A LOW MOLECULAR WEIGHT FIBRINOLYTIC ENZYME FROM POLYMORPHONUCLEAR LEUKOCYTES (PMN). L.A. Moroz, Division of Clinical Immunology, McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada.

Despite evidence implicating PMN in fibrinolysis, the enzymes involved are incompletely characterized. PMN were prepared from normal blood by dextran sedimentation and fibrinolytic activity assayed by ^{125}I-fibrin solid phase assay (Blood 46:543, 1975). More than 80% of activity was associated with intact PMN, was stimulated by Na salicylate (+65%, 20 mg/100 ml) and inhibited by α_1-anti-trypsin (α_1AT, -48%, 2 x 10^{-6} M). Similar activity was found in a PMN membrane fraction prepared by homogenization, differential centrifugation and Sepharose 4B gel filtration, from which fraction it was released by freeze-thawing and/or 1M KCl treatment. Soluble enzyme activity was inhibited by α_1AT (-80%, 10^{-6} M), PMSF (-98%, 10^{-3} M), $FeCl_3$ and $ZnCl_3$ (-100%, 10^{-2} M), vitamin E (-38%, 10^{-4} M) and trypan blue (-40%, 10^{-3} M), but not by EACA (10^{-2} M), tranexamic acid (10^{-2} M), TLCK (10^{-3} M) or TPCK (10^{-3} M). This activity had an alkaline proteinase pH-activity profile, was localized to a single cationic protein band on acid polyacrylamide disc gel electrophoresis, with pI of 8.6-8.7 by isoelectric focussing, and eluted between lysozyme and myoglobin on Bio-Gel P-10. On Bio-Gel A 0.5m and P-10, ^{125}I-fibrin degradation products eluted after myoglobin. These findings indicate the presence in PMN of a low molecular weight, membrane-associated fibrinolytic enzyme of alkaline proteinase and serine active site type.

FIBRIN AND FIBRINOGEN PROTEOLYSIS PRODUCTS: COMPARISON BETWEEN GEL FILTRATION AND SDS POLYACRYLAMIDE ELECTROPHORESIS ANALYSIS. N. Alkjaersig, A. Davies and A. Fletcher. Washington University School of Medicine, St. Louis, Missouri, U.S.A.

The proteolysis of purified human fibrinogen, stabilized and non-stabilized fibrin by plasmin were investigated by gel filtration analysis and SDS polyacrylamide electrophoresis of the reaction products. Plasmin proteolysis of fibrinogen followed the sequential steps previously reported and the two analytical methods yielded concordant results. Large molecular weight proteolysis products, of substantially greater molecular weight than native fibrinogen, were identified by gel filtration analysis following dissolution of stabilized and non-stabilized fibrin clots; with further incubation with plasmin, these proteolysis products gradually diminished in size. On the other hand, SDS polyacrylamide electrophoresis of these fibrin digests demonstrated that while non-stabilized fibrin yielded breakdown products similar in size to those obtained after proteolysis of fibrinogen, stabilized fibrin digests showed moieties of greater molecular size estimated to be of molecular weight 400,000 to 800,000. The final breakdown products of stabilized fibrin differed from those of fibrinogen and non-stabilized fibrin in that fragment D was present in the "double D" cross-linked form.

THROMBOGENIC CHARACTERISTICS OF ACTIVATED AND STANDARD CONCENTRATES OF PROTHROMBIN COMPLEX. J.A. Penner, K. Leach, and J. Rohwedder. University of Michigan Medical Center, Ann Arbor, Michigan, U.S.A.

The composition of a variety of prothrombin complex concentrates(PCs), including an "activated" product, was examined to determine thrombogenic potential. Small amounts of Xa activity were found to be present in all products surveyed, although much greater quantities were present in the activated one. Values for prothrombin factors VII, IX, and X activity varied over a limited range, from one product to another, and small quantities of factors XI and XII were identified in several concentrates. Animal recipients of each product, receiving a dose of 100 u/kg developed intravascular coagulation. Prior incubation of the products with Antithrombin III to eliminate activated factors did not alter the coagulopathy pattern.

It was concluded that the presence of factor Xa was not responsible for the observed intravascular coagulation syndrome, and that other factors, such as increased procoagulant levels in the circulation, or the presence of other prothrombin intermediates must be involved.