A. H. Drummond and J. L. Gordon (University Department of Pathology, Cambridge, CB2 1QP, England): **Receptors for 5-Hydroxytryptamine on Rat Blood Platelets. (287)**

When 5-hydroxytryptamine (5HT) or its analogue, 5-methoxy-α-methyltryptamine (5MOαMT) are added to rat citrated platelet-rich plasma (PRP), the platelets change in shape but do not aggregate. The response to both of these agents is inhibited by the 5HT antagonist, cinanserin ($IC_{50} = 3 \times 10^{-9}$ M). Cinanserin is at least 10,000 times less potent against the active uptake of 5HT ($IC_{50} > 5 \times 10^{-5}$ M). 5MOαMT is not actively transported by the platelet, although some instantaneous binding can be measured which is independent of temperature (4°–37°). 5MOαMT does not inhibit 5HT uptake over the concentration range at which it induces the shape change (10^{-8}–10^{-5} M). Binding of (^{3}H)-5HT to rat platelets at 4° indicates the presence of three binding sites, one of which is specifically blocked by cinanserin ($IC_{50} = 2.8 \times 10^{-9}$ M). Close correlation between the inhibitory potency of various drugs against (^{3}H)-5HT binding and 5HT-induced shape change suggests that this site is the 5HT receptor on the platelet which initiates the shape change. Our results indicate that 5HT induces the platelet shape change by combination with a specific cinanserinsensitive 5HT receptor, which is unconnected with the uptake site.

A. Hawiger, J. Hawiger, S. Timmons, S. Steckley and C. Cheng (Vanderbilt University School of Medicine and VA Hospital, Nashville TN., U.S.A.): **The Role of Membrane Receptors in Pathways of Human Platelet Activation by Endotoxin and other Microbial Products. (288)**

Endotoxin-producing gram-negative rods cause thrombocytopenia and contribute to shock and death of 25% of bacteremic patients. Cellular pathways of clot-promoting activity of endotoxin compared with other microbial products were studied. Membrane receptor for endotoxin was demonstrated using human platelets as target cells separated from plasma proteins by albumin gradient-Sepharose 2B gel filtration. Endotoxin receptor was distinct from thrombin-sensitive protein and from IgG Fc receptor. It was resistant to neuraminidase and trypsin. It was related to membrane hydrophobic region(s) as demonstrated with fluorescent probe. Endotoxin-receptor interaction triggered unmasking of platelet procoagulant (Platelet Factor 3) and selective release of ^{3}H-serotonin. A similar receptor was found for histoplasmin (yeast antigen). In contrast, staphylococcal protein A (cell wall antigen) required IgG Fc fragment and complement for its interaction with platelet membrane.

Thus in addition to the well known indirect pathway of activation of human platelets by microbial antigens through receptors for IgG Fc fragment and complement there is a direct pathway of activation due to the existence of specific antigen receptors on human platelets.

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N. O. Solum (Institute for Thrombosis Research, Rikshospitalet, Oslo, Norway): **Effects of Bovine Factor VIII-Related Protein on Human Platelets and Isolated Human Platelet Membranes. (289)**

The first phase of aggregation of human platelets induced by bovine factor VIII-related protein does not require an intact energy metabolism and probably represents a passive agglutination phenomenon due to receptors for the protein on the outside of the platelet cytoplasmic membrane. However, washed human platelets lost their ability to be aggregated by bovine factor VIII-related protein after freezing and thawing of the platelets. Furthermore, isolated human platelet membranes were not flocculated by the bovine protein in the absence of added calcium ions. Additions of calcium chloride (2.1 mM) alone flocculated the isolated membranes. The membranes used represented the material sedimenting between 10,000 and 100,00 g from a homogenate obtained by homogenizing washed platelets suspended in 0.27 M sucrose in an Aminco-French pressure cell (1.361 atm. 2×1 min). Subsequent sucrose density gradient centrifugation of the preparations did not reveal bands of particulate matter at a higher sucrose density than 1.12. In