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0457 A SEROTONIN INDUCED BIPHASIC AGGREGATION BY PLATELETS FROM CATS WITH THE CHEDIAK-HIGASHI SYNDROME.

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Platelets from cats with the Chediak-Higashi syndrome (CHS) have an intrinsic platelet defect similar to storage pool deficiency in man and there is a virtual absence of secretable serotonin and ADP. Serotonin-induced platelet aggregation responses were monitored in four cats with the CHS. Low serotonin concentrations (0.5 µg/ml) induced a monophasic reversible aggregation response. When the concentration was increased (1-2 µg/ml) a biphasic aggregation response was observed and at higher concentrations (4-8 µg/ml) a monophasic irreversible aggregation wave was observed. The concentration dependent aggregation responses can be progressively inhibited with increasing concentrations of the serotonin antagonists, SQ-10,631 and cyproheptadine. The secondary aggregation wave is inhibited by both aspirin and indomethacin. Arachidonate (0.5mM) induces a monophasic irreversible CHS platelet aggregation response which is also inhibited aspirin and indomethacin. These findings suggests that the secondary aggregation wave is due to an arachidonate metabolite formed in response to serotonin. While the metabolite has not been directly identified serotonin induces formation of thromboxane B_2 (T_XB_2) by CHS cat platelets and in all but one animal increasing serotonin increased T_XB_2 formation. In addition, the cat whose platelets exhibited the weakest serotonin-induced aggregation response produced the smallest amount of T_XB_2.

3-004 0458 HIGH RESOLUTION TWO DIMENSIONAL GEL ELECTROPHORESIS OF SURFACE LABELLED NORMAL AND THROMBASTHENIC PLATELETS.

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Glycoproteins and proteins on the surface of human platelets were labelled by techniques specific for sugar or protein moieties. Such labelled samples were separated by a high resolution two dimensional gel technique (O'Farell's technique). Platelets were surface-labelled with sodium periodate + NaB^3H_4, galactose oxidase + NaB^3H_4, neuraminidase + galactose oxidase + NaB^3H_4 and lactoperoxidase ^{125}I. Comparison with one dimensional separation (Laemmli system) allowed the identification of the major glycoproteins (Ia, Ib, IIb, IIIa, IIIb). In general the labelling patterns were very similar to those obtained with stainning techniques. Following neuraminidase treatment glycoproteins Ib and IIb moved to more basic pH regions while the others were largely unaffected. Analysis of platelet membranes from patients with Glanzmann's thrombasthenia by these techniques showed the absence of glycoproteins IIb and IIIa or their presence in greatly reduced concentrations. In addition both glycoproteins Ib and IIIb were much more basic than in normals and appeared to have a reduced amount of sialic acid. The use of surface labelling and two dimensional electrophoresis provides additional evidence on membrane defects of Glanzmann's thrombasthenic platelets.

3-005 0459 PLATELET FUNCTIONAL DEFECT CAUSING GLAZMANN'S THROMBASTHENIA

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Glanzmann's thrombasthenia is an inherited bleeding disorder characterized by an absence of two membrane glycoproteins and by defective platelet aggregation. This defect appears to be restricted to an inability of the platelets to interact since their other functions are normal. We previously showed that platelets generate lectin activity in response to thrombin and that this lectin appears to mediate at least the initial phases of platelet aggregation by specifically binding to receptors on adjacent platelets. The lectin and lectin receptor activities of thrombasthenic platelets were characterized to determine if either function was missing. Control and thrombasthenic platelets were washed and the lectin activity determined by their ability to agglutinate either bovine erythrocytes or formaldehyde fixed human platelets. The lectin activities of normal and thrombasthenic platelets were indistinguishable. Normal and thrombasthenic platelets were fixed with formaldehyde to facilitate the independent assay of lectin receptor activity. The fixed platelets lacked platelet lectin activity. The control fixed platelets had normal lectin receptor activity. Fixed thrombasthenic platelets, however, lacked platelet lectin receptor function in our assay. Therefore thrombasthenic platelets fail to aggregate because they lack the platelet lectin receptor function.

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