EFFECTS OF PYRAZOLONE DRUGS ON SYNTHESIS OF PROSTAGLANDIN D_2 AND THROMBOXANE B_2 BY PLATELETS. M. Ali, J. Zamecnik, and J.W.D. McDonald. University of Western Ontario. London, Ontario. Canada.

The principle products of arachidonic acid (AA) in platelets are hydroxylated fatty acids and thromboxane B_2 (TXB_2). Prostaglandin D_2 (PGD_2) has been considered to be a nonenzymatic degradation product of prostaglandin H_2 formed in the presence of plasma albumin. Using ^{14}C AA as substrate and thin layer and silicic acid chromatography, we have demonstrated PGD_2 synthesis by washed (albumin-free) human platelets. The identity of PGD_2 was confirmed by gas chromatography-mass spectrometry. In platelets lysed by freezing and thawing synthesis of TXB_2 and PGD_2 was approximately equal and equally inhibited by pyrazolones.

	nMoles/min/10^9 platelets	
	TXB_2	PGD_2
Control (10 µM Arachidonate)	0.772 ± 0.053	0.582 ± 0.145
+ 200 µM Sulfinpyrazone	0.410 ± 0.076	0.215 ± 0.037
+ 200 µM Phenylbutazone	0.335 ± 0.06	0.165 ± 0.028

Synthesis of PGD_2 by platelets is enzymatic and may contribute to bronchoconstrictor, vasomotor, and inflammatory effects induced by platelet aggregation. Pyrazolones appear to inhibit cyclooxygenase activity rather than the breakdown of cyclic endoperoxides as previously postulated.

ARACHIDONIC ACID METABOLITES NOT ALWAYS CRUCIAL FOR THROMBOREGULATION? G. Hornstra and E. Haddeman. Unilever Research, Vlaardingen, The Netherlands

When blood platelets are triggered for aggregation, phospholipase A_2 is activated resulting in the release of arachidonic acid (AA) from cell membrane phospholipids. AA is converted into prostaglandin endoperoxides (EP) by the cyclo-oxygenase enzyme system (CO). From EP's, Thromboxane A_2 (TxA_2) is formed enzymatically. There is now considerable evidence that the AA-EP-TxA_2 axis is of major importance for thromboregulation.
However, recent findings indicate that under certain conditions an alternative regulatory mechanism may exist.
In essential fatty acid (EFA-) deficient rats, platelets contain hardly any AA, the efficiency of which as a CO-substrate is severely depressed because of the competition of a fatty acid, specific of EFA-deficiency (C 20:3 n-9). The collagen- and thrombin-stimulated production of thrombogenic AA-metabolites (measured as malondialdehyde) is almost absent in EFA-deficient platelets. Nevertheless, platelet release is normal to increased.
This finding points to a thromboregulatory mechanism different from the AA-EP-TxA_2 axis. This, probably secondary, mechanism may be of significance for the prevention and therapy of thrombosis.

INDEPENDENT INDUCTION OF PLATELET AGGREGATION AND SECRETION BY PROSTAGLANDIN ENDOPEROXIDE ANALOGUES AND THROMBOXANE A_2-LIKE MATERIAL. I.F. Charo, R.D. Feinman, T.C. Detwiler and J.B. Smith. State University of New York, Downstate Medical Center, Brooklyn, N.Y., and the Cardeza Foundation, The Thomas Jefferson University, Philadelphia, PA. U.S.A.

We have investigated the mechanims of platelet activation by two prostaglandin endoperoxide analogues (U-46619 and U-44069) using a new instrument that simultaneously monitors platelet aggregation and secretion. Low concentrations of these compounds induce platelet aggregation without secretion (i.e., primary aggregation), while slightly higher concentrations induce biphasic aggregation with secretion paralleling the second phase. At still higher concentrations, aggregation and secretion begin simultaneously, and in the absence of stirring there is secretion but no aggregation. A critical concentration of endoperoxide analogues can often be found that will induce 2 waves of secretion, a phenomenon not seen with other stimuli. Similar results were obtained with thromboxane A_2-like material that was generated by incubation of dog platelets with Na-arachidonate. In contrast, when platelets are stimulated with Na-arachidonate, the precursor of the endoperoxides and thromboxanes, we never observe significant aggregation without secretion, and even at the lowest concentrations secretion is independent of aggregation. We conclude that both the prostaglandin endoperoxide analogues and thromboxane A_2-like material induce platelet aggregation independent of released ADP and only at higher concentrations can directly induce secretion, whereas Na-arachidonate induces aggregation and secretion in parallel.