EVALUATION OF CYCLO-OXYGENASE PATHWAY IN PLATELETS OF THE NEWBORN. D.G. Corby, W.C. Goad, J. Barber and T.P. O'Barr. Clinical Investigation Service, Fitzsimons Army Medical Center, Denver, CO., USA

A possible deficiency of cyclo-oxygenase in platelets of the newborn infant has been considered as an explanation for their impaired aggregation to stimuli which function by promoting the release of ADP. (Corby and Zuck, Thrombos. and Haemostas., USA 36:201-207, 1976). Cyclo-oxygenase activity was evaluated in washed platelets from paired mother and cord blood samples by monitoring the incorporation of radioactivity into metabolites during incubation with (1-^{14}C) arachidonic acid. Platelets from both the mothers and newborns showed normal aggregation to arachidonic acid. Thin layer radiochromatograms of methylated incubation products were essentially identical. Three main peaks of radioactivity, which corresponded to identified arachidonic acid metabolites, were noted (Malmsten et al. Proc. Natl. Acad. Sci., USA, 72:1446-1450, 1975). Platelets from mothers and newborns incorporated similar amounts of radioactivity into 8-(1-hydroxy-3-oxopropyl)-9,12L-dihydroxy-5-10-heptadecadienoic acid (PHD) and 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT). Since these two compounds are derived from the endoperoxide prostaglandin G_2 (PGG_2), which is believed to initiate the release reaction, the pathway leading from arachidonic acid to PGG_2 is probably fully developed in platelets of the newborn infant. It may be speculated that the lack of response by these platelets to external stimuli is related to either the availability or the formation of metabolically available arachidonic acid.

A NEW PATHWAY FOR ARACHIDONIC ACID METABOLISM IN HUMAN PLATELETS. S. Rittenhouse-Simmons, F.A. Russell, D. Deykin. Boston VA Hospital, Boston, Massachusetts.

We are reporting a novel pathway of arachidonic acid metabolism in the phosphatides of thrombin-activated platelets. For kinetic studies of arachidonic acid turnover, platelet phosphatides were labeled by incubation of platelet rich plasma with (^{3}H)-arachidonic acid for 15 min. Unincorporated isotope was removed during subsequent gel-filtration. Platelet phosphatides were resolved and quantitated following two-dimensional silica paper chromatography of chloroform/methanol extracts of incubated platelets. Plasmalogen phosphatidylethanolamine (PPE) was examined on paper chromatograms after its breakdown to lysoPPE with $HgCl_2$. In other experiments, gel-filtered platelets were incubated with (^{14}C)-glycerol to monitor de novo phosphatide synthesis. (^{3}H)-Arachidonic acid was released from phosphatidylcholine and phosphatidylinositol of pre-labeled platelets exposed to thrombin and appeared increasingly in PPE in acyl linkage at glycerol-C-?. (^{3}H)-Arachidonic acid was not found in PPE of resting cells. Maximum transfer occurred with 5 U/ml of thrombin and 15 min. of incubation, with $t_{\frac{1}{2}}$ of 2½ min., and was Ca^{+2} dependent. The presence of aspirin, indomethacin, or eicosatetraynoic acid did not prevent the thrombin-activated transfer of (^{3}H)-arachidonic acid to PPE. The stimulated incorporation of (^{3}H)-arachidonic acid into PPE was not accompanied by a stimulation of (^{14}C)-glycerol uptake into this phosphatide. We suggest that perturbation of the platelet may activate a phospholipase A_2 leading to turnover of arachidonic acid in PPE, which is rich in this fatty acid. Such turnover may provide substrate for conversion by cyclo-oxygenase and lipoxydase to biologically active metabolites, and therefore, may offer a locus for regulation of prostaglandin synthesis in the human platelet.

DIHOMO-Y-LINOLENIC ACID METABOLISM IN MAN: ITS RELEVANCE TO PLATELET FUNCTION. K.J. Stone, A.L. Willis, M. Hart and P. Marples. Roche Products Ltd., Welwyn Garden City, England; P.B.A. Kernoff and G.P. McNicol. University Department of Medicine, The General Infirmary, Leeds, England.

Prostaglandin (PG) metabolites of dihomo-Y-linolenic acid (DHLA) inhibit platelet aggregation (Kernoff et al, this meeting). In vivo, antithrombotic effects of DHLA might be limited by its rate of Δ^5-desaturation to arachidonic acid (AA), which enhances platelet aggregation when administered to man. In the rat and mouse desaturation is particularly active but in the guinea-pig, cat and rabbit it appears insignificant*. We now report on the metabolism of single oral doses of DHLA in 3 human volunteers. Lipids were extracted from blood fractions and separated into different lipid classes before estimation of fatty acid composition by gas-liquid chromatography. P.G.'s, generated from platelets by maximal stimulation with thrombin, were isolated by argentation chromatography and estimated by bioassay. Maximal plasma DHLA/AA ratios (0.2-0.5) occurred at 3-4 hrs when the DHLA/AA ratio in triglycerides had increased 9-12 fold. Between 5-24 hrs DHLA accumulated in phosphatidyl choline. There were no consistent increases in the AA content of blood lipids and no increases in platelet production of its metabolite PGE_2. Small increases in platelet PGE_1 synthesis occurred, indicating that orally-administered DHLA reaches PG precursor pools in man. These results support our hypothesis that chronically-administered DHLA may be of therapeutic value as an antithrombotic agent.

*Willis, Stone, et al (1977). In: Prostaglandins in platelets and other blood cells, Ed. Silver, Smith and Kocsis, Spectrum Publications, Holliswood, N.Y.