Time
16.00
cont.

0817 THE RELATIONSHIP OF THE QUATERNARY STRUCTURE OF FACTOR VIII TO ITS BIOLOGICAL ACTIVITIES

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Purified factor VIII exists as a homologous series of multimers in which the protomeric unit is a dimer of the basic subunit. On reduction by 2-mercaptoethanol, clotting activity (VIII:C) persists at 90% of the initial activity when the only species present are monomers and dimers. In contrast, Von Willebrand activity (VIII:VWF) is lost concurrently with the disaggregation of multimers and is decreased to 5% of the starting activity when only monomers and dimers remain. VIII:Ag reactivity with rabbit antibody is likewise lost on reduction, but the VIII:C activity of the reduced protein is inhibitable by hemophilic a-VIII antibodies. Unreduced VIII binds to fresh or formalin-fixed platelets in the presence of ristocetin or vancomycin. Although the apparent Ka for binding is high, approximately $5 \times 10^8\ M^{-1}$, binding is dependent on an excess of ristocetin. On removal of ristocetin or vancomycin by serial dilution, platelet-bound factor VIII readily dissociates. Reduced factor VIII also binds to platelets in the presence of ristocetin with a Ka of approximately $10^8 M^{-1}$, but does not cause platelet aggregation. These data suggest that the multimeric structure of factor VIII is not necessary for VIII:C activity nor for binding to platelets but may be important for VWF platelet-aggregating activity.

0818 ENZYMATIC DEGRADATION OF THE FACTOR VIII-VON WILLEBRAND PROTEIN: A UNIQUE TRYPTIC FRAGMENT WITH RISTOCETIN CO-FACTOR ACTIVITY.

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Highly purified von Willebrand protein was obtained from pooled human cryoprecipitate using polyethylene glycol precipitation followed by agarose gel chromatography, and concentrated by dialysis against 20% polyethylene glycol-20,000. Subsequent partial hydrolysis with trypsin destroyed all of the procoagulant activity and 97% of the ristocetin co-factor activity of the original purified material. Such digests were gel filtered and then analyzed by determinations of biological activities and by SDS-polyacrylamide gel electrophoresis. All of the ristocetin co-factor activity present in the digest was found in a 100,000 dalton fragment. Larger fragments with molecular weights 150,000-260,000 daltons as well as smaller fragments (< 50,000) lacked ristocetin co-factor activity. Fragments smaller than 50,000 daltons did not react with heterologous antisera. These observations suggest that the ristocetin co-factor activity of the intact von Willebrand molecule is located in one or more specialized regions, partially retained in this unique intermediate-sized tryptic degradation product.

0819 FACTOR VIII CLOTTING ANTIGENS STUDIED BY IMMUNORADIOMETRIC ASSAY

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Antigens related to procoagulant factor VIII (FVIIIC) were detected and measured using an immunoradiometric assay based on an inhibitor to FVIIIC which arose in a polytransfused severe haemophiliac. Good agreement between FVIIIC and FVIIIC antigen (FVIIICAg) is seen in normal and mild von Willebrand's disease (vWd) plasma while in severe vWd levels of FVIIICAg up to 5% are recorded. In haemophilia, FVIIICAg levels are reduced generally to a level below that of FVIIIC. FVIIICAg is also present in serum and is stable at 37° for 24 hours in plasma. Thus its measurement has considerable potential for the diagnosis of haemophilia, particularly in blood samples obtained from the young infant, umbilical cord, or fetus. FVIIICAg was assayed in several factor VIII concentrates and the ratio of FVIIICAg to FVIIIC correlated with the degree of purification of the concentrate. Unlike FVIII related antigen (FVIIIRAg) FVIIICAg was not detected in human cord endothelial cell culture medium, and was present in only low levels in washed platelets. Results of gel filtration of plasma FVIIICAg depended on the anticoagulant used but in general FVIIICAg was often detectable without FVIIIC or FVIIIRAg in the included fractions. The results confirm the existence of two broad types of factor VIII antigens and are consistent with the two molecule hypothesis on the nature of factor VIII.