

DEMONSTRATION OF TRANSGLUTAMINASE ACTIVITY IN LIMULUS LYSATE, S.I. Chung, R.C. Seid, Jr.\* and T.-Y. Liu\*, National Institute of Dental Research and Bureau of Biologics, F.D.A.\*

When purified coagulogen (Mol. wt. 24,500) is acted upon by the Limulus clotting enzyme (Tai, J.Y., and Liu, T.-Y., (1977) *J. Biol. Chem.* in press), a soluble C-peptide (Mol. wt. 6,000) and an insoluble coagulin (Mol. wt. 20,000) are formed. The latter interacts in a non-covalent fashion to form the soft-clot (Tai, J.Y. *et al.*, (1977) *J. Biol. Chem.* in press). The possibility of stabilizing the coagulin by a cross-linking process similar to that in the mammalian system has been examined. Transglutaminase activity was demonstrated in the freshly prepared Limulus lysate by its ability to incorporate primary amines into peptide- and protein-bound glutamine residues of B-chain of oxidized insulin, casein and limulus coagulin. The enzyme was activated by  $\text{Ca}^{2+}$ ,  $\gg$  by  $\text{Mn}^{2+}$  or  $\text{Sr}^{2+}$  and totally inhibited by sulphydryl reagents such as iodoacetamide, mercury benzoate and S-methyl-methyl sulfonate, but not by inhibitors to serine proteinases. Dansylcadaverine was incorporated into several proteins with differing molecular weights during the gelation of the whole lysate. Part of the clots formed were insoluble in 30% acetic acid. A high concentration of amines inhibited the formation of insoluble clot. Examination of the insoluble clot by SDS-gel electrophoresis, as well as by exclusion chromatography in 6M guanidine after reduction and carboxymethylation revealed the presence of high molecular weight polypeptide materials (>200,000). These results are strongly indicative of transglutaminase catalyzed covalent cross-linking of polypeptide chains during clot formation.

SPECIES DIFFERENCES IN PLATELET GLYCOPROTEINS. A. T. Nurden, Hopital Lariboisiere, Paris, France, and the Nuffield Institute of Comparative Medicine, London, England.

Reports of considerable species differences in the mammalian erythrocyte membrane glycoprotein composition led us to conduct a preliminary investigation of the platelet membrane glycoprotein composition in a range of mammals (15 species). Electrophoretic studies of detergent solubilized membranes and protease released acidic glycopeptides suggest considerable species differences in the extent of glycosylation of surface orientated proteins, with differences observed even between closely related species of primates. The failure to locate the large PAS-staining GP I band following SDS-polyacrylamide gel electrophoresis of cat platelet membranes, even though cat platelets have a comparable sialic acid content (21.3 $\pm$ 2.3  $\mu\text{g}$  per  $10^9$  platelets) to human platelets, may have implications concerning the proposed roles for this glycoprotein in platelet function. It is suggested that account should be taken of possible species differences in platelet surface structure during the interpretation of platelet function tests using animal models.