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CALCIUM-INDUCED FORMATION OF FIBRINOGEN -- FIBRIN COMPLEXES. M. Kaminski (1), and J. McDonagh (2). Dept. of Medicine, University of Wisconsin, Madison WI 53706, U.S.A. (1) and the Dept. of Pathology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02215, U.S.A. (2).

Presence of calcium chloride (8.3 mM) at u = 0.1 enhanced precipitate formation in several different fibrinogen preparations. The precipitate had gel like properties and demonstrated syneresis when disturbed. Precipitation proceeded in the time dependent manner with a plateau after 30 min. The amount of precipitate ranged from 4-24% of total protein content of different preparations. Precipitates did form at u = 0.1 without calcium ion; however, the amount of protein was 3 to 7 fold less than when calcium ion was present.

SDS gel electrophoresis of calcium precipitates demonstrated cross-linking of gamma chains. Cross-linking, but not precipitate formation was blocked by parachloromercuriebenzoate (PCMB). The precipitates were redissolved in 2M urea, treated with thrombin, and analyzed for fibrinopeptide content by HLPC. Precipitate formed in absence of calcium ion contained 19% of FPA and 89% of FPB. Precipitates formed in the presence of calcium ion contained 49% of the expected amount of FPA and 98% of expected FPB. The precipitation phenomenon could be reproduced after fibrin monomer-free fibrinogen (prepared by dialysis of calcium supernatants against calcium free buffer) was treated briefly with thrombin.

The above finding suggests that calcium ion enhances polymerization of intact fibrinogen and molecules missing one or two FPA's.

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THE PEPTIDE β_{43-47} WHICH INCREASES MICROVASCULAR PERMEABILITY IS RELEASED BY PLASMIN DURING CLEAVAGE OF FRAGMENT Y OF FIBRINOGEN. C.S. Cierniewski, J. Poniatowski, and J. Urbanczyk. Department of Biophysics, Medical School of Lodz, Poland.

Pentapeptide *Ala-Arg-Pro-Ala-Lys* corresponding to β_{43-47} is known to increase microvascular permeability and is supposed to be involved in the establishment of pulmonary edema in conditions with fibrin accumulation in the lungs (Belew, et al., Thromb. Res. 13, 983-994, 1978). This peptide also induced dilation of bovine mesenteric arteries and caused a release of prostacyclin as well as an increase in cyclic AMP in blood vessels (Anderson, et al., Thromb. Res. 30, 213-218, 1983). The aim of this report was to demonstrate at which step of plasmin degradation this physiologically active peptide is released from fibrin(ogen) to the environment. For this purpose the pentapeptide and its analog *Tyr-Ala-Arg-Pro-Ala-Lys* were synthesized and used to produce specific antibodies in three rabbits. Antibody titers were determined by radioimmunoassay using radioiodinated *Tyr-Ala-Arg-Pro-Ala-Lys* and fibrinogen and the highest titer antisera from each rabbit were selected. Antibodies raised against β_{43-47} recognized during immunoblotting intact fibrinogen, fragments X and Y as well as the Bβ chain. Since fragment Y is the last product which reacts with anti-β_{43-47} antibodies, splitting of fragment Y into fragment D and fragment E must be accompanied by plasmin cleavage of the peptide bond βLys_{47}-Ala_{48}. Anti-β_{43-47} antibodies precipitated also radioiodinated fibrinogen which was not exposed to sodium dodecyl sulfate confirming our previous observations (Cierniewski and Edgington, Thromb. Res. 14, 747-764, 1979) that sequence 43-53 of the β chain is exposed on the hydrated surface of intact fibrinogen molecule.

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PROPERTIES OF A HUMAN FIBRINOGEN A α-CHAIN DEGRADATION FRAGMENT. H.K.F. LAU. Department of Biochemistry, University of Hong Kong, Sassoon Road, Hong Kong.

A fragment of human fibrinogen was obtained by a 30-min digest of fibrinogen with plasmin. The reaction was terminated by 10 mM diisopropyl fluorophosphate and the fragment obtained by sequential gel filtrations on Sephacryl S-200 and Bio Gel P-30. This fragment displayed a single band on both reduced and nonreduced SDS polyacrylamide electrophoreses with a relative Mr of ~25,000. It did not crossreact with antisera against fibrinogen, fragment D or fragment E and suggested its sequestered location in native fibrinogen. An antiserum raised against this fragment reacted with the fragment itself as well as fibrinogen. Its amino acid composition and other properties suggested that it was derived from the Aα -chains of fibrinogen. The fragment had anticoagulant activity as it was able to inhibit thrombin time in a dose-dependent fashion and it could also inhibit the polymerization of fibrin monomers. However, radioactively labelled fragment was not able to bind calcium ions in equilibrium dialysis experiments, nor was it able to bind plasma fibrin clot, fibrinogen- or fibrin-bound Sepharose columns. The activation of plasminogen by streptokinase was inhibited by the fragment. On the other hand plasminogen activation by melanoma tissue plasminogen activator and urokinase were both enhanced in the presence of the fragment.

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THE DYSFIBRINOGEN OF CHILDHOOD NEPHROSIS. T. Abshire, L. Fink, J. Christian, J. O'Connell and W. Hathaway. Dept of Pediatrics, Univ of Colorado and Dept of Pathology, VA Hosp, Denver, CO, USA.

An abnormal fibrinogen (Fib) related to increased sialic acid (SA) has been described in adults with liver disease. This dysfibrinogen (Dysfib) seems most like fetal Fib. A review of 11 patients with nephrosis revealed an unexplained prolonged thrombin time (TT) and otherwise normal coagulation studies. Based on these observations, we sought to answer whether the prolonged TT defined a Dysfib and if this abnormal Fib was similar to fetal Fib. Pooled adult, fetal plasma, and the plasma of 3 patients with nephrosis were studied with TT and reptilase times (RT). Fib was measured by functional (Fib-act) and immunologic (Fib-ag) assays. An enzyme linked immunosorbent assay (ELISA) was established using antifibrinogen as the first antibody and either peroxidase conjugated Fib or a lectin (Limulus Polyphemus) specific for SA as the second antibody. The optical density was recorded per μgm Fib for both conjugated antifibrinogen or lectin and the ratio compared in order to estimate SA reactivity. Patient 3 was also studied by: 1) crossed immunoelectrophoresis (CIE) employing lectin in the first dimension and 2) polyacrylamide gel electrophoresis (PAGE) with transfer to nitrocellulose paper using Western Blot technique.

Patients	TT(sec)	RT(sec)	Fib-act (mg/dl)	Fib-ag (mg/dl)	Lectin/Fib
1	17.1	14.5	660	1232	0.04
2	17.3	16.0	740	829	0.06
3	17.4	16.9	361	526	0.05
Fetal	22.5	21.0	157	274	0.07
Adult	12.4	12.0	280	280	0.11

Results of the CIE showed patient 3 and fetal plasma were similar in electrophoretic pattern and different from adult plasma. The PAGE with Western Blot revealed a similar pattern of Fib for patient 3, fetal and adult plasma. We conclude that the prolonged TT and RT, the greater amount of Fib-ag when compared to Fib-act in patients 1-3 and fetal plasma and the absence of evidence for Fib degradation products, support the diagnosis of Dysfib. The similarity of the CIE for patient 3 and fetal plasma and the difference between ELISA lectin/Fib ratio of patients 1-3 and fetal compared with adult plasma suggest that the Dysfib of nephrosis may be similar to fetal Fib.