Time

09.15 **0641** THE PRIMARY STRUCTURE OF HORSESHOE CRAB (Tachypleus tridentatus) COAGULOGEN AND ITS HOMOLOGIES WITH PLATELET FACTOR 4

T. Takagi[1], S. Nakamura[2]*, Y. Hokama[3], T. Miyata[3], M. Niwa[4], and S. Iwanaga[3], [1]Biological Institute, Tohoku Univ. [2]Primate Research Institute, Kyoto Univ. [3]Dept. of Biolo[illegible], Faculty of Science, Kyushu Univ. [4]Osaka City Univ. Med. School, Japan.

Horseshoe crab coagulogen consists of a single basic polypeptide chain having the total 175 amino acid residues. Upon gelation of this protein, 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 two disulfide linkages. The sequence studies on peptide C, A chain and B chain provided that their sequences have a significant homology partly with primate fibrinopeptide B and largely with human platelet factor 4. The facts suggest that these are derived from a common ancester or that the coagulogen is a prototype of platelet factor 4. The whole sequence of coagulogen was as follows: 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-Ser-His-His-Pro-Val-Phe-Arg-Glu-Cys-Gly-Lys-Tyr-Glu-Cys-Arg-Thr-Val-Arg-Pro-Glu-His-Ser-Arg-Cys-Tyr-Asn-Phe-Pro-Pro-Phe-Thr-His-Phe-Lys-Leu-Glu-Cys-Pro-Val-Ser-Thr-Arg-Asp-Cys-Glu-Pro-Val-Phe-Gly-Tyr-Thr-Val-Ala-Gly-Glu-Phe-Arg-Val-Ile-Val-Gln-Ala-Pro-Arg-Ala-Gly-Phe-Arg-Gln-Cys-Val-Trp-Gln-His-Lys-Cys-Arg-Phe-Gly-Ser-Asn-Ser-Cys-Gly-Tyr-Asn-Gly-Arg-Cys-Thr-Gln-Gln-Arg-Ser-Val-Val-Arg-Leu-Val-Thr-Tyr-Asn-Leu-Clu-Lys-Asp-Gly-Phe-Leu-Cys-Glu-Ser-Phe-Arg-Thr-Cys-Cys-Gly-Cys-Pro-Cys-Arg-Ser-Phe.

09.30 **0642** SYNTHESIS OF AN ARGININE ANALOG OF THE FRAGMENT 58-68 OF HUMAN PLATELET FACTOR 4 HAVING SIGNIFICANT ANTIHEPARIN ACTIVITY

S.Bajusz*, I.Fauszt, É.Barabás and D.Bagdy, Institute for Drug Research, Budapest, Hungary

Of the 70-residue polypeptide human platelet factor 4, which binds heparin stoichiometrically, the C-terminal fragment 50-70 /Tc-3/ showed a reduction of the heparin induced prolongation of thrombin time /Deuel et al., Proc.Natl.Acad.Sci.USA., 74:2256, 1977/.
The arginine analog of the fragment 58-68 of Tc-3, which comprises the unique lysine rich region /Lys-Lys-Ile-Ile-Lys-Lys/, was prepared: Pro-Leu-Tyr-Arg-Arg-Ile-Ile-Arg-Arg-Leu-Leu-NH_2. The aim of replacing of lysine residues of the native molecule by arginine was to increase the interactions between the basic side chains of the peptide and SO_3^- and/or COO^- groups of heparin by introducing additional H-bridges and strengthening the ionic bonds. The new undecapeptide was synthesized by the standard solid phase method using Boc amino acids and dicyclohexylcarbodiimide condensation.
1 nmole /1.8/ug/ of synthetic peptide completely inhibits the action of 0.3 units of heparin which corresponds a heparin/peptide molecular ration of about 1:6.

09.45 **0643** PURIFICATION AND PROPERTIES OF A LOW MOLECULAR WEIGHT PROTEIN FROM HUMAN PLATELETS AND ITS RELATIONSHIP TO BETA-THROMBOGLOBULIN

Daniel A. Walz* and Stefan Niewiarowski, Wayne State University, Detroit, Michigan and Temple University, Philadelphia, Pennsylvania, U.S.A.

Activated human platelets secrete specific intracellular proteins which have anti-heparin activity. These proteins include platelet factor 4 (PF-4), beta-thromboglobulin (β-TG), low-affinity platelet factor 4 (LA-PF-4), and platelet growth factor. Outdated platelet concentrates were initially heat-treated for 10 minutes at 70°C, centrifuged, and the supernatant fractionated on Sephadex C-50. The fraction eluted with 0.5M NaCl contained the majority of the anti-heparin activity. Subsequent gel filtration resulted in a final product which was homogeneous on SDS electrophoresis (9,000 daltons). Amino terminal analysis of this fraction gave the sequence: Asn-Leu-Ala-Lys-Gly-Lys-Glu-Glu-Ser-. Residues 5-21 were identical to residues 1-16 of β-TG. This protein shared common antigenic determinants with β-TG in a radioimmunoassay. Isoelectric focusing of this material resulted in the majority of protein recovered at an isoelectric point of 8.0 (β-TG = 7.0). This protein fraction possessed four times the specific molar activity of insulin in stimulating the mitogenic expression of Balb 3T3 cells; however, additional protein fractions from the gel filtration procedure were far more potent, though less homogeneous. This protein, therefore, appears identical to LA-PF-4, and is apparently the precursor of β-TG; it is not the most potent of platelet growth factors. (Support: MI Heart; NIH 14217).