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ALBUMIN AND PLATELETS. F. Forestier*, G. Cherbit**, Y. Solé* M.H. Tersarkissian*. *Service de Biologie Hôpital ND. de B. Secours, 75014 Paris, **Université Paris VII, France.

We set up to point a double antibody radio-immuno-assay to quantify platelet albumin content.

We used commercial reagents such as an antibody of rabbit reacting against human albumin and a highly purified human albumin antigen, labelled with ^{125}I./Chloramine.

The main results were:

- 10^9 platelets contained approximately 1 mg of albumin
- Zonal ultra-centrifugation (105.000 g) in saccharose gradients showed that albumin was located in non-lysosomal α granules.
- The release of albumin occurred at the same time as β Thrombo-Globulin and Platelet Factor 4.

We studied the serum albumin (SA), platelet albumin (PA) and the number of platelets on 144 normal individuals aged from 20 to 90 years.

Our data show that:

- PA decreases in correlation with the platelet count
- the ratio $\frac{PA}{SA}$ increases with age
- a correlation between the level of SA and the platelet counts exists.

These data suggested a regulation mechanism between serum albumin and platelet levels. On the other hand our findings proved an active transport of albumin through the platelet membrane.

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THE INFUSION STUDY OF NEURAMINIDASE: AN ANIMAL MODEL FOR THE STUDY ON DISIALYLATION OF PLATELET AND THROMBOSIS. T. Meguro, K. Yamada, Y. Miyaji and M. Inagaki. Department of Pediatrics, St. Marianna Medical School, Kawasaki, Japan and Department of Pediatrics, School of Medicine, Keio University, Tokyo, Japan

In order to detect the influence of neuraminidase on thrombotic tendency, the infusion studies of neuraminidase were carried out in animal experiments. Recent studies in our laboratories has revealed higher activity of neuraminidase in plasma during acute stages of some inflammatory diseases in which thrombosis tended to be noticed with complications. New Zealand rabbit (N.Z.W.) were used for the animal experiments. Cl. Perfrigens type V or VI of neuraminidase (Sigma) was dissolved in saline and infused to the rabbits intravenously. In one group, one infusion of neuraminidase ranged from 0.5 U per kg to 1.0 U per kg per body weight was carried out. In other group, a continuous drip infusion with 2 U per kg of neuraminidase was given to the animal during 24 hours. In both groups, the blood specimens were obtained at every 24 hours and the examinations were sustained during more than 7 days after the infusions respectively. The neuraminidase activity of plasma was assayed by fluorescent method using synthetic substrate which was kindly made available by Dr. M. Flashner in Syracuse. The sialic acids of platelet was assayed by the method of M. A. Madoff, et al.

In the group of drip infusion, hyperfibrinogenemia and leukocytosis were observed in early stage, followed by thrombocytosis and acceleration of platelet aggregation, in accordance with decreased level of sialic acids of the platelet and increased level of free sialic acids in plasma. In the group of one infusion, most animals died with episodes of thrombocytopenia. Our results conclude that the drip infusion method devised by us would be useful and more applicable in the study of disialylation of platelet and hyperaggregablity and indicated the pathophysiology of some inflammatory diseases with thrombosis, more clearly compared with the one infusion technique.

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MECHANISM OF REGULATION OF PLATELET AMP DEAMINASE. B. Ashby and H. Holmsen. Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA, U.S.A.

Metabolic ATP is converted rapidly to IMP during platelet secretion. The conversion is rate limited by deamination of AMP to IMP. AMP deaminase has been purified to homogeneity from human platelet concentrates by phosphocellulose chromatography. Kinetic studies showed sigmoidal behavior as a function of AMP with $K_{0.5}$ values of 3.5 and 4.0 mM in 0.1 M NaCl and 0.1 M KCl, respectively, at pH 6.5. Activation by saturating ATP converted the velocity versus substrate plot to hyperbolic with a Km of 1.2 mM in either salt. Addition of increasing concentrations of GTP in the presence of NaCl led to activation followed by weak competitive inhibition whereas GTP in the presence of KCl gave strong competitive inhibition with no apparent activation. Consequently, enzyme activity measured at 100 μM AMP in the presence of 10 μM GTP was 22 times greater in NaCl than in KCl. Studies on the effects of pH revealed pH optima around 6.5 in NaCl and 6.7 in KCl. Activation by ATP or GTP shifted the optima to lower pH values whereas inhibition by GTP shifted the optima to higher pH values. All of these results may be described by a model that does not invoke subunit-subunit interactions to explain cooperative behavior but requires obligatory binding of AMP to a distinct activator site on the same subunit before AMP can bind to the catalytic site. ATP and GTP bind to the same activator site as AMP and GTP can also bind competitively to the catalytic site. Changes in activity produced by NaCl or KCl or pH are due to changes in the affinity of the two sites for the various nucleotides in media of different ionic composition and pH. Regulation of AMP deaminase in the intact platelet is thus envisaged as being the result of changes in ionic composition and pH that may accompany platelet stimulation rather than changes in the concentration of effector nucleotides.

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ENERGY CONTROL DURING PLATELET SECRETION: PREDOMINANT ROLE OF ATP-TURNOVER. A. Deijns and J.W.N. Akkerman. Department of Haematology, University Hospital Utrecht, The Netherlands.

Platelet aggregation and secretion of granular contents require metabolic energy. This implies the existence of a control mechanism that adjusts the rate of energy producing pathways to the energy need of these functions. Such a control function has been attributed to the level of metabolic ATP, to the adenylate energy charge (AEC =(ATP + ½ADP)/(ATP + ADP + AMP) and to the velocity of energy generation and consumption (ATP-turnover). In this study we investigated which factor dominates in control of dense granule (^{3}H-serotonin)-, α-granule (β thromboglobulin)- and lysosomal granule (N acetyl β glucosaminidase) secretion. Human gel-filtered platelets were incubated in glucose-free, CN containing medium. Under these conditions ATP-generation only took place in glycogenolysis, which alone was unable to maintain ATP homeostasis. Consequently, metabolic ATP and AEC fell. Addition of glucose restored glycolytic ATP resynthesis wich restored the AEC but the loss of ATP was for the main part irreversible due to hypoxanthin formation. With this system a broad range of metabolic ATP levels and AEC's could be obtained both at decreasing ATP turnover (before glucose addition) and at increasing ATP turnover (after glucose addition). Analysis of secretion velocity of dense-, α- and lysosomal granules after initiation with thrombin (5 U/ml) showed that at decreasing turnover the secretion velocity of all types of granules depended on both metabolic ATP level and AEC. In contrast, at increasing ATP turnover the correlation between secretion velocity and metabolic ATP level or AEC was lost. Instead, dense granule secretion was faster and lysosomal granule secretion was slower than expected on the basis of metabolic ATP level or AEC, whereas α-granule secretion showed intermediate levels. The data indicate that the three types of granule secretion differ in their dependence on metabolic energy and that apart from metabolic ATP and AEC, ATP turnover is of crucial importance for secretion of granular contents.