

HUMAN BASEMENT MEMBRANE COLLAGENS DO NOT ELICIT PLATELET AGGREGATION. R.L. Trelstad and A.C. Carvalho. Harvard Medical School, Pathology Department and Hematology Unit, Shriners Burns Institute/Massachusetts General Hospital, Boston, Massachusetts, U.S.A.

The immediate subendothelial connective tissue matrix consists of the basement membrane, a collagenous felt-like cell surface coat. The collagen from basement membranes has been isolated from human lung, skin, and kidney using a new fractionation method which separates native forms of collagen Types I, II, III, and IV. The Type IV collagens from the basement membranes have been characterized in respect to amino acid and carbohydrate composition, molecular size, charge and native structure. Antibodies prepared against the Type IV collagen reacted with both epithelial and vascular basement membranes as judged by immunofluorescence. Platelet-rich plasma (250,000/ μ l) from 5 normal subjects were tested for aggregation and 14 C-serotonin release with human collagen Types I, II, III, and IV. Complete aggregation (100%) and 14 C-serotonin release (80-100%) was obtained when Types I, II, and III were used. Human kidney, lung, and skin collagen Type IV (10-100 μ g/ml) did not aggregate platelets nor cause release of their contents. Pre-incubation of platelets and human collagen Type IV for periods of 30 minutes did not result in inhibition of platelet aggregation by Types I, II, or III.

These data indicate that the collagenous component of the basement membrane, the first extravascular collagen to which a platelet is exposed, does not elicit aggregation as do the fibrillar collagens in the perivascular matrix.

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POSTER SYMPOSIUM VII

Platelets: ADP Receptors.

BINDING OF ADENOSINE DIPHOSPHATE (ADP) TO ISOLATED HUMAN PLATELET MEMBRANES. J.Lips and J.J.Sixma. Dept. of Hematol., Univ. Hosp., Utrecht, The Netherlands.

Human platelet plasma membranes were isolated according to the glycerol loading technique of Barber and Jamieson. The binding of 14 C ADP was studied with Millipore filtration in a Ca^{2+} and Mg^{2+} containing buffer at pH 7.4. At least two types of binding sites were found: A high affinity system with a maximum binding of 160 pMoles/mg protein and an association constant of $1.1 \times 10^6 \text{ M}^{-1}$; and a low affinity system with a maximum binding of about 4500 pMoles/mg protein and an association constant of $0.6 \times 10^4 \text{ M}^{-1}$. The binding according to the high affinity system showed little temperature dependency ($Q_{10} = 1.10$). The pH optimum was at 7.3. Ca^{2+} ions were an absolute requirement for binding.

Nucleoside diphosphokinase (NDPK) was found in the membrane vesicles. Evidence that this enzyme was not responsible for ADP binding was obtained. The enzyme is Mg^{2+} dependent and is inhibited by AMP, in contrast to ADP binding. The Q_{10} was 1.44.

ADP binding was inhibited by ATP, IDP and β,γ -imidoadenosine triphosphate.