using a highly purified preparation of F VIII C:Ag between 0.3-0.01 u of coagulant activity. This form of immunometric assay has the following advantages: 1. a source of highly purified antigen is not necessary; 2. no isolation or labelling of the F VIII antibodies is required; 3. the assay is sensitive, technically simple and rapid.

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CROSSED CONCANAVALIN A AFFINOIMMUNOELECTROPHORESIS OF FACTOR VIII - RELATED ANTIGEN IN WHOLE PLASMA.
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Normal human factor VIII is precipitated by the lectin concanavalin A (con A). In order to study this interaction pooled normal plasma has been subjected to crossed affinoimmunoelectrophoresis with con A in the first dimension and commercial antiserum to factor VIII in the second dimension. Both decreased anodal migration and decreased precipitin height of the factor VIII - related antigen (FVIIIR:ag) confirmed the precipation of FVIIIR:ag by con A.

This technique, performed in conjunction with crossed immunoelectrophoresis (CIEP) of FVIIIR:ag, should prove useful in the analysis of von Willebrand disease (vWd) variants whose FVIIIR:ag has decreased carbonhydrate and, therefore, is poorly precipitated by con A.