MEASUREMENT OF NUCLEOTIDE POOLS IN PLATELETS AND RED BLOOD CELLS USING HIGH PRESSURE LIQUID CHROMATOGRAPHY. L. D'Souza and H. I. Glueck. University of Cincinnati College of Medicine, Cincinnati, Ohio U.S.A.

Release of adenine nucleotides during platelet aggregation is well established. The released adenosine diphosphate (ADP) is thought to cause secondary platelet aggregation. A high pressure liquid chromatographic technique has been used to measure accurately the various nucleotide pools in intact platelets, as well as their levels during various stages of aggregation. Accurate and highly reproducible measurements of 10^{-12} moles of all nucleotides are possible using the bonded microparticulate, Partisil -10 SAX column, a strong anion exchanger (Whatman Inc., Clifton, N.J.) and an electronic integrator. The method is a modification of an earlier method of P. Brown (1973), and G. Rao et al (1975). Using this technique we are able to obtain within 75 minutes a single step gradient separation of cyclic nucleotides (cAMP, cGMP) as well as mono-, di-, and tri-phosphonucleosides. Sample sizes of 0.5 ml of platelet rich plasma are adequate for at least 3 estimations. Normal donor platelets show the following adenine nucleotide levels; * AMP 0.13 (0.14); ADP 1.64 (0.81); and ATP 4.90 (0.42) nanomoles per 10^{11} platelets. The method has certain advantages over the enzymatic or luciferase assays, since all the nucleotides can be simultaneously measured in a single step from a very small sample and there is no cross reactivity with any of the nucleotides. Similar studies of plasma and red blood cell nucleotides are possible with this technique.

*Mean (standard deviation)

RELATIONSHIP BETWEEN AGGREGATION AND PROSTAGLANDIN (PG)-BIOSYNTHESIS IN RAT PLATELET RICH PLASMA (PRP). H. Bult, P.C. Bragt and I.L. Bonta. Erasmus University, Department of Pharmacology, Rotterdam, The Netherlands.

The importance of PG biosynthesis in aggregation has been studied by comparing PRP from normal and essential fatty acid deficient (EFAD) rats. The latter show a marked lack of the PG precursor, arachidonic acid (AA).

No differences were observed in aggregation with collagen (from 10 μ g/ml), ADP (from 0.8 μ M) or the PG-endoperoxide PGH_2 (1 μ g/ml). PGH_2 induced only a small, transient aggregation. However, with a collagen dose that produced a 50% response in normal PRP, the aggregation of EFAD PRP was 94% reduced. This correlated with reduced (84-89%) biosynthesis of thromboxane A_2 and PGE-like material (PGE), both assessed by bioassay. The PG synthetase of EFAD PRP was unaltered, since PGE and malondialdehyde production were normal when exogenous AA was added. AA, up to 0.33 mM, did not aggregate normal PRP, but high amounts of PGE were formed.

These results indicate that internal PG biosynthesis is only essential for aggregation with threshold doses of collagen. At higher collagen doses endogenous PG production does not seem to be important for aggregation. It is unlikely that residual formation of PG-endoperoxides in EFAD PRP, at high doses of collagen, is sufficient for a normal aggregation, since normal rat platelets form PG-endoperoxides from exogenous AA without aggregation.