

FURTHER EVIDENCE OF THE INVOLVEMENT OF THROMBIN IN PLATELET RELEASE BY ADP AND OTHER AGENTS. N.G. Ardlie and Huzoor-Akbar. Department of Clinical Science, Australian National University, Canberra, Australia.

There is evidence of the involvement of coagulation factors in platelet aggregation and the release reaction caused by ADP and collagen but this has been challenged. This report concerns further experiments which can explain the apparently conflicting observations of various laboratories and which provide additional evidence of the involvement of coagulation factors and thrombin in the platelet release reaction caused by ADP and other agents.

Washed platelets suspended in a buffer solution responded poorly to ADP with no second phase aggregation or release of [ $^3\text{H}$ ]5HT. In contrast, washed platelets suspended in dialysed plasma underwent second phase aggregation and released radioactivity. This response depended on calcium. Dialysed plasma deficient in factors XI or X did not restore second phase aggregation or the release reaction. Hirudin and heparin inhibited second phase aggregation and release by ADP and epinephrine. However, the inhibitory effects of heparin and hirudin on ADP, epinephrine and collagen were not observed when citrate was present. To explore the possibility that a reduction of the free calcium concentration accounts for the inhibition of action of these antithrombin agents by citrate experiments with EGTA were carried out. This alternative chelating agent also prevented the inhibitory actions of heparin and hirudin.

These observations support the view that platelets and clotting function cooperatively in platelet reactions involved in haemostasis. We suggest that small amounts of thrombin formed prior to fibrin clotting mediate platelet reactions in haemostasis initiated by collagen and ADP and that secondary aggregation does not represent a citrate artifact.

DE NOVO SYNTHESIS OF PURINE NUCLEOTIDES IN HUMAN PERIPHERAL BLOOD PLATELETS. Z. Jerushalmy, M. Patya and O. Sperling. Tel-Aviv University Medical School, Rogoff-Wellcome Medical Research Institute, Beilinson Medical Center, Petah Tikva, Israel.

Human blood platelets were studied for the presence of the pathway of de novo synthesis of purine nucleotides.  $8 \times 10^5$  cells were incubated for 2.5 h at  $37^\circ\text{C}$  in 2 ml of Eagle's Minimal Essential Medium containing Earle's Balanced Salt Solution, 15% fetal calf serum and 20  $\mu\text{Ci}$  sodium [ $^{14}\text{C}$ ] formate (59 mCi/mmol). Platelets were found to incorporate [ $^{14}\text{C}$ ] into total purines at a slow but detectable rate of 50–70 pmoles/ $8 \times 10^5$  cells/2.5 h. This incorporation was inhibited by approximately 80% at 10 mM azaserine and by 60% at 0.1 mM adenine. Adenine is known to affect the rate of purine synthesis de novo through the activity of phosphoribosylpyrophosphate amidotransferase, the first committed enzyme of this pathway. The results suggest the presence of the complete pathway of de novo synthesis of purine nucleotides in normal human peripheral blood platelets.

3'5'-ADENOSINE DIPHOSPHATE (3'5'ADP): A PHYSIOLOGICAL INHIBITOR OF PLATELET AGGREGATION AND PLATELET RELEASE REACTION. K. Subbarao and K. Jaya. Temple University School of Medicine and the University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Certain analogues of adenosine have been shown to inhibit ADP-induced platelet aggregation. We therefore studied the *in vitro* effect of 3'5'ADP and coenzyme A on human platelet aggregation and [ $^{14}\text{C}$ ]-serotonin release reaction induced by the addition of ADP, thrombin, collagen and epinephrine to human platelet rich plasma (PRP). It was found that coenzyme A  $\text{Li}_3 \cdot 2\text{H}_2\text{O}$  at a concentration of 0.12 mM strongly inhibited ADP-induced platelet aggregation of PRP but did not show similar effect on the aggregation of platelets induced by other aggregating agents. The 3'5'ADP which is a part of coenzyme A structure, on the other hand, inhibited both ADP and thrombin induced platelet aggregation. The extent of inhibition of platelet aggregation by coenzyme A and 3'5'ADP was found to depend upon the concentration of the inhibitor and the incubation time. Whereas 3'5'ADP  $\text{Li}_2 \cdot 3\text{H}_2\text{O}$  at a concentration of 10  $\mu\text{M}$  produced about 70% inhibition of ADP-induced platelet aggregation of human PRP, total inhibition of thrombin induced platelet aggregation was observed when platelets were incubated with 60  $\mu\text{M}$  of 3'5'ADP. The 3'5'ADP also inhibited the [ $^{14}\text{C}$ ]-adenosine uptake by platelets in a concentration dependent manner. The inhibitory potency of 3'5'ADP on platelet aggregation was found to be 10-fold higher than that of  $\text{N}^6\text{-}2'\text{-O-dibutyryl-cyclic } 3'5'\text{-adenosine monophosphate}$ . The inhibition of platelet aggregation by coenzyme A and 3'5'ADP was always accompanied by the inhibition of [ $^{14}\text{C}$ ]-serotonin release reaction. If coenzyme A and 3'5'ADP are indeed physiological inhibitors of platelet aggregation, then aggregation of platelets should depend on metabolic events that regulate the concentration of these agents in blood.