ACQUIRED DYSFIBRINOGENEMIA IN ACUTE AND CHRONIC LIVER DISEASE. D.A. Lane, V.V. Kakkar, I.L. Woolf and R. Williams. Thrombosis Research Unit and Liver Research Unit, King's College Hospital Medical School, London.

Recent work with the thrombin-like snake venom Reptilase has suggested that the incidence of dysfibrinogenemia in liver disease may be much higher than has been recognised previously. In the present study, nine patients with fulminant hepatic failure and three with chronic liver disease, all exhibiting prolonged thrombin and ancrod clotting times, were examined for evidence of dysfibrinogenemia with a variety of physiochemical and clotting techniques. Sodium dodecyl sulphate polyacrylamide gel electrophoresis revealed normal fibrinogen polypeptide chains, polypeptide chain crosslinking by Factor XIII, and in vitro generated plasmin degradation products of fibrinogen (FDP) in the plasma of these patients. Isolated and purified fibrin monomers were prepared by clotting plasma with Reptilase (Reptilase monomer) and thrombin (thrombin monomers). This enabled fibrin monomer polymerisation to be studied free of interference from plasma inhibitors such as FDP, and independent of any abnormal fibrinopeptide release. Delayed polymerisation of both Reptilase and thrombin monomers was demonstrated, providing an explanation for the observed prolonged clotting times. These results confirm that an abnormal fibrinogen may be produced during acute and chronic liver disease.

LOCALIZATION OF CROSSLINK SITES IN α-CHAIN OF HUMAN FIBRIN. L.J. Fretto, E.W. Ferguson and P.A. McKee. Duke University Medical Center, Durham NC, U.S.A.

To determine the position of potential crosslink acceptor sites in the α-chain, fibrin was specifically labeled with the fluorescent pseudo-donor monodansylcadaverine (MDC) and then digested to obtain labeled α-chain peptides. These were isolated and characterized by sodium dodecyl sulfate (SDS) gel electrophoresis, amino acid analysis, amino-terminal sequence analysis and ultracentrifugation. The α-chain of MDC-labeled fibrin was selectively cleaved to a series of fluorescent peptides of molecular weights 45,000 to 21,000 daltons using plasmin and 34,000 to 12,000 daltons using ancrod. A labeled plasmic peptide of molecular weight 23,000 displayed all the fluorescence of the MDC-labeled α-chain and when digested to the plasmin-resistant peptide of 21,000 daltons (P21), an amino-terminal segment having a fraction of the fluorescence was released. SDS-gel analysis of the residual, particulate plasmin-treated fibrin showed that the disulfide-bound amino-terminal 25,000-dalton portion of the α-chain was not MDC-labeled. However, the residual fibrin from ancrod digestion had an amino-terminal 38,000-dalton α-chain derivative which contained two MDC-labeled sites that were separable by CNBr cleavage. Taken together with the sequence data, the above findings established the existence of three acceptor sites located approximately at residues 260, 315 and 390 of the Aα-chains. That the physiological crosslink acceptor sites also reside in the middle 1/3 of the α-chain was demonstrated by plasmin digestion of highly crosslinked fibrin; a peptide having electrophoretic properties and an amino acid composition very similar to P21 was isolated which also contained at least one crosslink as determined by cyanoethylation. These findings combined with pre-existent information about fibrin structure permitted construction of a model for α-chain polymerization.

DISTINCTIVE NEOANTIGENIC MARKER ASSOCIATED WITH A PLASMIC DERIVATIVE (FRAGMENT D-D) of CROSS LINKED HUMAN FIBRIN. A.Z. Budzynski, V.J. Marder, M.E. Parker, P. Shames, Depts. of Medicine and Biochemistry and the Specialized Center for Thrombosis Research, Health Sciences Center, Temple University, Philadelphia, Pennsylvania, U.S.A.

Fragment D-D is a plasmic degradation product of crosslinked fibrin consisting of 2 Fragment D molecules linked by Factor XIII-induced ε(γ-glutamyl)lysine bonds. In this study, the antigenic markers of Fragment D-D were compared with those of Fragment D obtained from fibrinogen and non-crosslinked fibrin, as well as with those of fibrinogen Fragments X, Y and E. An antiserum against Fragment D-D raised in chickens and absorbed with fibrinogen and Fragment D showed immunoprecipitation with Fragment D-D but not with fibrinogen or with the other derivatives. In a double antibody radioimmunoassay system the antiserum bound 125-I labeled Fragment D-D but not labeled fibrinogen or Fragment D from fibrinogen and non-crosslinked fibrin. The binding of labeled Fragment D-D was inhibited by non-labeled Fragment D-D at concentrations of 10-10,000 ng/ml and was equally reliable in a plasma milieu. Purified plasmic degradation products of human fibrinogen inhibited binding less effectively. The increased assay sensitivity for Fragment D-D appears to be related to specific neoantigenic sites associated with the overall structure of this degradation product and provides molecular basis for the differentiation between fibrinogenolysis and thrombolysis.