

GLUTATHIONE PEROXIDASE AND MALONDIALDEHYDE PRODUCTION IN THE ARACHIDONIC ACID METABOLISM OF HUMAN PLATELETS. G.C. Guidi, R. Schiavon, G.L. Avventi and G. Perona. Division of Clinical Chemistry and Division of Haematology-Institute of Patologia Medica II, University of Padova, Verona, ITALY.

A series of functional parameters, including the aggregability triggered by various agents, the *in vitro* malondialdehyde production and the glutathione peroxidase activity, has been investigated in platelets from normal blood donors. Glutathione peroxidase activity assays showed a significant inverse correlation with malondialdehyde induced by arachidonic acid but not with aggregation data and malondialdehyde induced by thrombin. Moreover, arachidonic acid generates in human platelets lysates large amounts of hydrogen acceptor substrate(s) for the glutathione peroxidase with peculiar kinetic features. These are related to malondialdehyde production and to partial inhibition by acetyl-salicylic acid and are likely connected with prostaglandin metabolism. Our data suggest that physiological variations in glutathione peroxidase activity are important in human platelet arachidonic acid metabolism, because they modulate the biosynthesis of key end-products, as thromboxane  $A_2$ , whose malondialdehyde is an index.

## 1296

THE DENSITY DISTRIBUTION OF INTACT PLATELETS FOLLOWING THEIR ISOLATION FROM ALL OTHER BLOOD CONSTITUENTS. T. Shaw, J.F. Martin, C.N. Chesterman, D.G. Penington Melbourne University Department of Medicine, St. Vincent's Hospital, Melbourne, Australia.

A method for measuring the buoyant density of platelets which function normally after the procedure, has been developed and critically compared to published methods.

Following velocity sedimentation into a gradient of polyvinylpyrrolidone-coated colloidal silica particles (Percoll) at physiological pH and osmolarity, 90% ( $n=10$ ,  $SD=10$ ) of the whole blood platelet population was recovered with leukocyte contamination of .002% ( $n=10$ ,  $SD=.001$ ); erythrocyte contamination was less.

After centrifugation to equilibrium through a second continuous linear Percoll gradient, platelet density showed a Gaussian distribution about a mode of 1.0645 g/ml. ( $n=13$ ,  $SD=.0015$ ). Leakage of  $\beta$  thromboglobulin ( $\beta$ TG), lactic dehydrogenase (LDH) and serotonin into the gradients was negligible. Intracellular LDH, serotonin and total protein correlated closely with platelet count, but  $\beta$ TG distribution was skewed towards the denser fractions.

Platelets recovered from the second gradient showed normal aggregation patterns and produced thromboxane  $B_2$  and secreted  $\beta$ TG when stimulated by thrombin or arachidonic acid. Addition of plasma was necessary to produce ristocetin- and ADP-induced aggregation, and significant radioactivity was not associated with platelets isolated from blood to which  $^{125}I$  had been added.

Under physiological conditions, arabinogalactan II (Stractan) caused more spontaneous leakage of  $\alpha$ - and dense-granule markers than did Percoll. Platelets taken from different interfaces of a discontinuous Stractan gradient were seen to contain platelets of the full density range when they underwent equilibrium centrifugation in linear continuous Stractan or Percoll gradients.

True buoyant density of minimally altered platelets has been measured by this new technique.

## 1295

APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) TO THE STUDY OF PLATELET PHOSPHOLIPID METABOLISM. I. Alam, J.B. Smith and M.J. Silver. Cardeza Foundation and Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

A method for the separation of phospholipids using HPLC has been developed. Different radioactive prostaglandins and phospholipids are detected by passing a portion of the effluent from the HPLC column directly through a Radio Flow-1 detector. The remainder of the effluent is collected for phosphate and fatty acid determinations using a fraction collector. This system eliminates the solvent-quenching limitation associated with U.V. detection and offers many advantages over thin layer chromatography (TLC) which frequently requires two dimensional development and scraping of zones from the TLC plate. Separations of phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, spingomyelin and platelet activating factor (PAF) have been achieved. The system has been applied to the study of thrombin-induced changes in the phospholipids of human platelets prelabeled with  $^{14}C$ -arachidonic acid. After incubation of prelabeled platelets for 10 min. at  $37^\circ$  with 5 U/ml thrombin, a 30% decrease in radioactive phosphatidylcholine was detected.

## 1297

PROTEIN SYNTHESIS BY PLATELETS IN VITRO T. Shaw and C.N. Chesterman, Melbourne University Department of Medicine, St. Vincent's Hospital, Melbourne, Australia.

The ability of platelets to synthesise proteins *in vitro*, in particular the  $\alpha$ -granule secretory proteins, has been investigated following platelet isolation by a method which causes minimal leakage of granule contents.

Platelets were isolated by three cycles of Percoll density gradient centrifugation from citrated blood taken from normal donors. Platelet suspensions prepared by this method represent over 90% of the starting population, show negligible (<.002%) leukocyte and plasma protein contamination, and remain capable of aggregation and secretion.

When 1 ml. aliquots of platelet suspension containing  $\approx 10^8$  platelets were incubated at  $37^\circ C$  in a humid 5%  $CO_2$ /95% air atmosphere with  $1\mu Ci$   $^3H$  leucine or  $^3H$  protein hydrolysate, incorporation of label into TCA precipitable material proceeded linearly over a 3 hour period, plateauing after 4 hours. Inhibition of label incorporation was produced by addition of cold amino acids, cycloheximide, puromycin, chloramphenicol, ethidium bromide, fluoride or homologous platelet free plasma which had been exhaustively dialysed to remove free amino acids, but not by addition of albumen,  $\gamma$  globulin, actinomycin D or vincristine sulphate.

A differential release of label paralleling granule secretion could not be demonstrated when, after incubation, thrombin was added at concentrations known to produce a graded dose/response in terms of granule secretion without cell lysis. To date, no peaks of radioactivity have been found to correspond with elution patterns of granule proteins when release produces have been separated by affinity column chromatography following labelling.

The limited capacity of platelets for protein synthesis may be controlled by (a) plasma factor(s), and, at least in normal individuals, does not appear to include the ability to synthesise secretory proteins.