

Supplementary Abstracts

Factor IX

1054

HEATED LYOPHILIZED FACTOR VIII AND FACTOR IX CONCENTRATE — PRELIMINARY IN VITRO STUDIES. A. Rubinstein. Department of Clinical Pathology, Cedars-Sinai Medical Center, Los Angeles, California.

It has been known that albumin and plasma protein fraction (PPF) do not transmit hepatitis since these products are heated at 60°C for 10 hours. This heating is sufficient for inactivation of hepatitis virus. It has been previously tried to heat the Cohn Fractions leading to Factor VIII and Factor IX Concentrates in solution, however, this has resulted in significant loss of Factors VIII and IX.

We have taken lyophilized Factor VIII Concentrate powder and heated it in a waterbath at 60°C for 10 hours and have demonstrated no significant change in in vitro recovery of Factor VIII activity four hours following the heating. The same was shown for recovery of Factor IX activity after heating lyophilized Factor IX concentrate for 10 hours at 60°C. In addition Factor IX activity was not destroyed following heating of the lyophilized powder for 20-30 minutes at 100°C. This is significant because now potentially this heated lyophilized powder of Factor VIII and Factor IX is free of previous risk of transmission of hepatitis. However, the effect of heating on the thrombogenic effects in vivo of the concentrates and complications was not assessed in this study. Chimpanzee studies are planned to determine whether the heating has inactivated the hepatitis virus.

It is possible that a longer heating time will be needed to sufficiently inactivate the hepatitis virus in the lyophilized state.

1055

PREPARATION OF FACTOR IX-DEFICIENT HUMAN PLASMA USING RFF-IX/1 MONOCLONAL ANTIBODY. F. Rotblat, A.H. Goodall, G. Janossy, G. Kemble, D.P. O'Brien, E. Rawlings, G. Russell, and E.G.D. Tuddenham. Katharine Dormandy Haemophilia Centre & Dept. of Immunology, Royal Free Hospital, London, UK.

A cell line that secretes a monoclonal antibody to factor IX has been produced by fusing spleen cells from a mouse that had been hyperimmunised to purified factor IX with mouse myeloma cells (line P3-NS1/1-Ag4-1). Hybrid cells were selected and a monoclonal cell line has been established in culture. This cell line secretes an IgG₁(k) antibody (RFF-IX/1) with high affinity for a site related to the coagulant function of factor IX.

Monoclonal antibody was partially purified from ascitic fluid from mice implanted with the RFF-IX/1 secreting cells by precipitation at 50% saturation with ammonium sulphate. This fraction has typically 630 NIH units/ml anti IX activity and 13.5 mg/ml protein. It was coupled to cyanogen bromide activated Sepharose 2B in the ratio of 9 mg. protein/1 ml gel. A column containing 10 ml of this gel removed all the assayable factor IX from the first 280 ml of normal citrated plasma that was passed over it. After that volume small amounts of factor IX could be detected in the effluent. Subsequently 10-20% of the factor IX activity adsorbed could be recovered by eluting the column with 3 M potassium iodide.

Immuno-affinity depleted plasma could be used as substrate in a one-stage factor IX assay under routine laboratory conditions and was undistinguishable for that purpose from severe Christmas disease plasma.

1056

ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF ALLOANTIBODIES TO FACTOR IX IN HEMOPHILIA B. K. H. Ørstavik and I. Ørstavik. Institute of Medical Genetics, University of Oslo, Oslo, Norway and Ullevål Hospital, Oslo, Norway.

A solid phase enzyme-linked immunosorbent assay (ELISA) was developed for the detection and quantitative determination of acquired inhibitors to factor IX. Wells of polystyren Micro-ELISA plates were coated with the IgG fraction of a sheep antiserum to human factor IX. After incubation with pooled normal plasma as a factor IX source, the wells were incubated with test plasma. The binding of alloantibodies to the factor IX-sheep-anti-factor IX complexes was then detected by incubation with alkaline phosphatase conjugated antiserum to human IgG. As substrate was used p-nitrophenyl phosphate.

Plasma samples from five patients with severe hemophilia B and acquired inhibitors to factor IX were examined. All samples gave a positive reaction in the ELISA. The titers as determined in the ELISA were in good agreement with the titers as determined in a coagulation assay (0.1-800 U/ml). Plasma from 13 patients with hemophilia B and no detectable inhibitor in a coagulation assay all gave a negative reaction in the ELISA. A negative reaction was also found in plasma from four patients with hemophilia A and acquired inhibitors to factor VIII, and in plasma from 15 healthy persons.

It is concluded that the ELISA is a simple and sensitive technique for the determination of acquired inhibitors to factor IX in hemophilia B.