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PARTIAL PLATELET FUNCTION DEFECT IN A VARIANT OF GLANZMANN'S THROMBASTHENIA WITH INTERMEDIATE LEVELS OF GP IIb/IIIa.
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A 17-year-old Italian boy has had a lifelong bleeding tendency, with frequent epistaxes and gum bleeding. The bleeding time is prolonged and the platelet count low normal. Electron microscopy showed a wide diversity of platelet size with many giant forms. In citrated PRP, ADP and other agonists induce slow and incomplete aggregation. The response of washed platelets varied with the agonist but ranged from subnormal to almost normal. Fibrinogen binding to washed platelets occurred slowly in response to ADP but eventually approached normal levels. No significant abnormality was observed of 5HT uptake, adenine nucleotide content, platelet factor-3 availability, β-thromboglobulin content or release, or malonyldialdehyde production. Clot retraction was normal. SDS-PAGE showed reduced amounts of GPIIb and GPIIIa. Crossed immunoelectrophoresis of Triton X-100 extracts of washed platelets showed the presence of GPIIb/IIIa complexes at 25-50% of normal levels. SDS-PAGE combined with an immunoblot procedure confirmed unchanged mobilities of GPIIb and GPIIIa and a normal proportion of GPIIb to GPIIIa. However, binding studies with radiolabelled monoclonal antibodies showed that intact washed platelets expressed only 12-20% of the normal binding sites for M148, AP-2 and Tab. These antibodies recognize different epitopes on GPIIb/IIIa complexes. Similar levels of these glycoproteins were detected by autoradiography after SDS-PAGE of radio-iodinated patient's platelets. GP Ib was normally present. A possible defect in the exposure of fibrinogen binding sites might contribute to the altered platelet function. Meanwhile, the patient appears to be a unique variant of Glanzmann's thrombasthenia with GP IIb/IIIa complexes at the borderline of those able to support platelet aggregation.

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GLANZMANN'S THROMBASTHENIA: HEMOSTATIC EFFECTIVENESS OF PLASMA CRYOPRECIPITATE TRANSFUSION.
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A 39-year-old 75 kg man with Type I Glanzmann's thrombasthenia developed recurring severe therapy-refractory nosebleeds, associated with nasal septal deformity, synechial scarring, and previous radiotherapy. During a 21 month period he required 16 hospitalizations for treatment of severe epistaxis. At initial hospitalization it was observed that his epistaxis ceased shortly after transfusion of 10 bags (units) of plasma cryoprecipitate (cryo), although transfusion with HLA-matched apheresis platelets from 3 donors, and topical therapy (cautery, packing), had been ineffective. Serum anti-platelet antibodies were not detectable by indirect immunofluorescence. During 9 subsequent hospitalizations his epistaxis stopped promptly (usually within 1 hour) following cryo transfusion (10 or 20 bags), including 6 occasions when cryo was the only therapy. On 6 additional occasions his epistaxis did not stop following cryo transfusion, but did stop after subsequent platelet transfusions or topical therapy. Eventually he underwent nasal septal reconstructive surgery surgery, and severe epistaxis has not recurred. Hemostatic studies, before and after cryo transfusions on 5 occasions, did not show improvement of platelet aggregation defects nor of Ivy bleeding times, although occasionally the volume of blood emanating from bleeding time punctures appeared decreased following cryo transfusion. Platelet glycoprotein (GP) IIb/IIIa antigen was measured in aliquots of 10 of the pools of cryo received by the patient (representing 100 bags of total volume 2200 ml), using an immunoradiometric assay (Nichols et al, Blood 68:300a, 1986). On average, the transfused cryo pools contained GP IIb/IIIa equivalent to 1.1×10^9 platelets/ml (range $0.6\text{-}1.7 \times 10^9$/ml. Our recently reported studies of blood bank cryo documented similar GP IIb/IIIa levels, and revealed that $\geq$ 93% of GP IIb/IIIa in cryo is present in the form of sedimentable membranous platelet macroparticles and microparticles. We hypothesize that the GPIIb/IIIa-bearing platelet particles in transfused cryo might account for the improvement in hemostasis we frequently observed. We conclude that cryo transfusion deserves further study as a potentially useful therapeutic adjunct in promoting hemostasis in individuals with Glanzmann's thrombasthenia.

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ROLE OF RELEASED ADP IN THE STABILIZATION OF PLATELET AGGREGATES: STUDIES IN PATIENTS WITH DEFICIENCY IN AMINE STORAGE GRANULES CONTENTS.
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Human platelets aggregated by thrombin (T) under conditions in which the release reaction (RR) occurs to only a small extent can be deaggregated by agents that dissociate ^{125}I-fibrinogen bound to platelets. In contrast, when platelets undergo the RR, they cannot be readily deaggregated even though combinations of inhibitors cause ^{125}I-fibrinogen to dissociate. Therefore, material released from platelet granules seems to stabilize aggregates. T-induced aggregates of washed platelets deficient in fibrinogen or von Willebrand factor cannot be deaggregated readily by deaggregating agents, implying that released fibrinogen or von Willebrand factor do not have a major role in stabilizing aggregates. To examine the role of platelet δ-granule contents in stabilizing platelet aggregates, aggregation and deaggregation were studied with platelets from patients with δ-Storage Pool Deficiency (δ-SPD). Platelet aggregation and the release of β-TG in response to T (1 U/ml) were similar for platelets from patients and controls. Platelets from patients (but not from controls) could be deaggregated by PGE_1 (10 uM) plus chymotrypsin (10 U/ml), with hirudin (5 U/ml) added to block further effects of T. Addition of ADP (20 uM) to the δ-SPD platelets 5 sec after T abolished the ability of this combination of inhibitors to deaggregate the platelets. The addition of serotonin (2 uM) 5 sec after T did not prevent inhibitors from deaggregating δ-SPD platelets. When apyrase was added to normal platelets immediately before they were aggregated by T, the combination of inhibitors readily deaggregated the platelets. Therefore, released ADP may stabilize platelet aggregates through a mechanism that could be independent of released fibrinogen and von Willebrand factor.

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MEMBRANE SIGNAL TRANSDUCTION IN PLATELETS WITH ALTERED RELEASE REACTION.
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The process of signal transduction during thrombin-induced activation was studied in pathological platelets characterized by a defect in a specific storage granule, i.e. from Hermansky-Pudlak syndrome (HPS) and from Grey-Platelet Syndrome (GPS). HPS platelets exhibited an apparently normal ultrastructure except for a decreased number of dense bodies. Grey platelets showed marked vacuolization and an almost total absence of alpha-granules. During thrombin stimulation both types of platelets showed the same tendency of centralization of the organelles present indicating that neither type of granule is a prerequisite for this ring-like structure. However this granule centralization was clearly delayed in GPS where it occurred 15 sec after thrombin addition instead of 5 sec in normal platelets. The transducing system involving phosphoinositides specific phospholipase C was observed in platelets lacking dense bodies (HPS) but the phosphatidyl 4,5 bisphosphate(PIP_2) breakdown in ^{32}P-prelabelled platelets was measurable at 20 sec instead of 10 sec in normal platelets. No such activity was detectable at any time in grey platelets. ^{32}P-phosphatidate (PA) formation was subnormal in HPS platelets and normal in grey platelets. Phosphorylation pattern of myosin light chain (P20) and of 43K protein (P43) were normal in HPS platelets and markedly reduced in grey platelets, being less than half of the normal during the first 15 sec and remaining subnormal even after complete aggregation. The release of constituents from the present granules and the thromboxane formation were lower than in normal platelets in all cases. In conclusions, (i) alpha-granules but not dense bodies may play a key role in the activation of the PIP_2 specific phospholipase C, (ii) PA formation does not always correlate with phosphoinositide metabolism and could originate from another pool of diacylglycerol, (iii) complete phosphorylations of both P20 and P43 may not be sufficient to stimulate a normal release, and (iv) end products such as thromboxanes and released ADP accelerate and reinforce platelet responses.