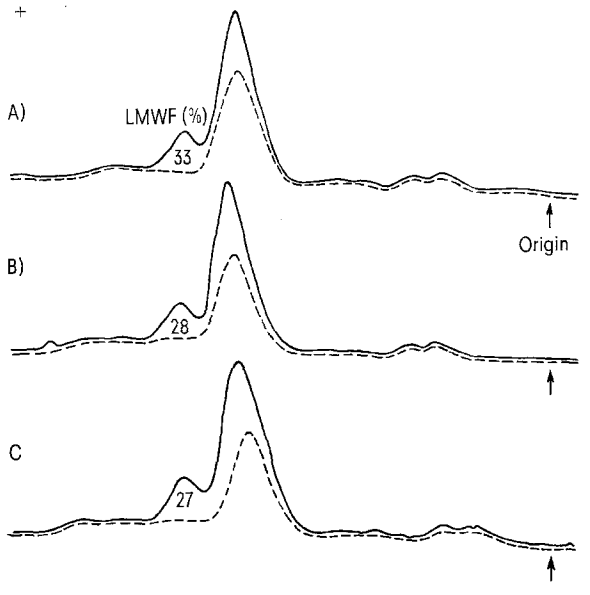
Fibrinolysis Versus Fibrinogenolysis in Man: Resistance of Fibrinogen to Breakdown by Fibrinolytic Activity Induced by Venous Occlusion

Despite numerous studies on the degradation of fibrinogen by plasmin in vitro, little is known about the mechanism of fibrinogenolysis in vivo. The presence of fibrinogen/fibrin degradation products (FDP/fdp) which are found in blood in both physiological and pathological states does not provide the answer, since currently available methods fail to distinguish fibrin from fibrinogen degradation products. Human fibrinogen has been shown to be heterogeneous with respect to solubility[1] and to molecular weight[2]. In recent studies, MOSESSON et al.[3] demonstrated a number of structural differences between the high and lower molecular weight fibrinogens (LMWF). The findings were consistent with the notion that fibrinogen is progressively catabolized by plasmin into various intermediate clottable proteins prior to digestion to the unclottable fragments known as FDP. Similarly, SHERMAN et al.[2] concluded from findings in experimental animals that lower molecular weight fibrinogen was a catabolic derivative of high molecular weight fibrinogen.

The concept of in vivo degradation of fibrinogen by fibrinolytic enzymes was recently questioned by GALLIMORE et al.[4] who found that urokinase added to human plasma or infused into monkeys at doses sufficient to lyse clots failed to lyse fibrinogen. They concluded that fibrinogenolysis was an unlikely event and that FDP/fdp in the circulation are probably derived from fibrin as previously suggested by LIPINSKI et al.[5].

No data has been presented on the in vivo conversion of fibrinogen into its early catabolic intermediates (LMWF) in man. The purpose of the present study was to determine whether any alteration of fibrinogen occurs under conditions of activated fibrinolysis induced by venous occlusion which is known to result in the release of plasminogen activator from the vessel wall[6].

The subjects under investigation included 10 healthy individuals and one patient in whom ^{125}I-labelled fibrinogen (100 μCi) had been administered 15 min previously for the purpose of routine post operative peripheral venous scanning. A sphygmomanometer cuff was placed on the upper arm and inflated to a pressure midway between systolic and diastolic for a period of 20 min. Blood sampels were taken immediately before inflation of the cuff and again just before it was deflated. The following determinations were made on each sample: hematocrit, fibrinogen[7], and euglobulin clot lysis time[8]. Dilute clot lysis time was determined according to FEARNLEY[9] except that ammonium oxalate (9:1) was used. 2 ml of whole blood diluted with 0.85% NaCl (1:10) was clotted with 0.1 ml of a mixture of thrombin (50 U/ml) and $CaCl_2$ (0.025 *M*). FDP/fdp in serum were determined by the staphylococcal clumping test method[10]. Electrophoresis of plasma samples was carried out in 3.5% polyacrylamide gel in sodium dodecyl sulfate (SDS)[11]. After electrophoresis and staining with Coumassie Brilliant Blue R the gels were scanned using the Densicord Model 542A (Photovolt, New York). All the oxalate blood samples were diluted 1:10 with 0.85% NaCl and incubated for 24 h at 37 °C. The purpose of this dilution was to make the



Densitometric scans of the fibrinogen bands in polyacrylamide-SDS electrophoretic gels (3.5%) of plasma (solid line) in comparison with serum (broken line) obtained from a representative subject. A, before venous occlusion; B, after occlusion; C, post-occlusion sample 24 h after incubation at 37 °C. The percentage of lower molecular weight fibrinogen (LMWF) is indicated for each sample.

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Table I. Laboratory findings (mean and range) before and after occlusion in 10 subjects and in 1 patient given ^{125}I-labelled fibrinogen

	Dilute clot lysis time	Euglobulin clot lysis time (min)	Serum FDP/fdp (μg/ml)	Fibrinogen (mg/100 ml)	Thrombin time (sec)	Radioactivity in serum (cpm/ml)	Hematocrit (%)
Before occlusion	>24 h (10–48 h)	138 (93–260)	2	235 (160–290)	32 (22–34)	2510	44 (41–48)
After occlusion	146 min (60–300 min)	24 (15–62)	2	315 (200–390)	28 (20–29)	2620	53 (48–57)

Table II. Laboratory findings (mean and range) before and after incubation of the post-occlusion blood samples in 5 subjects and in 1 patient given ^{125}I-labelled fibrinogen

	Serum FDP/fdp (μg/ml)	Fibrinogen (mg/100 ml)	Thrombin time (sec)	Radioactivity in serum (cpm/ml)
Before incubation	<10	305 (210–375)	28 (19–33)	1900
After incubation	<10	310 (210–380)	27 (19–31)	1940

conditions comparable to those of the dilute clot lysis. The determinations listed in Table II and the electrophoretic studies were performed on plasma obtained from blood before and after incubation. In the samples containing ^{125}I-fibrinogen, the radioactivity was determined in the serum after clotting the plasma with thrombin.

The mean values and range for the laboratory determinations before and after venous occlusion are shown in Table I. Significant shortening of euglobulin lysis time and of the dilute whole blood clot lysis time occurred in each of the post-occlusion samples. These findings are in accordance with those previously reported[12]. However, the level of FDP/fdp in serum did not change, being 2 μg/ml, in all samples. Similarly, the radioactivity in the serum remained unchanged, confirming the fact that no unclottable fibrinogen derivatives were formed as a result of the fibrinolytic activity induced by venous occlusion. The fibrinogen concentration was increased following occlusion, being out of proportion to the degree of hemoconcentration as measured by the hematocrit, an observation previously made by NILSSON and ROBERTS[12].

The effect of incubation of plasma samples on these measurements is shown in Table II. No changes in fibrinogen, thrombin time, FDP/fdp or serum radioactivity occurred. These findings indicate that the fibrinolytic activity did not result in any degradation of fibrinogen into its unclottable derivatives.

It has been shown in our laboratory that electrophoresis of plasma in SDS-acrylamide gel (3.5%) results in separation of fibrinogen into 2 bands, the major portion being of higher molecular weight, the LMWF representing 25–35% of the total clottable protein[13]. The proportion of LMWF was not affected either by venous occlusion nor by incubation of the blood samples in vitro. The Figure shows the densitometric scans of gels after electrophoresis of plasma and serum from a single representative subject. The pattern before occlusion (A), after occlusion (B) and after incubation of the post-occlusion blood (C). No increase in the percent of LMWF to the clottable protein occurred, in fact a slight decrease was found. The mean percentage values of LMWF in plasma of all subjects before and after occlusion was 32% (range 31–34%) and 28% (range 26–30%) respectively. The mean percent of LMWF in plasma after incubation for 24 h at 37 °C was 27% of the total fibrinogen (range 19–31%). These findings indicate that the fibrinolytic activity did not result in any conversion of high into LMWF even after 24 h of incubation of the plasma rich in plasminogen activator. The heterogeneity of fibrinogen in man, represented by the presence of LMWF, does not seem to be the result of direct fibrinogenolysis and may rather be due to an indirect pathway of fibrinogen catabolism such as 'endocytosis' postulated by REGOECZI[14].

The difference in susceptibility of fibrinogen and fibrin to proteolytic degradation have been attributed to several factors. The binding of plasmin by antiplasmins, such as α_2-macroglobulin, was shown to inhibit the enzymatic activity with respect to fibrinogen but not fibrin degradation[15]. Secondly, the type of plasminogen activator appears to be a significant factor by which fibrinolysis rather than fibrinogenolysis occurs as demonstrated by CAMIOLO et al.[16] in a study comparing tissue activator with urokinase and streptokinase. By whatever mechanism, circulating fibrinogen appears to be effectively protected from proteolysis induced by naturally occurring plasminogen activators. It is therefore likely that FDP/fdp in serum are the products of fibrin breakdown as previously suggested[4, 5].

Zusammenfassung. Nachweis, dass nach Venenokklusion bei 10 gesunden Probanden erhöhte Fibrinolyse, nicht aber Fibrinogenolyse auftrat.

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The Protective Effect of L-DOPA on Heinz Body Formation in G6PD Deficient Red Cells

A number of chemicals including Acetylphenylhydrazine (APH) have been found to produce Heinz body formation in normal human red cells, and in erythrocytes of glucose-6-phosphate dehydrogenase (G6PD) deficient patients. The same compounds were shown to generate free hydrogen peroxide (H_2O_2) in red cells[1]. It is generally accepted that Heinz body formation involves the oxidative denaturation of hemoglobin mediated by H_2O_2[2]. On the other hand, the red cell enzymes, catalase and glutathione peroxidase, play a major role in protecting hemo-