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9.30

0212 AUTOMATED MICRODENSITOMETRY AND PROTEIN ASSAYS AS MEASURES FOR PLATELET ADHESION AND AGGREGATION ON COLLAGEN COATED SLIDES UNDER CONTROLLED FLOW CONDITIONS.

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Microscope slides were homogeneously coated over a length of 2 cm with a mixture of soluble and fibrillar collagen and exposed at 37°C and under laminar flow to citrated whole rabbit blood at a flow-rate of 100 ml/min. Surface coverage with platelets (adhesion) and platelet accumulations higher than about 5 µm in height (aggregation) were determined by automated microdensitometry of fuchsine stained 'en face' preparations. The platelet mass per unit surface was measured with a modified Lowry technique whose sensitivity was equivalent to $5x10^5$ platelets. Platelet number, amount of protein and surface coverage with platelet accumulations correlated. After a perfusion time of 10 min thrombi up to 30 µm in height and oriented in the direction of flow had developed on the collagen coated area. Surface coverage with platelets was 75% and the amount of deposited protein 1.4 $\mu g/mm^2$ ($2x10^6$ platelets/mm^2). On the uncoated surface single platelets predominated; the surface coverage was 20% and the density of platelets $8x10^4/mm^2$. Acetylsalicylic acid at 100 µM decreased platelet aggregation by about 80% without affecting adhesion.
The new parallel plate perfusion system offers rapid quantitation of platelet-surface and platelet-platelet interaction after exposure to flowing blood and may also be diagnostically useful.

9.45

0213 ON THE RELEASE FUNCTION OF ADHERING PLATELETS.

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A method for measuring the continuous uptake and release of serotonin during 60 minutes by platelets adhering to a polymer surface is presented.

Heparinized human blood with autologous ^{51}Cr and ^{14}C-serotonin labelled platelets is exposed to the polymer in Chandler loops rotated slowly for different periods. Initially the adhering platelets take up serotonin from non-adhering aggregating platelets by an active process and then slowly begin to release serotonin. Acetylsalicylic acid treated platelets adhere and take up serotonin to the same extent as untreated platelets, but do not undergo a release reaction.

Coating of the polymer surface with fibrinogen prior to blood exposure or with fibrin (achieved by thrombin treatment of the fibrinogen adsorbate) does not affect the degree of adhesion and serotonin uptake, but inhibits the release reaction. When only fibrinopeptide A is split off with Defibrase from the fibrinogen layer the release function of the adhering platelets reappears.

It is concluded that the release mechanism of adhering platelets is not only dependent of the functional state of the platelet itself, but also on the ultimate composition of the protein layer to which the platelets adhere.

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0214 CLOT PROMOTING EFFECT OF PLATELET-VESSEL WALL INTERACTION

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When a piece of vascular tissue (rat,rabbit) is incubated in plasma, clotting occurs. The presence of platelets accellerates this clotting process.Inhibition of vascular prostacyclin (PGI_2) production,either by drugs or by inducing arachidonic acid deficiency,causes the platelets to become activated by the vessel wall,resulting in platelet shape change and -aggregation and in a further enhancement of the coagulation response. Hirudin very actively inhibits both aggregation and clotting whereas ADP scavenging has only a weak effect.This indicates that the aggregation response,occurring upon platelet-vessel wall interaction is for the greater part mediated by thrombin.
Saturated fat feeding increases the clot promoting effect of the platelet-vessel wall interaction.This appeared not to be due to differences in the production of vascular PGI_2 or platelet endoperoxides and thromboxanes (measured as malondialdehyde).
These findings indicate that:

1. Thrombin, locally generated upon the interaction between damaged vessel wall and blood constituents,contribute to arterial thrombus formation;
2. Prostacyclin regulates local thrombin formation via its inhibiting effect on platelet activation;
3. The existence of another, platelet bound affector is postulated.