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HEMOPHILIC DOG MODEL FOR EVALUATING HEMOSTATIC EFFICACY OF PLASMA PROTEIN FRACTIONS. H.S. Kingdon and T.M. Hassell. Departments of Medicine, Biochemistry, and Periodontics, the Dental Research Center, and Center for Thrombosis and Hemostasis, University of North Carolina, Chapel Hill, NC USA.

About 15% of patients with hemophilia A develop inhibitors to Factor VIII. Because in many cases the inhibitor renders the patient refractory to treatment with Factor VIII, plasma protein fractions designed to bypass the inhibitor have been developed. In the USA these are referred to generically as Anti Inhibitor Complex Concentrates (AICCs). Development of AICCs has been hampered by lack of a suitable model in which to judge hemostatic efficacy. Similarly, lack of a model has impeded research on the mechanism of action of AICCs. Therefore, we chose to evaluate AICCs in dogs with hemophilia A, reasoning that a material capable of bypassing a Factor VIII inhibitor should be effective in Factor VIII deficient recipients with or without inhibitors. Under local anesthesia a standardized gingival biopsy was performed using a flexible plastic template and a modified scalpel handle holding two #11 Bard-Parker blades. The parallel incisions were 5 mm long, 2 mm apart, and 1.5 mm deep. The tissue block thus defined was removed by sharp dissection. In normal dogs, bleeding from this wound ceased in 5 $\pm$ 2 min, the wound was filled with concave clot, and bleeding did not recur. In contrast, hemophilic dogs formed an abnormal (very large) clot, and rebled for several days if untreated. The hematocrit usually dropped by 2-10 percentage points in 24 hr of uncontrolled bleeding. An experimental AICC fraction under development by Cutter Laboratories was evaluated in 5 dogs, and shown to be hemostatically effective. The dose required to achieve hemostasis was 25-75 units/kg; in some dogs a second dose was required 6 hr after the first dose to maintain hemostasis. A single dog with a low titer inhibitor to Factor VIII was successfully treated with 39 u/kg, followed by a repeat dose of 39 u/kg 6 hr after the first dose. We conclude that this AICC preparation brings about hemostasis in Factor VIII deficient individuals with or without inhibitors to Factor VIII.

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A METHOD FOR RAPID VISUALIZATION OF THE SIZE-DISTRIBUTION OF FACTOR VIII-RELATED PROTEIN IN PLASMA. B.A. Perret, R. Felix, M. Furlan and E.A. Beck. Central Haematology Laboratory, Inselspital, Berne, Switzerland.

Factor VIII-related protein circulates in normal human plasma as a series of multimeric forms with apparent molecular weights ranging from 1 to 20×10^6. So far, combined electrophoretic and immunologic methods permitted demonstration of variable concentration and size-distribution of factor VIII-related protein in von Willebrand's disease as compared to normal. We now have devised a one-step method for determining the size pattern of this plasma protein. Fresh plasma, containing 1% SDS and 0.8M urea, was layered on top of 2.5% polyacrylamide gels with 2.75% by weight of methylene bisacrylamide/acrylamide. Following extended electrophoresis in 0.2% SDS-0.1M Tris/HCl (pH 7.4), the gels were soaked in 5% formaldehyde and then extensively washed with 10% ethanol. Proteins were visualized employing an ultrasensitive ammoniacal silver stain. This staining revealed a multimeric protein pattern in the upper part of the gel the distribution of which was recorded by densitometry. The protein was identified as factor VIII by two-dimensional immunoelectrophoresis. The method was reproducible and allowed densitometric evaluation within 24 hr.

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DECREASED POTENCY OF FACTOR VIII CONCENTRATES. C.K.Kasper. Department of Medicine, University of Southern California and Orthopaedic Hospital, Los Angeles, Calif., U.S.A.

Plasma factor VIII recoveries after infusions of factor VIII concentrates into patients with classic hemophilia have been measured in this laboratory for 14 years. Recently, we observed a decline in the in vivo recovery of factor VIII per factor VIII unit infused. In 1980, plasma factor VIII levels were measured by a one-stage APTT-based assay before and 10 min after 150 infusions of 46 lots of 3 brands of factor VIII concentrate produced in the U.S.A. Our pooled normal plasma reference was calibrated against WHO International Standard 2 and results expressed in International factor VIII units. Observed in vivo factor VIII recovery was compared to the value expected from calculations based on the unitage stated on the label. The ratio of observed/expected recovery averaged 56% per lot for brand A, 60% per lot for brand B, and 103% per lot for brand C. In vitro assays were performed on 22 lots on 36 occasions, and the ratio of observed/labelled units average 46% per lot for brand A, 53% for brand B and 75% for brand C. The two-stage factor VIII assay method of Pool and Robinson was also used to assay plasma samples from 18 infusions, and results averaged 135% of the one-stage values for infusions of brand A, 160% for brand B, and 109% for brand C. (Brand A is assayed by the manufacturer by a two-stage method, brands B and C by one-stage methods.)

Decreased clinical efficacy was observed when post-infusion plasma factor VIII levels were lower than customary. The decline in potency of brands A and B has necessitated more frequent assay of patients and use of larger amounts of concentrate, with greatly-increased expense. Investigation of the effect of different assay methods and different factor VIII standards and references on the apparent factor VIII content of concentrates has begun.

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PURIFICATION OF FACTORS IX AND X FROM CLINICAL CONCENTRATE. G.C. Russell, G. Kemble, and E.G.D. Tuddenham. The Royal Free Hospital, Katharine Dormandy Haemophilia Centre & Haemostasis Unit, Pond Street, London NW3 2QG, UK.

Human factors IX and X have been purified to homogeneity from clinical factor IX concentrate that had been rejected for therapeutic use due to particulate contamination. (It was necessary to start with this material since in the UK, plasma is not commercially available). The procedure involved barium citrate adsorption followed by ammonium sulphate elution, DEAE-cellulose chromatography, gel filtration on Sephacryl S-200 and affinity chromatography on heparin sepharose gel. The preparation of factor IX at this stage showed a single band on SDS-polyacrylamide gel electrophoresis, of molecular weight 58,000. No change in molecular weight was observed in the presence of 2-mercaptoethanol. A further affinity chromatography column - poly (homoarginine) Sepharose or dextran sulphate sepharose - was necessary to obtain homogeneous factor X. The preparation obtained showed a single band on SDS-polyacrylamide gel electrophoresis of molecular weight 67,000. In the presence of 2-mercaptoethanol, two bands were obtained of molecular weights 49000 and 17000 representing the heavy and light chains respectively of factor X. The purified coagulation proteins contained no activated species detectable by non-activated partial thromboplastin time or by chromogenic substrate (S2222) assay. Prothrombin protein S and protein C are by-products of this purification procedure.