MIXED POSTERS XI

Coagulation

PRODUCTION OF A HIGH-TITRED SHEEP ANTISERUM AGAINST HUMAN FACTOR IX AND IMMUNOLOGICAL DEMONSTRATION OF FACTOR IX AGGREGATES. K.H.Ørstavik, Institute of Medical Genetics and A.M.Vennerød, Institute of Pharmacy, University of Oslo, Norway.

Plasma factor IX was purified from a factor IX concentrate by a five step procedure including absorption onto aluminium hydroxide, affinity chromatography on heparin-coupled Sepharose 4B, preparative disc gel electrophoresis, affinity chromatography on an immunosorbent column with rabbit antiserum against factor X and chromatography on DE-52 cellulose. The pooled fractions had a specific activity of approximately 250 U/mg protein. A sheep was immunized with pooled and concentrated fractions. An antiserum was produced which gave one main precipitin band and occasionally an additional weak band against normal plasma in double immunodiffusion. At a dilution of 1/100-1/200 the antiserum neutralized 90% of the factor IX activity in an equal volume of normal plasma.

Polyacrylamide disc gel electrophoresis of the fractions from DE-52 cellulose revealed one major and three minor bands with lower electrophoretic mobility and intensity. The three minor bands disappeared on disc gel electrophoresis in the presence of 10 M urea. When the disc electrophoresis gel was submitted to electrophoresis into an agarose gel containing the sheep antiserum or a previously characterized rabbit antiserum against factor IX, four precipitin arcs corresponding to the four bands were seen. A reaction of identity was seen between the four arcs. This study demonstrates that a highly potent antiserum may be produced against factor IX in sheep.

PURIFICATION OF HUMAN FACTOR V. P.A. Bolhuis, T.B.M. Hakvoort, K. Breederveld, I.A. Mochtar and J.W. ten Cate. University of Amsterdam, Amsterdam, The Netherlands.

Human factor V has been purified from cryo-supernatant by fractionated precipitation with polyethylene glycol (6000, 16-24% w/v, yield 65%), followed by gelfiltration on AcA 44 in Michaelis buffer with 50 mM Ca^{++} (elution in V_o, yield 90%), adsorption of contaminating haptoglobinpolymers on hemoglobin bound to Sepharose-4B (yield 95%) and adsorption of the glycoproteins (mainly factor V) onto concanavaline A bound to Sepharose-4B, elution with α-methyl-pyranoside (4M) and gelfiltration of the factor V eluted. The purified factor V has a specific activity of about 28 u/mg and the total yield is 16%. The native protein and the subunits obtained by reduction in the presence of dodecyl sulphate and urea have been characterized by polyacrylamide gel electrophoresis.