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BLOOD PLATELET FUNCTIONS: INFLUENCE OF AGING.

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Aging has been associated with a high incidence of vascular diseases and it is suggested that platelet activation could contribute in the development of these diseases. The purpose of this study was to compare platelet functions from young adults (< 35 years) and elderly people (> 70 years). Aggregation of platelet rich plasma induced by arachidonic acid or epinephrine was significantly increased in the elderly. Similarly, an increase of platelet aggregation (platelets isolated from their plasma) induced by various agents (thrombin, U46619, arachidonic acid ...) was also noted. The same tendency was observed in whole blood aggregation. Platelet endogenous arachidonic acid metabolism under stimulation was evaluated. Production of thromboxane B2, measured by GLC, was significantly higher in the elderly people (510 ± 207 vs 242 ± 83 ng/10^9platelets, $p<0.02$). On the other hand, platelet vitamin E, quantified by HPLC, was significantly decreased in elderly people (0.92 ± 0.21 vs 1.41 ± 0.55 nmoles/10^9platelets, $p<0.05$). To further assess platelet and vascular function in vivo, we measured excretion of thromboxane B2 (TXB2), 2,3-dinor-TXB2 (M-TXB2), 6 keto-PGF1α and 2,3-dinor-6 keto-PGF1α (M-6-k-PGF1α) in urine. These four metabolites were nearly all significantly increased in the older population (TXB2: 24.3 ± 26.6 vs 3.1 ± 1.2 $p<0.05$; M-TXB2: 51.5 ± 43.2 vs 25.1 ± 14.5 NS; 6-k-PGF1α: 37.5 ± 29.3 vs 19.1 ± 4.2 $p<0.05$; and M-6-k-PGF1α: 193.6 ± 118.6 vs 116.4 ± 42.4 ng/mmole creatinine $p<0.05$). We conclude that changes in platelet functions reveal an enhanced platelet activity which may reflect a prethrombotic state in elderly people. The mechanisms of these modifications remain to be determined but the increased of specific peroxidation observed might be linked to the decrease of platelet vitamin E.

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THE EFFECT OF RED BLOOD CELLS ON PLATELET AGGREGATION IN NORMAL SUBJECTS AND MYELOFIBROTIC PATIENTS.

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We investigated the effect of red blood cells (RBC) on "in vitro" platelet aggregation by the use of the "Electronic Whole Blood Aggregometer" (Chrono-Log Corporation). Preliminary experiments, studying platelet aggregation in the same PRP by the simultaneous use of the optical method and the electronic method, demonstrated that the maximum rate of impedance changes correlated well with both the rate and the extent of platelet aggregation as measured by the optical method. Therefore, the maximum rate of impedance increase was chosen for the measurement of platelet aggregation in the presence of different concentrations of RBC. RBC, both at 40 and 60%, significantly inhibited platelet aggregation stimulated by low and high concentration of ADP and epinephrine. Platelet aggregation stimulated by collagen was slightly reduced only by the higher RBC concentration. The effect of RBC on platelet aggregation was also investigated in idiopathic myelofibrosis, a pathological condition characterized by both platelet and RBC alterations. While on the basis of PRP studies 5 out of 17 patients had hypo-aggregation and 12 had normal aggregation, whole blood studies evidentiated hypo-aggregation in 3 patients, normal aggregation in 4 and spontaneous platelet aggregation (SPA) in 10. SPA was a consequence of platelet abnormality, since it occurred also when platelets from patients were stirred with normal RBC.
In conclusion, RBC may exert different effects on the aggregation of normal and pathological platelets.

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THE BLEEDING TENDENCY OF PROGRESSIVE RENAL FAILURE IS NOT ASSOCIATED WITH DEFECTIVE PLATELET AGGREGATION.

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The bleeding tendency of uraemia may be related to reduction by anaemia of erythrocyte/platelet interaction, toxic inhibition of platelet aggregation and abnormal von Willebrand Factor (vWF) mediated platelet adhesion. Our aim in this study was to determine at what stage of renal failure bleeding time becomes prolonged and to investigate the mechanisms involved.

We have measured bleeding time (Simplate II), plasma levels of fibrinogen and vWF, and ex-vivo platelet responsiveness in 31 patients with chronic renal failure (CRF) of various degrees of severity and compared them with values obtained in 22 healthy controls. No patient was dialysed, nephrotic or suffering from immunological renal disease. Patients were divided into mild (plasma creatinine <300 umol/l), n=10, moderate (300-600 umol/l), n=14, or severe (>600 umol/l), n=7, CRF.

Bleeding time became significantly prolonged only in severe CRF ($p<0.005$). Haematocrit fell as renal failure advanced, and correlated with bleeding time ($r=0.40$, $p<0.05$). Platelet counts were normal. Platelet aggregation in response to ristocetin (mediated by vWF) and ADP increased progressively ($p<0.005$ in severe CRF), as did spontaneous aggregation ($p<0.005$ in severe CRF). This was associated with an increase in plasma vWF and fibrinogen ($p<0.005$ in severe CRF). Collagen induced aggregation was slightly, but not significantly increased. Thromboxane (TxB2) generation in clotting blood was the only measurement that showed a reduced platelet response ($p<0.025$ in severe CRF).

In summary, a bleeding tendency develops late in the course of progressive CRF when plasma creatinine has risen to at least 600 umol/l. Platelet aggregation is enhanced rather than reduced and platelet interaction with vWF is not defective. Anaemia appears more important than abnormal platelet aggregation in mediating uraemic bleeding, although reduced serum TxB2 generation suggests a defect in platelet response to endogenous thrombin which may also contribute. Increased platelet aggregation and fibrinogen concentrations might promote glomerular thrombosis and contribute to the progression of CRF.

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PLATELET ALPHA$_2$-ADRENERGIC RECEPTOR ABNORMALITIES IN PATIENTS WITH IDIOPATHIC SCOLIOSIS.

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Idiopathic scoliosis is a genetic multisystem disease involving skeletal, biochemical,central nervous system, muscle and blood platelet abnormalities. Platelets of patients with idiopathic scoliosis have been shown to have decreased adenosine diphosphate and epinephrine-induced aggregation. Similarities between the contractile protein system of platelets and muscle have made the platelet a popular model for certain aspects of muscle physiology. This study confirmed that 64% of the patient platelets tested exhibited a significantly decreased sensitivity to aggregation by epinephrine. In seven of the eleven patients studied, epinephrine induced aggregation was markedly decreased, i.e., the threshold of agonist was markedly elevated (> 11 uM). The geometric mean concentration of epinephrine required to produce complete second-wave aggregation in idiopathic scoliosis patients was 8μM, as compared to a control concentration of 1uM. We therefore examined the platelet alpha$_2$-adrenergic receptors of 17 patients with idiopathic scoliosis by measuring ligand binding using the selective antagonist, methyl yohimbine. Platelets from healthy individuals had 185 ± 16 sites per platelet with a K_d of 1.90 ± 0.32 nM, while patients with idiopathic scoliosis had 54 ± 22 sites per platelet with a K_d of 1.02 ± 0.03 nM. The number of binding sites per platelet in idiopathic scoliosis patients were significantly decreased ($p < 0.05$) as compared to controls, while the K_d was not significantly different ($p > 0.05$) between the two groups. Seven of these patients exhibited a significant decrease ($p < 0.05$) in the number of alpha$_2$-adrenergic receptors on their platelets while the binding in 7 additional patients was undetectable.Three patients exhibited normal receptor number and affinity as compared to normal individuals. This study indicates a profound alteration in the number and function of the alpha$_2$-adrenergic receptors in platelets of patients with idiopathic scoliosis and indicates the functional heterogeneity of the receptor disorder. Further investigation of platelet abnormalities may give insight into the putative muscle defects.