Time
14.30

0785 IMMUNORADIOMETRIC ASSAY FOR VIII:CAg BASED ON TWO NON-HAEMOPHILIC ANTIBODIES

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Antihaemophilic-factor-A-antibodies, which had spontaneously arisen in two patients, were used to develop an immunoradiometric method for measurement of antihaemophilic factor A antigen (VIII:CAg). One of the antibodies was used for coating plastic tubes. A second antibody formed a stable high molecular weight complex with a factor VIII-concentrate and could conveniently be isolated in labelled form. This antibody, which seemed to be bound to only one immunologic site of VIII:CAg, was used for detecting the VIII:CAg fixed to the tubes by the first antibody.
Thirteen patients with severe haemophilia A had VIII:CAg below the limit of detection (0.01 U/ml). Patients with moderate and mild haemophilia A either had VIII:CAg roughly equal to factor VIII clotting activity (VIII:C) or a not detectable VIII:CAg, suggesting two different molecular mechanisms in moderate and mild haemophilia. VIII:CAg could be detected in serum but in lower amounts than in plasma. In two patients with von Willebrand's disease VIII:CAg equalled VIII:C. The post-transfusional retarded increase of VIII:C in a patient with von Willebrand's disease was accompanied by a slight increase in VIII:CAg. Fetal plasma contained measurable amounts of VIII:CAg.

14.45

0786 NON-FUNCTIONAL FVIII MEASURED IN A GROUP OF HAEMOPHILIA A PATIENTS WITH A HOMOLOGOUS AND HETEROLOGOUS ANTI VIII:C SERUM

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After dissociation of the FVIII complex in the presence of high salt concentration a rabbit antiserum was developed against the low molecular weight fragment (LMW FVIII). The anti LMW FVIII had anti VIII:C activity (450 Bethesda units), but also precipitated against a FVIII concentrate. Adsorption of the antiserum with cryoprecipitate of a haemophilia A patient removed the precipitating properties (anti HMW FVIII), without affecting the anti VIII:C activity. The rabbit antibody and a human inhibitor were used in a fluid phase inhibitor neutralisation assay. The mean value of VIII:C:Ag in 23 normal persons was 83.5% (SD 25.9) when tested with the human antiserum; 90.1% (SD 27.1) when tested with the rabbit antiserum. The mean VIII:C was 86% (SD 23.3). (100% is the amount of VIII:C or VIII:C:Ag in 1 ml of pooled normal plasma.) 59 Patients with haemophilia A (severe, moderate, and mild) were tested. 46 Patients were A^+ when tested with the human antibody, 12 patients were A^+ when tested with the rabbit antibody. It is concluded that 1) heterogeneity among haemophilic patients exists, and 2) the rabbit antibody recognises more antigenic determinants on the VIII:C molecule than the human antibody used in this study.

15.00

0787 FACTOR VIII COAGULANT ANTIGEN (VIII:CAg) IN FETAL BLOOD SAMPLES

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Quantitative immunoradiometric assays (IRMAS) for the coagulant portion of the FVIII complex (VIII:CAg) have recently been described by several laboratories (Reisner et al. Thrombosis Research, in press). The antigen detected by these assays is absent or decreased in the plasma of most individuals with severe hemophilia A. Levels of VIII:CAg and FVIII related antigen (VIIIR:Ag) were measured in ten presumably non-hemophilic samples obtained either by fetoscopy or after abortion. Ratios of VIII:CAg to VIIIR:Ag ranged from .07 to .52 in four samples where both values could be determined. VIII:CAg could not be detected in four other samples. Three of these also lacked detectable VIIIR:Ag and were probably diluted with amniotic fluid which has neither VIII:CAg nor VIIIR:Ag. One sample had 80% VIIIR:Ag in the absence of detectable VIII:CAg. VIII:CAg was detected in six samples with a range of 4 to 50% of an adult normal pool. An aborted male fetus from a potential carrier of severe hemophilia A had an VIII:CAg to VIIIR:Ag ratio of .42 and was probably not affected. The low values of VIII:CAg seen in this preliminary study may be explained by the decrease in antigenicity seen in serum versus plasma samples in our assay. Hence, extreme care in sample preparation and storage must be exercised should this IRMA for VIII:CAg be used in fetal diagnosis of hemophilia A.