Time

14.30 **1022** THE D DIMER-E COMPLEX AND ITS AGGREGATES IN CROSSLINKED FIBRIN DIGESTS

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In vitro data have indicated that plasmin-mediated lysis of crosslinked (XL) fibrin in vivo yields only one distinct high molecular weight complex, which has the empirical formula, 2D-E. We have compared the compositions of lysates obtained from I^{125} labelled and unlabelled fibrin clots in buffer, human and animal sera, and trasylol, using conventional immunological, chromatographic and electrophoretic techniques. Both trasylol (10 KI units/ml) and various animal sera stabilised the D dimer-E complex following lysis of XL fibrin and only in buffer were free D dimer and E fragments observed. The D dimer -E complex was isolated by affinity chromatography and the expected polypeptide chain composition, including the crosslinked γ chain remnants, was confirmed. By combining various molar ratios of D dimer and E the expected equimolar nature of the complex was confirmed and the association sites between D dimer and E may be synonymous with the polymerization sites already shown to exist in the D and E domains of fibrinogen. Using I^{125} labelled fibrin clots, covalently linked high molecular weight complexes (up to 1 x 10^6MW) were observed during in vitro lysis with plasmin. At least one of these was identified as a crosslinked Y dimer while other larger fragments may be covalently linked complexes of the "D dimer-E subunit". A hypothesis for XL fibrin lysis in vivo is proposed which complements accepted ideas on fibrin clot formation.

14.45 **1023** LOCALIZATION OF THE α-CHAIN CROSS-LINKING SITES IN HUMAN FIBRINOGEN

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Antisera were raised in rabbits against the fragments Hi2-DSK and N-DSK obtained from cyanogen bromide treatment of human fibrinogen. Sensitive radioimmunoassay techniques were developed to quantitate substances sharing the same antigenic determinants as these fragments. We have found that the cross-linking process in fibrin formation involves the Hi2-DSK region which has its origin in the Aα-chain of fibrinogen. This relationship is based upon the following experimental data: - Fibrin samples produced in the presence and absence of Factor XIIIa and Ca^{++} and thus cross-linked and non cross-linked respectively were treated with cyanogen bromide. The resulting degradation products were chromatographed on Sephadex G-100, and radioimmunoassays for Hi2-DSK and N-DSK were performed on the eluates. The elution profiles of Hi2-DSK and N-DSK antigenic determinants demonstrated that Hi2-DSK eluated after N-DSK in non cross-linked fibrin samples but in the cross-linked samples Hi2-DSK eluated before N-DSK. These results demonstrate that the Hi2-DSK region is involved in the cross-linking process in the presence of Factor XIIIa and calcium ions. Fibrin samples produced in the coagulation of whole blood showed a similar cross-linking profile.

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15.00 **1024** FIBRINOGEN Bβ CHAIN PROTEOLYSIS BY THROMBIN AND PLASMIN - A REGULATORY MECHANISM IN HEMOSTASIS AND THROMBOSIS

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Thrombin cleaves fibrinogen in two steps producing fibrin I and fibrinopeptide A(FPA) and then fibrin II and fibrinopeptide B(FPB). Plasmin cleaves the Aα chain and the N-terminal Bβ chain to form fragment X. Radioimmunoassay of plasma FPA reflects fibrin I formation. A modified FPB assay detects plasmin-produced Bβ1-42, reflecting fragment X formation. Sequential changes have been studied in saline abortion and in venous thrombosis. Following intra-uterine infusion of hypertonic saline to interrupt pregnancy, FPA levels rose immediately from 1 to 18 pmol/ml in 60 minutes, and after 2 hours began to slowly decline to the baseline level. Bβ1-42 levels rose rapidly after the first hour to reach 67 pmol/ml at 4 hours. Thus fibrin I was formed and then fragment X. In post-surgical patients FPA levels rose and exceeded Bβ1-42 levels for several days preceding thrombosis indicated by ^{125}I-fibrinogen scanning and confirmed by venography. These results suggest that following injury thrombin forms fibrin I. Each fibrin I molecule is then either cleaved by thrombin to form fibrin II or by plasmin to form fragment X. The balance between thrombin and plasmin proteolysis of the N-terminal Bβ chain may be critical. Normally sufficient fibrin II formation occurs for effective hemostasis. Thrombosis occurs with excessive thrombin action and fibrin II formation. Slow thrombin formation as in hemophilia results in preferential formation of fragment X and ineffective hemostasis.