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THE THROMBOCYTE MEMBRANE IN CIRCULATORY DISEASES

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Summary

There is no certain evidence for the proposition, often put forward, that thrombocytes, or platelets, are involved in atherogenesis. On the other hand, thrombotic deposits in arteries as well as in the blood channels of artificial organs consist at first mainly of platelets. This contribution reviews evidence indicating that the formation of such thrombi depends on alterations in the platelet membrane and that these in turn depend on an agent, apparently ADP, which is released from the erythrocytes.

Zusammenfassung

Es gibt keine sicheren Beweise für die oft vertretene Annahme, dass Thrombozyten oder Plättchen an der Atherogenese beteiligt sind. Auf der anderen Seite bestehen thrombotische Massen in Arterien sowohl als in Blutkanälen künstlicher Organe zuerst hauptsächlich aus Plättchen. Der vorliegende Beitrag befasst sich mit Hinweisen dafür, dass die Bildung solcher Thromben auf Aenderungen in der Plättchenmembran beruhen und dass diese wiederum durch ein Agens ausgelöst werden, offenbar ADP, das von den Erythrozyten freigesetzt wird.

There is no certain evidence for the proposition, often put forward, that thrombocytes, or platelets, are involved in atherogenesis (see, for example, WALTON, 1975). On the other hand, thrombotic deposits in arteries as well as in the blood channels of artificial organs consist at first mainly of platelets. This contribution reviews evidence indicating that the formation of such thrombi depends on alterations in the platelet membrane.

Platelet adhesion and aggregation

(PACKHAM and MUSTARD, 1971; BORN, 1975)

Normally platelets ciculate singly with an average life time in man of about 9 days. Diffe-

rent naturally-occurring agents change platelets from a non-adhesive to an adhesive state. One such agent is collagen which is exposed when there are defects in the endothelial lining of blood vessels. Platelets colliding randomly with such defective areas adhere to the sub-endothelial collagen in the vessel wall. The adhering platelets then undergo a change which is associated with the release of other agents, notably ADP, capable of producing adhesiveness in platelets. This sequence of adhesion and release is, therefore, assumed to account for formation of platelet aggregates in acutely injured blood vessels or of platelet thrombi in vessels damaged by disease such as atherosclerosis. This is supported by clinical evidence (personal communication from Dr W.S. FIELD, Houston, Texas) showing that aggregation-inhibiting drugs prevent transient cerebral ischaemic attacks caused by platelet emboli which form in atheromatous carotid arteries.

The adhesion of platelets to collagen does not require calcium. Platelet aggregation requires calcium and fibrinogen as co-factors (BORN and CROSS, 1964; CROSS, 1964). Aggregation can be accounted for on the assumption of the formation of calcium bridges between sialic acid residues on fibrinogen and similar or other acidic groups on the platelet membrane (BORN, 1969). Recent evidence (MOTAMED, MICHAL and BORN, 1976) indicates that activation of platelets by ADP or other agents is associated with an increase in the glycoproteins exposed on the cell surface. It is conceivable that platelet aggregation may depend on the exposure of these glycoproteins if they should turn out to be the specific receptors for fibrinogen essential for the mutual adhesion of the cells.

The train of events just proposed can account for the growth of mural thrombi of platelets in injured or diseased blood vessels but not in the blood channels of artificial organs, including oxygenators, where there is no collagen. In such situations, the aggregation of platelets must clearly depend on their activation by something other than collagen. Amongst possible activators are the materials making up the channel walls; thrombin formed in the plasma; and/or activating agents released from other circulating cells.

Platelet activation by erythrocytes

There is much evidence that platelets can be activated by at least one agent, which is probably ADP, released from the erythrocytes which outnumber and surround them in the blood. Indeed, the discovery of the activation of platelets by ADP, which is highly specific amongst nucleotides and related substances, began with the demonstration that the adhesion of platelets in columns of small glass beads depended on the presence of red cells and varied in proportion to their concentration (HELLEM, 1961); the agent was identified as ADP (GAARDER,

JONSEN, LALAND, HELLEM and OWREN, 1961). Similar experiments showed later that platelet adhesiveness to glass was increased in the presence of red cells without any evidence of haemolysis (HARRISON and MITCHELL, 1966).

Clear evidence of increased platelet adhesiveness brought about by the operation of flow-mechanical factors on erythrocytes was provided by experiments in which blood was made to flow through branching channels in extra-corporeal shunts (ROWNTREE and SHIONOYA, 1927; MUSTARD, MURPHY, ROWSELL and DOWNIE, 1962). Chambers made of different plastic materials were introduced into a shunt through which haeparinised blood flowed from a carotid artery to a jugular vein of anaesthetised pigs. Deposits of platelets formed consistently on the shoulders of a bifurcation in the flow chamber but nowhere else in the channels. Clearly, therefore, this deposition did not depend on the properties of the materials from which the chambers were made. Furthermore, when the chambers were perfused not with blood but with platelet-rich plasma, no deposit formed, showing that red cells were essential for the increased reactivity of the platelets that resulted in their mural deposition. Although the publications on this do not appear to contain a statement that there was no haemolysis, there is no reason to believe that the experimental conditions caused haemolytic damage to the erythrocytes. The augmenting effect of red cells on the deposition of platelets can also be demonstrated with blood flowing through chambers of other geometrical conformations or other types of wall surface. For example, the endothelium can be removed by introducing a balloon catheter into rabbit aortas, exposing a subendothelial surface composed mainly of connective tissue; such a subendothelial surface can also be exposed to blood flowing in annular chambers of different diameters to provide a variety of shear rates at the blood-surface interface (BAUMGARTNER and HAUDENSCHILD, 1972; BAUMGARTNER, 1973; TURITTO and BAUMGARTNER, 1974). When blood was perfused over such a surface, platelets soon covered almost all of it and there were numerous platelet aggregates or thrombi on the adhering layer. When platelet-rich plasma was perfused instead of blood, very few platelets were deposited.

The increased deposition of platelets from flowing blood associated with the presence of the red cells could be caused by physical or chemical mechanisms or, of course, by both acting synergistically. A physical mechanism would depend essentially on an increase in the later diffusivity of platelets caused by the flow behaviour of the erythrocytes. Indeed, the diffusivity of platelets in flowing blood was estimated to be two orders of magnitude greater than that predicted for platelets diffusing in plasma (TURITTO, BENIS and LEONARD, 1972; TURITTO and BAUMGARTNER, 1975). This is consistent with the enhanced radial fluctuati-

ons of erythrocytes and latex microspheres (2 μm in diameter) in flowing suspensions of red cell ghosts (GOLDSMITH, 1971). High platelet diffusivity is required also to explain the growth of mural thrombi. This must depend on successful platelet-to-platelet collisions, the rate of which between platelets following streamlines near the walls would hardly be sufficiently high to account for the rapidity of growth observed in vivo (BEGENT and BORN, 1970; RICHARDSON, 1973).

There is evidence also, however, for a chemical mechanism in the increased adhesiveness of platelets in the presence of red cells, i.e. through their release of ADP. The concentrations of ADP required are very small (10^{-6}M or less) so that its direct demonstration in plasma would probably be impossible for two main reasons: first, because the cells contain so much ATP and ADP that the slightest damage to them would swamp the plasma with ADP; and secondly, because the outer surfaces of the cells as well as the plasma contain enzymes which catalyse the rapid breakdown of ADP (HASLAM and MILLS, 1967; BOLTON and EMMONS, 1967; PARKER, 1970). Therefore, the release of ADP into plasma has been inferred indirectly by demonstrating that the effect of red cells on platelets is prevented by the addition of enzyme systems capable of utilising ADP specifically. Thus, in the presence of the phosphoenolpyruvate kinase system which removes ADP by enzymic phosphorylation to ATP, the difference in the adhesiveness to glass of platelets from whole blood or from platelet-rich plasma is abolished and so is the increase in platelet adhesiveness caused by adding red cells to platelet-rich plasma (HARRISON and MITCHELL, 1966). Similar results are obtained with added apyrase which catalyses the hydrolysis of ADP to AMP. Indirect evidence of this kind is analogous to the conclusion that the abolition by atropine of, say, a secretion indicates that it is mediated physiologically by acetyl choline which, unless its destruction is prevented by an anti-cholinesterase, is too rapidly destroyed to be demonstrated directly.

There are in principle two other ways of inhibiting the effect of erythrocytes on platelets and so preventing or minimising thrombus formation in oxygenators and other artificial organs: first, by inhibiting the reactivity of the platelets; and secondly, by inhibiting the release of the activating agent(s) from the erythrocytes. In recent years rapid progress has been made with our understanding of platelet reactivity and with its inhibition by drugs so that it may suffice to refer to reviews (BORN, 1966; MILLS, SMITH and BORN, 1970; DE GAETANO, VERMYLEN and VERSTRAETE, 1969; BORN, 1975; SCRIABINE, 1975). Recent experiments of ours (BORN, BERGQUIST and ARFORS, 1976) suggest that it is possible to inhibit the release of platelet-activating agent, presumably ADP, from erythrocytes in flowing blood under conditions in which they are not demonstrably otherwise damaged. It seems pro-

bable that the reversible deformations which red cells undergo in the course of even slightly non-laminar blood flow suffice for the release of enough ADP (plus ATP rapidly dephosphorylated to ADP) to activate the accompanying platelets. The objection that a continuous release of this kind would deplete red cells of adenine nucleotides essential for their normal survival in the circulation is invalidated by the equally continuous two-way traffic in nucleotides between tissues and red cells (HENDERSON and LE PAGE, 1959; MAGER, HERSHKO, ZEITLIN-BECK, SHOSHANI and RAZIN, 1967; ADAMS and HARKNESS, 1973) whereby any leakage is apparently made good during the cells' life-span.

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