

INCORPORATION OF ^3H -GLYCEROL INTO PLATELET PHOSPHOLIPIDS DURING PLATELET AGGREGATION. R.A.Hutton and R.M.Hardisty Institute of Child Health, and Hospital for Sick Children, London, England.

In vitro incorporation of glycerol- $2\text{-}^3\text{H}$ into phospholipids of normal platelets was measured at rest and during platelet aggregation induced by adenosine diphosphate, epinephrine, collagen and ristocetin. Compared to the basal state, aggregating platelets showed a marked increase in ^3H glycerol uptake, although total platelet phospholipid content was unchanged. The most striking change was an increase in the proportion of the radioactivity incorporated into the phosphatidyl inositol fraction (from 11% to 36% after 30 minutes incubation with epinephrine), this being largely at the expense of the phosphatidyl choline fraction which decreased from 46% to 30%. The extent of the glycerol uptake correlated well with the degree of platelet aggregation observed ($p=0.05$ for adenosine diphosphate), but was not directly related to the speed of either aggregation or the release reaction. The rate of glycerol uptake paralleled the development of platelet factor 3 availability (PF3a) over the first 20-30 minutes of incubation, but thereafter, PF3a levelled off while glycerol uptake continued to rise for at least another 30 minutes. We conclude that the changes in platelet phospholipid turnover observed here during platelet aggregation, are of little direct consequence to the cells haemostatic functions. The increase in phosphatidyl inositol turnover may represent part of the cells' response to membrane distortion or damage during the secretory process, as has been documented in other secretory processes.

ENHANCEMENT OF 5HT UPTAKE BY PICRYL SULFONIC ACID AMINO GROUP MODIFICATION IN THE PLATELET MEMBRANE. C.B. Harbury, M.E. Janszen, S. Rutherford. Stanford University School of Medicine.

The main purpose of this experiment was to assess the functional importance of platelet membrane amino groups (NH_2). Picryl Sulfonic Acid (PSA) is a known NH_2 modifier. Platelets were harvested, exposed to 0.25 - 32 mM PSA for 15 minutes at 22°C . They were then washed and functionally tested. The release was measured by ^3H 5HT release. The release reaction was induced by 0.1, 1, and 10 u/ml Thrombin (THR), Trypsin (TRY), Collagen (COL), and Latex particles (LAT). There was gradual but never complete inhibition of the release reaction. At 16 mM PSA, the platelets started to lose small amounts of 5HT (4%). Clot retraction was not affected by 1 to 32 mM PSA. 5HT uptake was enhanced at 0.1 to 4 mM PSA (paired t test, $p<0.01$) and maximal at 2 mM PSA. Pre-incubation with 0.25 and 0.5 mM PSA in the presence of calcium enhanced the PSA effect on rate of 5HT uptake (paired t test, $p<0.01$). This may be due to the enhanced availability of amino groups to PSA in the presence of Ca, speculated by Godin to be due to a lipid effect (1972). Incorporation of PSA into the membrane was increased in the presence of Ca (O.D. at 335 nm). In summary, PSA modification of platelet membrane NH_2 groups partially but never completely interferes with the induction of the release reaction. The inhibition was not specific with respect to the release inducing agent. Clot retraction remains intact. The observed significant enhancement in rate of 5HT uptake may be specific or may be due to neutralization of the charge on free amino groups, which repels 5HT.

TISSUE THROMBOPLASTIN IN LEUKOCYTES FROM VARIOUS LEUKEMIAS AND ITS LOCALIZATION BY IMMUNOPEROXIDASE STAINING METHOD. T. Maekawa, H. Gommori, N. Kobayashi, T. Ueno and H. Tanaka. Gunma University School of Medicine, Maebashi, Gunma, Japan.

Tissue thromboplastin (TTP) in the leukocytes from various types of leukemia was studied. Washed leukocyte suspensions were prepared by centrifugation and hypotonic lysis techniques from 11 acute myeloblastic leukemias (AML), 5 acute promyelocytic leukemias (APL), 6 chronic myelocytic leukemias and 20 healthy subjects. The TTP activity was estimated by Nemerson's two stage method and effects on clotting times of various deficient plasmas were studied by Quick's one stage method. The distribution of the activity in the homogenate of APL leukocytes was studied by differential centrifugation. Anti-placenta(P)TTP was produced and used for immunological studies and immunoperoxidase staining method (IPS). Homogenates of APL leukocytes showed significant TTP activity as much as 152 units per 10 million cells on the average, while homogenates of other blood cells, except a case of AML which had an activity of 3.8 units per 10 million cells, showed little activity. APL homogenates demonstrated striking shortening of the clotting times of Factor VIII and IX deficient plasmas but did not show this effect on Factor VII and X deficient plasmas. This activity sedimented mainly at 700 G in the centrifugation study and was neutralized time-dependently by anti-PTTP like brain(B) and PTTTP. By Ouchterlony method, desoxycholate extract of APL leukocytes showed single precipitin line against anti-PTTP, which fused into the lines formed by purified B- and PTTTP. APL leukocytes were deeply stained by IPS, while the other blood cells were not stained or, if stained, only faintly. In conclusion, APL leukocytes have strong TTP similar to B- and PTTTP in antigenicity. The development of DIC may be predicted by these techniques, especially by IPS.