ABSTRACTS OF CURRENT LITERATURE

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Editors

Isolation and some properties of thrombin-E and other prothrombin derivatives. WH Seegers, DH Walz, J Reuterby, LE McCoy. Department of Physiology, Wayne State University School of Medicine, Detroit, Mich. Thromb Res 4:829-860, 1974.

Prothrombin is a single-chain zymogen, of molecular weight 70,000, which must be systematically digested through at least two other nonenzymatic zymogen forms before the appearance of the proteolytic activity of thrombin. Autoprothrombin C or thrombin will remove the NH_2-terminal, carbohydrate-rich polypeptide referred to as the PR fragment. This fragment consists of 160 amino acids and has a molecular weight of 21,000. The new zymogen formed by the removal of the PR fragment is prethrombin (M.W. 50,000). The limited digestion of prothrombin by thrombin is based partly on the specificity of thrombin [Thromb Res 4:713-717, 1974]. Prothrombin can be converted to the active form of thrombin in a two-step reaction, only by Autoprothrombin C (Factor Xa). The first reaction removes a 109 amino acid NH_2-terminal polypeptide chain (0 Fragment) and its sequence has been reported [Thromb Res 4:885-890, 1974]. This produces the zymogen prethrombin-E, which is a single polypeptide chain, M.W. 38,000, and has an amino acid composition similar to that of thrombin. Prethrombin-E has only esterase activity. The second reaction results in the cleavage of a single peptide bond, generating thrombin (M.W. 38,000). Incubation of concentrated thrombin solutions at pH 8.2 resulted in the formation of two primary autolysis products, thrombin-E and the B1 portion of the B chain. Thrombin-E (M.W. 28,000) had only esterase activity and consisted of the intact A chain, bridged by a single disulfide bond to the B2 portion of the thrombin B chain. The B1 chain (M.W. 10,000) represents the first 77 amino acids of the B chain. The structural alignment of prothrombin is represented as follows: PR fragment + 0 fragment + A chain and B1 chain + B2 chain. The activities derived from prothrombin activation, in sequence, are as follows: esterase (prethrombin-E) →esterase + clotting (thrombin) →esterase (thrombin-E).–*GM*

Evidence for localization of polymerization sites in fibrinogen. BJ Kudryk, D Collen, KR Woods, B Blombäck. Department of Blood Coagulation Research, Karolinska Institutet, Stockholm, Sweden, and The New York Blood Center, New York, N.Y. J Biol Chem 249:3322-3325, 1974.

This report describes studies on the binding of fragment D (obtained by plasmin digestion of fibrinogen) to fibrinogen and derivatives thereof, conjugated to Sepharose and subsequently activated with thrombin or Reptilase. Three products were conjugated: (1) intact fibrinogen; (2) N-DSK—this is the NH_2-terminal CNBr dimeric fragment of fibrinogen (Aα 1-51; Bβ 1-115; $\gamma 1\text{-}78)_2$; and (3) E-knot—this is the NH_2-terminal CNBr dimeric portion of plasmic fragment E (Aα 1-51; Bβ 54-115; $\gamma 1\text{-}58)_2$. The results obtained indicate that: (1) none of the conjugates bind fragment D unless "activated" by either thrombin or Reptilase; (2) with the exception of activated conjugated E-knot, all of the activated conjugates bind fragment D, the strongest affinity being for the high molecular weight subspecies of fragment D; (3) intact fibrinogen also binds to the activated conjugates.

Using these findings, the authors propose that in the formation of fibrin, two binding domains are involved. One of these sites is in the fragment D structure and is apparently exposed in intact fibrinogen. The second domain is in the N-terminal portion of fibrinogen (N-DSK), and certain structures in this area are of particular importance for binding and/or activation. This second site(s) is apparently masked in fibrinogen but becomes readily available for binding following removal of fibrinopeptides. It is further suggested that in Fibrinogen Detroit, where a mutation has occurred in the N-terminal area of the Aα chain (Aα 19 ARG→SER), polymerization is inhibited by improper binding or lack of exposure of binding site(s).—*GM*

Preparation of heparin-linked Agarose and its interaction with plasma. I Danishefsky, F Tzeng. Department of Biochemistry, New York Medical College, Valhalla, N.Y. Thromb Res 4:237-246, 1974.

Basing their work on findings that uronic acid groups of heparin can be linked to amines, the authors have successfully covalently linked heparin to amino ethyl Agarose in the presence of carbodiimide. The procedure yields an adsorbant containing 130 μg of heparin/ml of packed Agarose—a substantial improvement over previous coupling methods (3 μg heparin/ml of adsorbant). Results of studies on plasma and serum interacted with heparin-linked Agarose indicate that coagulation Factors IX and XI "activities" are bound by the adsorbant and can be eluted by high salt buffers with a 22- and 15-fold respective purification over plasma. Assays for heparin cofactor activity indicated that this component is also bound to the matrix but not as efficiently (50%) as Factor IX. Selective fractionation procedures, coupled with the use of this adsorbant, should prove useful in the purification of Factors IX, XI, and heparin cofactor.—*GM*

Identification of two distinct heparin cofactors in human plasma: II. Inhibition of thrombin and activated Factor X. GF Briginshaw, JN Shanberge. Hemostasis and Research Laboratories, Department of Pathology, Mount Sinai Medical Center, Milwaukee, Wis. Thromb Res 4:463-477, 1974.

The kinetic data presented in this report provide evidence for the existence of two separate heparin cofactors: A and B. [Method of purification: Arch Biochem

Biophys 161:683, 1974.] The authors conclude that: (a) Heparin by itself can neutralize up to 40% of the active thrombin–a phenomenon reversible by polybrene or protamine–and that this must be taken into consideration when examining the system for "reversibility." They express the view that no true reversibility of cofactor-heparin inactivation of thrombin occurs, since this lost thrombin cannot be recovered by neutralizing with protamine. (b) Cofactor A requires heparin for its progressive antithrombin activity, and has no inhibitory influence on Factor Xa, with or without heparin. (c) Cofactor B has a two fold function: By itself it has a marked antithrombin activity, and up to a certain heparin concentration, the rate of thrombin inactivation can be further accelerated. Furthermore, in the absence of heparin, cofactor B will progressively, but slowly, inhibit Factor Xa activity. With heparin, cofactor B immediately neutralizes Factor Xa activity.–*GM*

Preparation of plasminogen activator from vascular trees of human cadavers: Its comparison with urokinase. N Aoki. Department of Medicine and Biochemistry, Toho University School of Medicine, Tokyo. Japan J Biochem 75:731-741, 1974.

A method of preparation and some chemical and biologic properties of a heretofore uncharacterized activator of plasminogen are reported. This protein, extracted from the *in situ* vascular trees of human cadavers, possesses no proteolytic activity on fibrin, casein or hemoglobin, but readily activates plasminogen to plasmin. Similar to urokinase, the Km value with plasminogen as substrate is 1.5×10^{-6} M, the pH optimum for its activity is about 8.5, and its activity is not effected by PCMB but destroyed by DTT. Distinguishing it from urokinase (M.W. 55,000), the molecular weight of this vascular activator is estimated at 80,000; its Km value with the synthetic substrate acetylglycyllysine methyl ester (AGLMe) is 5.7×10^{-3} M (for urokinase it is 5.9×10^{-4} M); it is stabilized by high salt concentrations–not a quality of urokinase; antibodies to human urokinase do not inhibit the activity of vascular activator. It is suggested that this plasminogen activator and urokinase are perhaps functionally similar but chemically not identical enzymes.–*GM*

A new approach in the treatment of peripheral arterial occlusions: Defibrination with Arvin. H Ehringer, R Dudczak, K Lechner. Division of Angiology of the First Medical Department, University of Vienna, Austria. Angiology 25:279-289, 1974.

A series of patients suffering from peripheral arterial occlusions were therapeutically defibrinated with the purified fraction of the venom of the Malayan pit viper (*Agkistrodon rhodostoma*)–Arvin. Hemorheologic and hemodynamic effects are reported. The data indicate that within 6 hours after the initial Arvin infusion (134 units) the blood viscosity was reduced by 20% and the fibrinogen concentration by 96%. Administration of Arvin (134 units/12 hours) for 10 days resulted in a concomitant decrease in hematocrit, decreasing blood viscosity by an additional 15%. Ten days after discontinuing treatment, viscosity was about 85%, fibrinogen about 70%, and hematocrit about 90% of pretreatment values. These

values, when compared to those obtained by treating with streptokinase (SK), indicate that Arvin induced a more pronounced and more sustained effect than SK. Also, no change in the general systemic systolic pressure, and a slight increase in distal perfusion pressure, probably due to improved collateral circulation, was observed. A slight increase in the rest flow, especially of the calf, and also of the foot in those patients without rest pain, and in 10 out of 13 extremities, the disappearance of rest pain was reported.

The authors recommend the therapeutic defibrination with Arvin in patients with ischemic rest pain. Also, they have evidence indicating that Arvin, although more dangerous with respect to bleeding complications, is superior to heparin in the changeover phase from SK to phenprocoumon, in thrombolytic therapy.—*GM*

Hemophilia A in a female: Use of Factor VIII antigen levels as a diagnostic aid. EE Czapek, LW Hoyer, AD Schwartz. Department of Pediatrics, Northwestern University, Chicago, Ill., Department of Medicine, University of Connecticut, Farmington, Conn., and Veterans Administration Hospital, Newington, Conn. J Pediat 84:485-489, 1974.

This is a case report of an eight month old female infant with severe AHF deficiency and a negative family history of bleeding. Diagnosis was established by determination of AHF procoagulant activity and AHF antigen activity. Chromosome analysis and buccal smear confirmed the infant as indeed female. Clotting studies on the propositus and her parents demonstrated AHF activity of less than 1% for the patient, 35% for the mother and 105% for the father. No inhibitor of AHF activity was demonstrated. The patient and both parents had normal bleeding times. Platelet adhesiveness and aggregation were normal in the mother. Both the propositus and mother had normal levels of Factor V activity. Basing their conclusions on the near normal AHF antigen levels (81% for the patient, 88% for the mother, 109% for the father) and the AHF activity levels, the authors established the father to be normal, the mother to be a carrier of hemophilia A, and that the infant synthesized an abnormal AHF molecule, resulting in hemophilia A. It is proposed that this rare occurrence of hemophilia A in a genotypic female is a consequence of inactivation of the paternal X chromosome during cell division in early embryonic life, resulting in somatic cells containing an active maternal chromosome carrying a gene for hemophilia.—*GM*

Surgical hemostasis in a hemophiliac using blockade of the inhibitor system. BH MacPherson, NE Drayton, W Reid. Department of Pathology, St. Joseph Mercy Hospital, Pontiac, Mich. Amer Surg 40:224-228, 1974.

This is a case report of a 62 year old male hemophiliac (Factor VIII titer was 4.8% of normal) who underwent excision of 12 polypoid, intradermal nevi of the face and neck, without need of transfusion and without complications. Prior to surgery, the patient was pretreated for two days with EACA (AMICAR®), orally, 5 gm every six hours, and the therapy continued through the seventh postoperative

day. No delayed bleeding and no thromboembolic phenomena were noted postoperatively. The authors theorize that treatment of a hemophiliac with EACA, an inhibitor of plasminogen activation, establishes a delicate balance between coagulation and inhibitor systems by blocking the dual action of phosphatidylserine. They have evidence that this compound, normally released from platelets during viscous metamorphosis, exerts an antithromboplastic action, and promotes the activation of the fibrinolytic system.–*GM*

Factor VIII-related antigen in platelets. MA Howard, DC Montgomery, RM Hardisty. Department of Haematology, Institute of Child Health, Hospital for Sick Children, Great Ormond Street, London, England. Thromb Res 4:617-624, 1974.

Factor VIII-related antigen (FVIII-RAg) was found to be closely bound to membranes of platelets, and was not removed by repeated washings. Quantitative immunoelectrophoresis against rabbit antihuman Factor VIII antibody was used to detect FVIII-RAg. Normal human platelets were found to contain FVIII-RAg in amounts equal to 5 to 15% of that in whole platelet-rich plasma. FVIII-RAg was not found on platelets of a patient with von Willebrand's disease, and was markedly reduced on platelets from a thromboasthenia patient. It was found on platelets from a patient with hemophilia, and was increased on platelets from patients with Bernard and Soulier giant platelet syndrome. Interchange between plasma and platelet FVIII-RAg was not found in normals. The role of platelet FVIII-RAg in hemostasis is not clear. It does not appear to be required for ristocetin-induced platelet aggregation, whereas plasma FVIII-RAg is normally required.–*RTW*

Endothelial injury and platelet aggregation associated with acute lipid mobilization. RD Maca, JC Hoak. Blood Coagulation Research Laboratory, Division of Hematology-Oncology, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa. Lab Invest 30:589-595, 1974.

Marked increases in plasma free fatty acid (FFA) concentration were induced in rabbits by subcutaneous adrenocorticotropic hormone (ACTH) injection. At least a four-fold increase in FFA was seen in each animal compared to control. ACTH increased significantly the number of circulating small and medium platelet aggregates, as compared to control, without causing internal platelet changes. This effect was not prevented by heparin. Electron microscopic examination of thoracic aortas revealed mitochondrial swelling and marked cytoplasmic vacuolation of endothelium in the majority of ACTH-treated rabbits. Cells resembling damaged endothelial cells were found in the aortic lumen of ACTH-treated rabbits. Some detachment of endothelium occurred in some rabbits with ACTH, with subsequent platelet attachment to subendothelium. The effects seen may represent the effect of increased plasma free fatty acid concentration. The authors suggest that this mechanism may have significant clinical importance, since several diseases associated with high FFA levels have an increased incidence of thrombosis.–*RTW*

Platelet secretion induced by divalent cation ionophores. RD Feinman, TC Detwiler. Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, N. Y. Nature 249:171-172, 1974.

Secretion of ATP from human washed platelets was induced by the divalent cation ionophores X-537A (Lasalocid, Hoffman-LaRoche) and A23187 (Lilly). The time course of ATP release by these antibiotics was nearly identical to thrombin-induced release. After ionophore-induced release, no further ATP was released by thrombin, and vice versa. These ionophores caused release of calcium and most likely act by disrupting divalent cation gradients. On a concentration basis, A23187 is a more effective calcium ionophore than is X-537A. The authors suggest that these ionophores directly release calcium from intracellular stores and that this internal calcium flux triggers secretion by platelets. *RTW*

Calcium-containing platelet granules. JH Martin, FL Carson, GJ Race. Department of Pathology, Baylor University Medical Center, Dallas, Texas. J Cell Biol 60:775-777, 1974.

Calcium was shown to be present in the dense 5-hydroxytryptamine (5-HT) granules of human blood platelets. By use of electron microscopy, microincineration, and X-ray spectroscopy, considerably more calcium was found to be present in the 5-HT granules than in other areas of the platelet cytoplasm. Although the functional significance of the calcium and its association with 5-HT in the granules is not known, the authors suggest it may act to increase the storage of 5-HT. -*RTW*

Phagocytosis of antibody-coated platelets by human granulocytes. RI Handin, TP Stossel. Department of Medicine, Division of Hematology, Peter Bent Brigham Hospital, The Division of Hematology, Children's Hospital Medical Center and the Departments of Medicine and Pediatrics, Harvard Medical School, Boston, Mass. New Eng J Med 290:989-993, 1974.

Using microscopy, ^{51}Cr-uptake, and nitroblue tetrazolium dye (NBT) reduction, the authors demonstrated human platelet phagocytosis by autologous granulocytes after pretreatment of platelets with serum containing antiplatelet antibodies. Phagocytosis and NBT reduction were increased by serum from 14 patients with idiopathic thrombocytopenic purpura (ITP), 13 patients that were refractory to platelet transfusion, and serum from rabbits injected with washed human platelets. NBT reduction was the most convenient means of assaying the rate of sensitized platelet ingestion. Calcium and magnesium were required for uptake of ^{51}Cr-labeled platelets and increased NBT reduction. The antiplatelet antibodies act as opsonins to promote phagocytosis. Observations suggest that platelets interact, in part, with F_c dependent antibody recognition sites on the granulocyte. The opsonic activity was present in IgG from positive serums and was resistant to 56°C heat treatment and barium sulfate adsorption. The serum opsonic activity persisted in ITP patients after treatment with steroids or splenectomy.

Although granulocytes have been shown here to ingest opsonized platelets *in vitro*, the participation of granulocytes in immune platelet clearance *in vivo* is not clear.–*RTW*

Role of platelets in pathogenesis of diabetic retinopathy. JG Dobbie, HC Kwaan, J Calwell, N Suwanwela. Departments of Ophthalmology and Medicine, Northwestern University Medical School and Veteran's Administration Research Hospital, Chicago, Ill. Arch Ophthalmol 91:107-109, 1974.

Eighty-seven males with diabetes mellitus which developed during maturity were compared with 62 normals by measuring mean platelet aggregation index (% change in optical transmission 4 minutes post-ADP addition). Those patients with retinopathy showed a 39.7%±2.8 S.D. increase in aggregation index over controls. This increased platelet aggregation may lead to development of ischemic retinal lesions. These patients showed platelet aggregation-enhancing activity in their plasma. This activity increased progressively with the severity of retinopathy. Two grams of aspirin/day blocked the platelet aggregation-enhancing activity of diabetic plasma nonspecifically, but did not affect the plasma level. Since the patients were mainly middle-aged men, the study is not representative of all diabetics.–*RTW*

Platelet factor 3 activity and platelet aggregation in patients submitted to coronarography. S Renaud, P Gautheron, R Arbogast, E Dumont. Research Department, Department of Medicine, Montreal Heart Institute and Department of Pathology, University of Montreal, Canada. Scand J Haemat 12:85-92, 1974.

Thirty-seven patients submitted to coronarography because of severe angina pectoris were compared with 19 controls having no risk factors for coronary heart disease, for platelet factor 3 (PF-3) activity and platelet aggregation. Plasma triglycerides and cholesterol levels were slightly higher in patients than controls, but were significant only when severe lesions were present. Platelet aggregation was increased slightly only in patients with severe lesions and only when thrombin-induced. A high percentage of patients did not present significant coronary lesions as visualized by coronarography. These patients and all others showed significantly increased PF-3 activity and *in vitro* hypercoagulability. The increased PF-3 activity appeared to be related to increased clotting activity of platelet phospholipids.–*RTW*

Kinetic evaluation of haemostasis during surgery and wound healing. SJ Slichter, DD Funk, LE Leandoer, LA Harker. Departments of Medicine and Surgery, School of Medicine, University of Washington, and The King County Central Blood Bank, Seattle, Wash. Brit J Haemat 27:115-125, 1974.

Platelet and fibrinogen kinetics were measured in 21 patients after uncomplicated elective surgical procedures. Patients were studied to the fifth day

post-surgery and in some cases to the twelfth postoperative day. Platelet counts remained constant except in a few cases, but all patients showed a progressive rise in fibrinogen concentration during the initial postoperative period. Both platelets and fibrinogen had similar increases in turnover ratio during the first postoperative day, which was due to a reciprocal decrease in their survival. The amount of platelets and fibrinogen consumed appeared to be proportional to the degree of tissue injury caused by operation. The fact that platelet and fibrinogen levels remained essentially unaltered during the postoperative period, while the demand for these factors was increased, suggests that storage pools of these factors are readily mobilized. Accumulation of radio-labeled platelets and fibrinogen at the incision site suggests their involvement in wound healing.–*RTW*

Purification of Factor VII from bovine plasma, reaction with tissue factor and activation of Factor X. J. Jesty, Y. Nemerson. Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn. J Biol Chem 249:509-515, 1974.

Factor VII, purified 330,000-fold from bovine plasma, has been shown to be a trace protein (150 μg/l). This protein, whether in its native form or in complex with tissue factor, is sensitive to the irreversible inhibitors DFP and PMSF. Coagulant activity (activation of Factor X to Xa) resides in the "complex," but the "active site" resides in the Factor VII moiety. The formation of the active complex does not appear to result in a reduction of the molecular size of Factor VII. The authors conclude that Factor VII most likely circulates as an active enzyme that has an absolute requirement for a specific lipoprotein (tissue factor) that serves to orient Factors VII and Factor X to facilitate proteolysis of Factor X. The authors have further noted that the proteolysis is restricted to the heavy chain of Factor .–*G.M.*

FOR THE SCHOLAR'S BOOKSHELF

The Biological Role of the Clot-Stabilizing Enzymes: Transglutaminase and Factor XIII. K. Laki (Ed.) Ann NY Acad Sci 202:1-348, 1972.

This publication consists of a series of articles elucidating aspects of synthesis, mode of action, and structural properties of the fibrin crosslinking enzyme and its precursor, both in physiologic and pathologic states. Of particular interest are the comparative studies on transglutaminase(s) from various tissues, and the documentation of its relationships to physiologic inhibitors, hereditary deficiencies, tumor growth, fibrinolysis, transfusion therapy, and oral contraception.

An abundance of molecular models appears throughout the text and will serve as an excellent source for orienting the reader in understanding the mechanics of fibrin stabilization.–*GM*

Thrombosis: Mechanisms and Control. KM Brinkhous, DM Stengle, S Hinnom, S Sherry, JM Stengle (Eds). Thromb Diathes Haemorrh Suppl 54:1-464, 1973.

The current status of work in the field of thrombosis is summarized in this series of reports. Emphasis is on basic control mechanisms of hemostasis, molecular aspects of fibrinogen, antihemophilic factor, and plasminogen, pathophysiology of thrombosis, and the application of various therapeutic agents in the control of thrombosis.

This volume also contains the summary reports of the activities of the International Committee on Haemostasis and Thrombosis defining basic methods, nomenclature and standards.–*GM*