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HORSESHOE CRAB COAGULOGEN: ITS STRUCTURE AND GELATION MECHANISM. S. Iwanaga, S. Nakamura, T. Takagi, T. Morita, J.S. Finlayson, K. Takahashi, and M. Niwa. Institute for Protein Research, Osaka Univ., Osaka, Primate Research Institute, Kyoto Univ., Inuyama, and Osaka City Univ. Med. School, Osaka, Japan

A clottable protein, isolated from the amoebocyte lysate of horseshoe crab (Tachypleus tridentatus) was a basic protein with a molecular weight of approximately 17,000. It contained a total of 155 (± 5) amino acid residues and had a single NH_2-terminal alanine and COOH-terminal phenylalanine. Upon gelation of the coagulogen by an endotoxin-activated clotting enzyme(s), a large peptide, named peptide C, which has 28 amino acid residues, was released, and the resulting gel protein consisted of A and B chains, bridged by disulfide linkages. The chemical analyses on these polypeptide segments indicated that the enzymatic formation of gel involves limited proteolysis of the Arg-46-Gly-47 and Arg-18-Thr-19 bonds located in the N-terminal portion of coagulogen, liberating peptide C. Moreover, the amino acid sequence studies on peptide C and A chain provided that their COOH-terminal octapeptide sequences have a remarkable homology with primate fibrinopeptide B. Up to now, the following NH_2-terminal sequence of whole coagulogen was established: NH_2-Ala-Asp-Thr-Asn-Ala-Pro-Ile-Cys-Leu-Cys-Asp-Glu-Pro-Gly-Val-Leu-Gly-Arg-Thr-Gln-Ile-Val-Thr-Thr-Glu-Ile-Lys-Asp-Lys-Ile-Glu-Lys-Ala-Val-Glu-Ala-Val-Ala-Gln-Glu-Ser-Gly-Val-Ser-Gly-Arg-Gly-Phe-Ser-Ile-Phe-Thr-His-Arg-Pro-Val-Phe-Arg-Glu-Cys-Gly-Lys-Tyr-Glu-Cys-Arg-Thr-Val-His-Arg-Pro-Glu-Ser-Arg-Cys-Tyr-Asn-Phe-Pro-Pro-Phe-Thr-His-Phe-Lys------------Phe-COOH.

A NEW PROCEDURE FOR PURIFICATION OF CROTALASE FROM CROTALUS ADAMANTEUS VENOM. F.S. Markland, J. Chou, Y. Shih. University of Southern California, School of Medicine, Los Angeles, California, and H. Pirkle. University of California, College of Medicine, Irvine, California, U.S.A.

A new procedure has been developed for large scale, rapid purification of crotalase, the thrombin-like enzyme from the venom of the eastern diamondback rattlesnake (Crotalus adamanteus). The three step procedure involves: (1) molecular sieve chromatography on Sephadex G-100 in 0.04 M Tris buffer containing 0.10 M sodium chloride, pH 7.1; (2) gradient elution from DEAE-cellulose with sodium acetate buffer, pH 7.0; and (3) affinity chromatography on p-aminobenzamidine Sepharose using a spacer of 6-aminohexanoic acid. Crotalase was eluted from the affinity resin by 0.05 M Tris buffer containing 0.10 M sodium chloride and 0.15 M benzamidine-hydrochloride, pH 9.0, after first washing with the Tris buffer containing 0.40 M sodium chloride. From the crude venom, pure enzyme was obtained with an overall recovery of 40-60% of clotting activity and a 90-100 fold increase in specific activity. Crotalase was shown to be pure by polyacrylamide disk gel electrophoresis which gave one band. The molecular weight was estimated to be approximately 31,000 by gel filtration on a calibrated Sephadex G-100 column. Amino acid analysis was performed and the composition was shown to be very similar to that reported earlier (F.S. Markland and P.S. Damus, J. Biol. Chem. 246: 6460, 1971). Clotting activity of the enzyme was not inhibited by heparin, either with or without plasma, whereas, thrombin was rapidly inactivated by heparin in the presence of plasma. In conclusion, we have developed a rapid and reproducible procedure for isolation in high yield of large quantities of the thrombin-like enzyme from the venom of the eastern diamondback rattlesnake. Studies are continuing on the primary structure and possible clinical applications of this enzyme.

VASOCONSTRICTOR AND VASODILATOR AGENTS IN DISSEMINATED INTRAVASCULAR COAGULATION. A.N.Whitaker, I.H. Bunce and B. Sleigh. Department of Medicine, University of Queensland, Brisbane, Australia

Modification of disseminated intravascular coagulation by vasculoactive agents has been evaluated in rabbits infused with thrombin, measuring the extent of renal capillary thrombosis, suppression of creatinine clearance and elevation of plasma creatinine and pl. haemoglobin. Control studies included the administration of each agent without thrombin. The addition of angiotensin (0.1 µg/kg/min) consistently increased the extent of thrombosis and enhanced the other sequelae of thrombin infusion. Angiotensin was more potent than either norepinephrine or epinephrine (1 µg/kg/min). Intravenously, 5-hydroxytryptamine (1-10 µg/kg/min) decreased the severity of renal involvement and thrombosis. In contrast, if given intra-aortically 5-HT (1 µg per kg/min) enhanced all sequelae, being comparable to angiotensin. Propranolol, to blockade β-adrenoreceptors, did not significantly modify the response to thrombin, whether given alone or in addition to epinephrine. Blockade of α-adrenoreceptors with phenoxybenzamine variably decreased both renal lesions and haemolysis produced by thrombin, and prevented the enhancement of these by epinephrine. Phenoxybenzamine blockade could be reversed by angiotensin. The addition of vasculoactive agents to thrombin only slightly influenced reductions in platelets and fibrinogen. While statistically significant, these small differences could not explain the differences in the pattern of thrombosis. α-adrenoreceptors in this model may be less critical than in the generalized Shwartzman reaction, but chemical mediators in general may be important in the expression of disseminated intravascular coagulation and thrombus localization.