

MORPHOLOGIC PLATELET CHANGES IN CITRATE BLOOD AND PRP. R. Wiedemann, K. Breddin and H. Grün. Department of Angiology, Medical Center, University, Frankfurt a.M., Germany.

Using high power Nomarski optics the film presents the shape change of thrombocytes in vitro. Their native shape can be studied by immediate fixation of blood at venepuncture. Platelets appear as flat discs. Less than 25% show pseudopodes. In citrate blood and PRP a progressing shape change occurs depending on time after blood sampling and on incubation temperature. Platelets tend to swell and tentacle formation continues with time. At room temperature 90% of thrombocytes have undergone these changes within 60 min. The addition of ADP (10^{-6} molar) to PRP induces the formation and rupture of large vesicles in single platelets. Thrombocytes are aggregating, many of the aggregated platelets show the same large vesicles. They are bursting and the release of a granular material can be observed. The remaining platelet material is fusing to an unstructured mass. Bencyclan (2×10^{-4} molar) affects platelet morphology by inducing a spherical transformation, which is paralleled by the inhibition of platelet adhesiveness, spreading and aggregation. Finally a platelet is shown spreading on a glass surface. The morphologic changes of single platelets (primary shape change) demonstrated in the film probably represent basic processes in hemostasis and thrombus formation.

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THE RELATIONSHIP AMONG PLATELET AGE, VOLUME AND ELECTROPHORETIC MOBILITY. T. Motomiya, H. Yamazaki, Y. Sawasaki and O. Nakajima. Tokyo Metropolitan Institute of Medical Science and Tokyo Medical and Dental University, Tokyo, Japan.

The platelet carries a net negative charge and its change may be an important factor in platelet adhesion and aggregation. Platelet age and volume are also believed to be important factors in its functions. Six male rabbits were injected with 50 μ Ci of 75 Selenomethionine intravenously and EDTA-blood was drawn 3 days after. Platelets were washed 3 times and suspended in 3 mM triethanolamine acetate carrier buffer. Platelet electrophoresis was performed using with a Continuous Free-Flow Electrophoresis VAP-5 (Bender & Hobein, Munich). Electrode buffer used was 100 mM triethanolamine acetate buffer pH 7.4 and electrophoresis was performed at 17.5°C, 1250V and 80mA. Approximately 5×10^8 platelets were electrophoresed per hour into 90 fractions. The fraction #1 is the anode side and #90 cathode side. Platelets in each fraction obtained as a suspension in the buffer were studied for platelet count and volume determinations using with a Coulter Counter ZBi and a Channelyzer C-1000. The radioactivity was measured by a well-type scintillation counter. Platelets were separated well making a single peak at the 27 ± 3.4 fraction. The radioactivity of platelet increased as the fraction number increased but platelet in bilateral side fractions was too small in number and determination of the radioactivity was difficult and the peak radioactivity was at the measurable extreme right fraction (68 ± 3.8). The largest platelet volume also appeared at the measurable extreme right (cathode side) fraction. In conclusion, Continuous Free-Flow Electrophoresis separated platelets well by the difference of cell surface potentials. 75 Selenomethionine labeled platelets which have been thought to be young seem to have low electrophoretic mobility and relatively large volume.

ULTRASTRUCTURAL STUDY OF PLATELET AGGREGATION BY MOUSE 15091A TUMOR CELLS. G.J. Stewart*, R.A. Kuprionas*, G.J. Gasic†, J. Catalfamo† and G.P. Gasic†. *Temple University Health Sciences Center and †University of Pennsylvania Medical School, Philadelphia, Pennsylvania, U.S.A.

Most tumor cells cause aggregation of platelets in heparinized plasma via material shed into culture medium. In this study we investigated the events by transmission electron microscopy. Freshly washed cells were covered with closely spaced microvilli, many of which pinched off during 1 hour of incubation at 37°C. Both cells and shed microvilli were membrane enclosed. Shed microvilli became spherical vesicles containing cytoplasm. Platelets aggregated when stirred with incubated tumor cells or shed material. The aggregates were composed of platelets that showed pseudopod formation, centralization of granules and increase in the open channel system. Platelets around the periphery of aggregates had bulbous portions free of granules (ballooning) but many granules remained in platelets in the interior of aggregates suggesting that release of lysosomal enzymes may have been somewhat limited. Aggregates resembled those induced by ADP rather than by thrombin. Tumor cells were not incorporated into the aggregates. Vesicles were not selectively associated with platelets prior to or during aggregation. While some vesicles were incorporated into aggregates, it appeared that this was a consequence rather than the cause of aggregation. Therefore, vesicles may have produced soluble material that induced platelet aggregation.