

significant difference compared to fibrin formed by reptilase. The segment length of reptilase fibrin are reduced to 25% and the thickness of the fibers are reduced to 75% in comparison with thrombin fibrin.

The thickness of the fibrin meshwork obtained by thrombin and/or reptilase in the presence of dextran (Mw 40,000) is significantly diminished compared to saline controls ($p < 0.001$). Segment length and thickness of fibers in the clots with dextran showed different results when thrombin or reptilase were used as enzyme.

The investigation indicates that the fibrin meshwork formed by thrombin and/or reptilase in the presence or absence of dextran result in a significantly different fibrin morphology. The results are different to the so far descriptive electron microscopic studies.

A. Albert, M. R. Ayuso and P. Usobiaga (Instituto de Quimica Fisica "Rocasolano". C. S. I. C. Serrano, 119, Madrid, Spain): **Structural Studies of Fibrinogen by Ultrasound Irradiation.** (231)

Low frequency ultrasound irradiation of purified fibrinogen solutions produces a precipitation of protein and a loose on clottability due to molecular fragmentation, both depending in different ways on the irradiation dose. Precipitation seems to be closely related to free radical formation, the fragmentation being due mainly to the shearing forces appearing during cavitation.

The high molecular weight fragments obtained by ultrasound irradiation of fibrinogen have been isolated by gel filtration and studied by disc gel electrophoresis and analytical ultracentrifugation. They show molecular properties similar to those of the fragments obtained by endopeptidase incubation and the protein degradation proceeds in a similar way. All that can be interpreted according to a trinodular model for the fibrinogen molecule, with two elongated fibrous zones more easily cleaved by mechanical forces and less compact parts of the molecule that are mostly constituted by the α -chains.

A. Hurllet, C. De Beys, M. Moriau, A. Monsieur, E. Schulzen and R. Masure (Cliniques Universitaires St.-Pierre, B 3000 Louvain, Belgium): **Critical Analysis of Platelet Factor 4 (Antiheparin Activity) Assays.** (232)

Antiheparin activity is usually achieved by a heparin-thrombin time assay. Platelet free substrate plasma is adsorbed or not, calcium chloride used or not. Those technical conditions are analysed.

Results are expressed by the amount of heparin units neutralized in the assay. With non adsorbed plasma substrate, calcium chloride generates thrombin, increasing antiheparin like activity. Lower antiheparin like activity is also observed with BaSO_4 plasma substrate and with thrombin free of factor Xa. The amount of heparin units neutralized varies from 2.9 to 1.2 according to the system used.

Tested without calcium, with Xa free thrombin and BaSO_4 adsorbed substrate plasma, there exists a good relationship between the amount of heparin neutralized and various dilutions of serum, platelet rich plasma and platelet extract.

An assay based on anti-Xa effect of heparin, set up according to the heparin assay of Yin, is compared to the method described above.

L. Mester, B. Kraska, J. Crisba and M. Mester (Institut de Chimie des Substances Naturelles, 91190 - Gif s/Yvette, France): **Sugar-Amine Interactions in the Blood Clotting System and their Effects on Haemostasis.** (233)

N-Glycosides and 1-Desoxy-1-amino-2-keto-sugar derivatives (Amadori compounds) are formed from reducing sugars and amino groups of amino acids, proteins or simple amines in the first step of the Maillard reaction leading to the non enzymic browning of foodstuffs. Very little if any attention has been devoted to this reaction in the blood clotting system. Two examples are given to illustrate the possible role of this reaction in haemostasis.

Poly-L-lysine, often considered as a model for collagen, reacts easily with D-glucose or D-galactose to form Amadori compounds with the ϵ -amino group of poly-L-lysine (mol.

w. from 2,000 to 30,000). As a consequence, poly-L-lysine loses its ability to aggregate platelets. Serotonin reacts with D-glucose to form a stable and strongly reducing Amadori type compound. No change in the platelet aggregating ability of this compound has been observed, but other biological properties of the amine are highly affected.

R. Castillo, S. Maragall, J. A. Guisasola, F. Casals, C. Ruiz and A. Ordinas (Hospital Clínico y Provincial, Barcelona 11, Spain): **Inhibition of Human Platelet Aggregation by the Proteolytic Effect of Streptokinase (SK). Role of the Factor VIII Related Antigen.** (234)

Defective ADP-induced platelet aggregation has been observed in patients treated with streptokinase. This same effect appears "in vitro" when adding SK to platelet rich plasma (PRP). Classic hemophilia and normal platelet poor plasmas (PPP) treated with SK inhibit the aggregation of washed platelets; plasmin-treated normal human serum also shows an inhibitory effect on platelet aggregation. However, von Willebrand SK-treated plasmas do not inhibit the aggregation of washed platelets. The same results appear when plasmas are previously treated with a rabbit antibody to human factor VIII.

This confirms that the antiaggregating effect is mainly linked to the digested factor VIII related antigen.

The inhibition of ADP-induced platelet aggregation has been proved in gel filtration-isolated and washed platelets from SK-treated PRP.

Defective ristocetin-induced platelet aggregation has also been observed. This action does not appear in washed platelets from SK-treated PRP in presence of normal PPP, but it does in presence of SK-treated PPP, which suggests that the inhibition of the ristocetin-induced aggregation is due to the lack of factor VIII and not to the factor VIII-related products.

Heparin, either "in vivo" or "in vitro", has corrected the antiaggregating effect of SK.

J. L. Gordon, D. E. MacIntyre, A. H. Drummond (University Department of Pathology, Cambridge CB2 1QP, England): **Experimental Modification of the Platelet Release Reaction Induced by Collagen.** (235)

Collagen-induced release of platelet constituents can be divided into two kinetically-distinct phases, only one of which is associated with platelet aggregation, and the aggregation-independent release is less susceptible to inhibition by pharmacological agents (Drummond and Gordon, 1974). Minor variations in experimental conditions alter the release reaction profile. Collagen was incubated with platelet rich plasma (PRP) for one minute at 37°, under conditions in which no aggregation occurred (+ 3nM EDTA or in absence of stirring). The reaction was 'terminated' by addition of ice-cold EDTA-saline and samples were then centrifuged (14,800 g) under the conditions described in the table.

Time of Centrifugation	Centrifugation Temperature	Storage Time	Storage Temperature	Release of 5HT
30 s	4°	2 hours	4°	—
120 s	4°	2 hours	4°	—
30 s	20°	2 hours	4°	—
120 s	20°	2 hours	4°	+
30 s	4°	1 Min	20°	—
30 s	4°	5 Min	20°	+

No significant release was detected under any of the above conditions if saline were substituted for collagen suspension.

These results indicate that the storage temperature and time after centrifugation are as important as the centrifugation conditions themselves, and suggest reasons for discrepancies reported in previous studies of collagen induced release kinetics.

Drummond, A. H. and Gordon, J. L. (1974). *Brit. J. Pharmacol.* 52, 130 P.