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MINIREVIEW

The Behavior of Platelets at Foreign Surfaces (42807A)

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Artificial Surfaces Exposed to Blood in Vivo. As medical technology develops, circulating blood is being exposed more and more often to foreign surfaces. The artificial surfaces that are contacted by blood *in vivo* include those in hemodialysis devices, shunts for dialysis, cardiopulmonary oxygenators, circulatory support devices, artificial valves, artificial hearts, artificial bypass grafts in large- and small-diameter vessels, apheresis equipment, and vascular catheters. None of the surfaces in these devices has the nonthrombogenic characteristic of the normal endothelium and, to varying extents, the foreign surfaces activate the coagulation and complement systems and promote platelet adhesion and aggregation. The consequences of the interaction of platelets with the surfaces of these devices include formation of thrombi and thromboemboli, thrombocytopenia, reduced platelet survival due to removal by the reticuloendothelial system, and reduced platelet function in response to hemostatic stimuli, resulting in bleeding (1–4). After blood has been exposed to an artificial surface, substances released from the platelet granules, such as platelet factor 4 and β-thromboglobulin, and products formed by activated platelets (thromboxane B_2) have been detected in the circulation. It is not surprising that there is widespread interest in determining how blood interacts with these surfaces and in defining the characteristics of surfaces that are most thromboresistant.

Early Studies. Some of the first experiments on platelet adhesion to a foreign surface were done by Wright in 1941 (5) with a rotating glass flask containing anticoagulated whole blood. In 1960, Hellem (6) introduced the technique of pumping whole blood through a column of glass beads and measuring the reduction in platelet count. This test was later refined by Salzman (7) and Bowie *et al.* (8) as a means of identifying patients with von Willebrand disease. In these glass bead columns, not only platelet adhesion but also the release of platelet granule contents and platelet aggregation occur, so the test is termed a "platelet retention test," rather than a test of adhesiveness. Further *in vitro* experiments explored the effect on platelet adhesion of coating glass surfaces with various plasma proteins, leading to the demonstration that platelets readily adhered to surfaces on which fibrinogen was adsorbed, but did not tend to adhere to surfaces that had been coated with albumin (9–14). Many *in vivo* investigations have been done with experimental animals in which blood has been permitted to flow past various artificial surfaces, and information about the effects of such surfaces on the constituents of the blood and on the formation of thrombi is also available from studies of patients whose blood has been exposed to artificial surfaces in a variety of devices.

Measurement of Adhesion. The wide variety of *in vitro* methods that have been used to measure platelet adhesion, both static and under conditions of flow, have been reviewed by others (15–17). The two main methods of quantitating platelet adhesion are the morphometric technique of Baumgartner and his colleagues (18) and isotopic techniques with platelets labeled with chromium-51 or indium-111 (19). Although Baumgartner's method (which was originally developed for the subendothelium) is time consuming and permits the examination by transmission electron microscopy of only a small surface area, it does distinguish between the adhesion of single platelets and platelet thrombi, and between platelets that have contacted the surface but maintained their disk shape and platelets that have spread on the surface. The isotopic methods do not distinguish single adherent platelets from platelet thrombi, but have the advantages that adhesion over a large area can be readily measured and the method can be ap-

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plied to hard artificial surfaces that cannot be cut to prepare sections for transmission electron microscopy. In addition, the isotopic method can be used in man since the radioactivity can be quantitated by external detection devices. Recently, for *in vitro* studies, a radioimmunoassay using a monoclonal antibody to the glycoprotein IIb/IIIa complex on the platelet membrane has been developed to avoid the manipulations involved in labeling platelets and permit the determination of platelet adhesion from whole blood (20).

McManama *et al.* (15) have outlined the characteristics that an ideal adhesion measurement system would have. These include the use of nonanticoagulated whole blood, flow conditions that mimic those in the part of the vascular tree in which the surface is to be incorporated, body temperature, a well-characterized and uniform surface, and both short and long periods of exposure to blood. Ideally, platelet adhesion should be visualized directly, and platelet aggregation, release of granule contents, and fibrin formation should be readily measurable.

Properties of Artificial Surfaces. Attempts to develop nonthrombogenic materials have led to many studies of the properties of artificial surfaces that affect the rate of activation of coagulation and the interactions of platelets with them. These have been discussed in several recent reviews (21–24). The thrombogenicity of artificial surfaces varies widely according to the physical and chemical properties of the materials (25, 26). Thrombogenicity depends to a great extent upon the relative adsorption of certain plasma proteins, especially fibrinogen and albumin, to surfaces. A number of techniques, both physicochemical and biological, have been used to modify surfaces to lessen their thrombogenic properties. The biological modifications include adsorbing or bonding to the surface materials such as heparin, albumin, or prostaglandin E_1 (PGE_1) which inhibit coagulation or platelet adhesion (26–30). Another approach is to seed prostheses with endothelial cells to promote the formation of an endothelial surface on them (31–34). Short-term studies of platelet adhesion to biomaterials in an *in vitro* flow chamber do not predict the results of long-term studies of events occuring on the same materials when they are implanted in the arteries of experimental animals. This lack of correlation is probably due to the fact that long-term events such as pseudointimal hyperplasia and calcification are governed by control mechanisms different from those that modulate the short-term thrombotic response (35, 36).

Blood Flow. Platelet adhesion has been shown to be a direct function of blood flow and shear rate (37–39). The hematocrit influences platelet adhesion, mainly because of the physical effects of the red blood cells (40–43), although there may be some contribution to thrombus formation from the platelet-aggregating agent ADP that is lost from them under conditions of high shear (44). Several reviews of the effects of flow patterns on bringing platelets into contact with surfaces, platelet adhesion, and platelet aggregation are available (40, 45–47).

Adsorption of Plasma Proteins to Artificial Surfaces. *In vivo,* platelets probably never come into direct contact with an artificial surface that is exposed to circulating blood, because plasma proteins adsorb almost instantly to such surfaces. In the process, some of these proteins may be denatured or, at least, their conformation may be altered or the proteins that adhere initially may be replaced by others. When surfaces are exposed to complex mixtures of proteins, the extent to which the various proteins are adsorbed is not a simple function of their concentration, and the proportions may differ with different physical and chemical characteristics of the surfaces and change with time. In considering the formation of platelet aggregates on artificial surfaces, one must really concern oneself with the interaction of platelets with the adsorbed plasma proteins.

Early studies showed that precoating glass surfaces with albumin inhibited platelet adhesion to them, whereas precoating with fibrinogen promoted platelet adhesion (9, 10). Further studies have shown that many artificial surfaces preferentially adsorb fibrinogen from plasma or whole blood (48–51). This adsorption occurs within 2 sec and is generally limited to a layer about one molecule thick. Salzman and Merrill (22) have con-

cluded that for platelets to adhere to a surface, a film of adsorbed fibrinogen must have formed. Salzman's group (51–53) used polyclonal and monoclonal antifibrinogen antibodies to obtain evidence that the extent of platelet adhesion to a fibrinogen-coated surface is less well correlated with the total concentration of adsorbed fibrinogen than with the concentration of "native" fibrinogen, which would presumably be recognizable by the platelet fibrinogen receptors. These findings are contrary to earlier suggestions that changes in the conformation of adsorbed fibrinogen molecules may lead to platelet adhesion and activation (54).

Vroman *et al.* (55) have pointed out that fibrinogen deposited from plasma onto hydrophobic substrates remains unchanged, but if fibrinogen is deposited from plasma onto hydrophilic surfaces (e.g., glass) it is quickly replaced by high-molecular-weight kininogen and, to a smaller extent, by Factor XII. Brash *et al.* (56) have presented results indicating that this "Vroman effect" may be attributable to the active cofactor form of high-molecular-weight kininogen that is generated by exposure to kallikrein during Factor XII-dependent contact and activation.

Under conditions in which fibrin forms on an artificial surface, platelets will adhere to the polymerizing fibrin (57) but they do not adhere readily to fully polymerized fibrin, providing thrombin has been removed or inactivated (58). The fibrin surface that develops on implanted vascular prostheses is less thrombogenic than the prosthetic surface immediately after implantation (3).

The adsorption to artificial surfaces of other proteins that may influence platelet adhesion has been studied less thoroughly and it does not appear that any of them are likely to be adsorbed from normal plasma or blood in sufficient amounts to have a major influence on platelet adhesion to artificial surfaces. However, a number of studies of individual proteins adsorbed to artificial surfaces indicate that platelet adhesion can be promoted by proteins other than fibrinogen (59).

Although it is known that under conditions of high shear, von Willebrand factor is involved in platelet adhesion to the subendothelium (60, 61), most studies of the influence of von Willebrand factor on platelet adhesion to foreign surfaces have involved the platelet retention test in glass bead columns. Platelet retention is reduced in von Willebrand's disease, and under carefully controlled conditions, platelet retention is proportional to the concentration of von Willebrand factor in blood (7). It should be emphasized, however, that this test involves both platelet adhesion to the beads and the formation of platelet aggregates, and it appears to be the later stage of platelet-to-platelet adherence that requires von Willebrand factor, released ADP, and the glycoprotein IIb/IIIa complex on the platelet surface (62). From the results of experiments with monoclonal antibodies against von Willebrand factor or the platelet glycoprotein IIb/IIIa complex, McPherson *et al.* (62) have concluded that "von Willebrand factor is altered during rapid passage of blood through the glass bead column so that it attaches to glycoprotein Ib, exposing glycoprotein IIb/IIIa which then binds to the altered von Willebrand factor or fibrinogen, either of which can induce platelet aggregation and retention in the column." The theory that von Willebrand factor is altered upon exposure to an artificial surface has also been used by Furlan *et al.* (63) to explain their findings that large (2.02 μm in diameter) latex particles coated with von Willebrand factor agglutinate formalin-fixed platelets in the absence of other aggregating or agglutinating agents; they concluded that the adsorption of the von Willebrand factor to the surface exposed binding sites for platelets. They also suggested that the large von Willebrand factor miltimers were involved since exposure of smaller beads (0.312 μm) to von Willebrand factor did not produce a surface that would agglutinate platelets. These changes in von Willebrand factor that occur when it interacts with this surface apparently alter it in such a way that ristocetin becomes unnecessary as a cofactor for platelet agglutination by von Willebrand factor.

This effect of an artificial surface on von Willebrand factor apparently did not occur in Santoro's experiments in which he used a static test of adhesion of human platelets to polystyrene dishes coated with von Willebrand factor (64). He reported that the adhe-

sion of unactivated platelets was very limited over a 1-hr period. Thrombin or ristocetin greatly enhanced adhesion; this enhancement was attributed to the binding of von Willebrand factor to the platelet glycoprotein IIb/IIIa complex in the case of thrombin-activated platelets and to glycoprotein Ib in the case of ristocetin-mediated adhesion. Platelets adherent by means of the ristocetin-mediated mechanism were much more flattened and fully spread on the von Willebrand factor-coated surface than were platelets activated with thrombin. It may not be appropriate, however, to compare results with isolated platelets in a static system with results in flowing blood in which red cells and shear effects influence adhesion.

Experiments with blood from pigs with von Willebrand factor deficiency have shown that von Willebrand factor plays an essential role in the events leading to occlusion of porous, woven Dacron vascular graft fabric by aggregated platelets (65).

In studies with canine von Willebrand factor precoated onto plasticized polyvinyl chloride arteriovenous shunts in dogs, Lambrecht *et al.* (59) found that it was more active than fibrinogen in promoting platelet adhesion and in anchoring thrombi to the surface of biomaterials. Caution must be exercised, however, in extrapolating results from different species to man since there are variations among species in surface-induced thrombogenicity (66).

Other proteins coated onto surfaces, which promote platelet adhesion, are fibronectin (59, 67–71), vitronectin (72), thrombospondin (73), α_2-macroglobulin (70), γ-globulin (9,70), and laminin (69). Not all of these proteins, however, lead to the full sequence of platelet changes; for example, when platelets adhere to a surface coated with laminin, little change in their shape occurs, indicating very little activation, and when they adhere to a surface coated with fibronectin, they extend pseudopodia and spread, but they do not release their granule contents (69). In addition, changes in the conformation of the adsorbed proteins may be influenced by the type of surface to which they have adsorbed, and these changes will affect the extent of platelet adhesion (71).

Adhesion of thrombin-activated platelets to a polystyrene surface coated with fibronectin, fibrinogen, or von Willebrand factor is inhibited by the tetrapeptide Arg–Gly–Asp–Ser (74), which has been identified as the cell adhesion domain of these proteins (75).

Surfaces coated with thrombin also promote platelet adhesion (15).

Platelet Membrane Glycoproteins. Platelets probably interact with surfaces through some of their membrane glycoproteins, but this interaction is incompletely understood. If a surface is coated with fibrinogen, it seems likely that the platelets would adhere through the fibrinogen receptors (the glycoprotein IIb/IIIa complex) on their surface. The difficulty with this concept, however, is that this receptor does not become available to bind to fibrinogen unless the platelets have been stimulated. In addressing this dilemma, Lindon *et al.* (52) have calculated that the effective concentration in the layer of fibrinogen that adsorbs to glass can be as high as 350 mg/ml, which is 100 times the concentration in plasma. They point out that, "Such a bound array might behave as a multivalent ligand with the fixed fibrinogen molecules acting to crosslink fibrinogen receptors in the platelet membrane and inducing platelet activation in a manner similar to the 'mobile receptor' hypothesis of Jacobs and Cuatrecasas [(76)]. Low fibrinogen binding affinities of nonactivated platelets would not preclude such a model."

Under some conditions *in vivo,* particularly if blood flow is turbulent and some hemolysis occurs, ADP from the red blood cells could stimulate the platelets so that their fibrinogen receptors become available and the platelets become able to bind to the fibrinogen adsorbed to the surface (2). In addition, if coagulation has been activated with the formation of thrombin, it too will make the fibrinogen receptors on the platelets available.

Early studies showed that thrombasthenic platelets, which lack the glycoprotein IIb/IIIa complex, are not retained in glass bead columns (7, 77, 78). On activated platelets, this complex can function as a receptor for von Willebrand factor as well as for fibrinogen. A monoclonal antibody that prevents the binding of both fibrinogen and von Willebrand

factor to this receptor strongly inhibited platelet retention in these columns (62). Thus it would appear that the presence on platelets of an uninhibited GPIIb/IIIa complex is required for their adhesion to glass beads in the platelet retention test. In contrast, thrombasthenic platelets have been shown to adhere to the subendothelium (79). In one *in vivo* study in baboons, administration of monoclonal antibodies against the glycoprotein IIb/IIIa complex was reported to reduce platelet thrombus formation on Dacron vascular grafts (80), but an investigator from the same group later showed no effect of one of these monoclonal antibodies on platelet deposition on Gore-Tex grafts in baboons (81). Thus the role of the glycoprotein IIb/IIIa complex in platelet adhesion to the surfaces of these grafts is not clear. It should be emphasized that since both adhesion and aggregation would have been involved, the effect of the antibodies on adhesion per se cannot be assessed in these *in vivo* experiments.

The membrane glycoprotein that has been most frequently implicated in the binding of platelets to the subendothelium is glycoprotein Ib because the absence of this glycoprotein on the platelets of patients with the Bernard–Soulier syndrome has been shown to result in defective adhesion of platelets to the subendothelium, although this is demonstrable only at high shear rates. Studies of the interaction of Bernard–Soulier platelets with artificial surfaces are rare. Weiss *et al.* (82), however, did observe that platelet retention in glass bead filters, measured in either directly sampled or heparinized blood, was decreased in the presence of normal concentrations of von Willebrand factor. More recently, two groups of investigators have shown that a monoclonal antibody (6D1) directed against glycoprotein Ib partially inhibits platelet retention in glass bead columns (62, 83). It must be emphasized, however, that this test is affected by a number of reactions in addition to the adhesion of the platelets to the surface of the glass.

The effect of other platelet membrane components on platelet adhesion to artificial surfaces does not appear to have been assessed. At present, the glycoprotein IIb/IIIa complex is recognized as having the major role, although glycoprotein Ib may exert some effect if von Willebrand factor is involved.

Morphological Changes. The initial adherence of platelets to an artificial surface that has been exposed to blood occurs within 1 min, but as indicated above, proteins from the plasma (mainly fibrinogen) adsorb to the surface before the platelets reach it. Much of the experimental work has been done with citrated blood, in which the low concentration of ionized calcium may influence platelet adhesion and subsequent events. It has been suggested that the initial adhesion of platelets does not involve the expenditure of metabolic energy and hence is a relatively passive process (15, 84). According to Turitto and Baumgartner (40), the behavior of the platelets when they interact with artificial surfaces differs from their behavior when they react with the vascular subendothelium. A platelet monolayer forms, but the surface becomes saturated, with less than 30% of it covered by platelets within 10 to 40 min. Many of the platelets are not closely attached to the surface and have not spread out on the surface or lost their granule contents. These authors point out that few studies have been done with nonanticoagulated blood at high shear rates, but that under these conditions, a limited platelet monolayer also develops and is stable for 1 to 3 hr. Most investigators, however, describe extensive changes in platelet morphology upon contact with an artificial surface, regardless of whether it has been coated with a plasma protein, although the extent of the changes may be influenced by the type of surface (85). The platelets change from their normal disk shape to a more rounded form, extend psuedopodia, and spread out, and the originally raised central region (granulomere) gradually flattens as the granule contents are released. According to Goodman and Albrecht (85), who studied human platelets in an artificial medium containing albumin, but no other proteins, three distinct cytoskeletal zones develop, each surrounding the central, raised granulomere zone. These have been termed the inner filamentous zone, the outer filamentous zone, and the peripheral web. Loftus and Albrecht (86) have used colloidal gold-labeled fibrinogen to study the redistribution of the platelet

fibrinogen receptors during these changes. They observed that fibrinogen was initially distributed uniformly over the platelet surface, including the pseudopodia and the periphery of the platelets, and then, as spreading progressed, the bound fibrinogen migrated toward the granulomere and eventually became localized over it and the inner filamentous zone; the peripheral areas were then relatively free of bound fibrinogen. Further experiments with a monoclonal antibody–gold conjugate directed against the fibrinogen receptor (the glycoprotein IIb/IIIa complex) showed the same receptor distribution. This movement of the fibrinogen receptors probably involves reorganization in the underlying cytoskeleton to which these receptors appear to be attached in aggregated platelets (87, 88). Myosin redistributes during platelet spreading and becomes more centralized; redistribution of actin has also been observed (86). Olorundare *et al.* (89) have shown that inhibition of myosin light-chain kinase with the calmodulin antagonist trifluoperazine, which prevents the phosphorylation of myosin and blocks its interaction with actin, inhibits the redistribution of the bound, gold-labeled fibrinogen.

Allen *et al.* (90) used differential interference contrast microscopy with cinematographic recording to study the details of the shape change and release of granule contents of human platelets in citrated platelet-rich plasma upon adhesion to siliconized glass. They observed that pseudopodia that extended into the medium could be retracted, but those that extended in contact with the substratum were unlikely to retract. Spreading occurred in at least three different ways: (a) The hyalomere spread either symmetrically or asymmetrically without any preceding pseudopodial activity; (b) the hyalomere spread radially as a "web" between two pseudopodia that were already extended; (c) the hyalomere spread laterally from a thick pseudopodium. As the platelets spread, their rounded central granulomere slowly flattened. The particles in the granulomere remained clustered and as the dense bodies were discharged, they left craters behind in the surface of the platelet. Small cavities or pits were also observed on the surface of the platelets; these may represent sites of discharge of the α granules of the platelets.

Under conditions of laminar blood flow which prevent the accumulation of aggregating agents released or formed by the platelets, only a monolayer of platelets will adhere to a surface. If blood flow is disturbed so that aggregating agents can accumulate, platelets in the circulation near the adherent platelets will be stimulated and will form aggregates with the adherent platelets.

Submicroscopic Changes during Adhesion. At a submicroscopic or biochemical level, many reactions underlie the visible platelet changes. The reactions that platelets undergo following their adhesion to a fibrinogen-coated surface (and other surfaces to which they adhere) require metabolic energy and the operation of the contractile mechanism (15), and appear to be similar to those that result from their stimulation by aggregating agents such as thrombin (13, 91). Evidence that supports this supposition is the observation by Salzman *et al.* (51) that when platelets loaded with aequorin and suspended in a medium containing fibrinogen were passed through a column of glass beads, a rise in cytosolic calcium occurred that resembled the rise that follows stimulation of platelets with thrombin. However, even for extensively studied soluble aggregating agents such as thrombin, the transduction of the signal from the membrane glycoproteins to the interior of the platelet is not fully understood. Analogy with other cells and studies with permeabilized platelets indicate that guanine nucleotide-binding proteins (G proteins) are probably involved in the activation of phospholipase C (92, 93). When phospholipase C is activated, it acts on the minor membrane component phosphatidylinositol 4,5-bisphosphate, with the production of the second messengers inositol trisphosphate and diacylglycerol. Diacylglycerol decreases the concentration of Ca^{2+} required for the activation of protein kinase C, which phosphorylates a 47-kDa protein that is involved in triggering the fusion of granule membranes with either the plasma membrane or the membrane of the open canalicular system, resulting in the secretion of the contents of platelet-dense granules and α granules. The α granules con-

tain a number of proteins, including fibrinogen, fibronectin, thrombospondin, von Willebrand factor, β-thromboglobulin, platelet factor 4, and platelet-derived growth factor (94). Some of the proteins of the α granules may remain attached to the platelets at the point of discharge (95) and may take part in platelet adhesion to the surface and in stabilizing the platelet aggregates. The dense granules contain the platelet-aggregating agent ADP and they also contain serotonin, which by itself is a very weak aggregating agent, but can potentiate the effects of other aggregating agents.

In thrombin-stimulated platelets, the 20-kDa light chain of myosin is phosphorylated by myosin light-chain kinase, an enzyme that is regulated by Ca^{2+} and calmodulin. The Ca^{2+} is considered to be mobilized from the vesicles of the dense tubular system by inositol trisphosphate, which raises the concentration of Ca^{2+} in the cytosol from about 1 to 100 μM (92). It is not known whether an influx of Ca^{2+} from the external medium also occurs because platelet adhesion requires extracellular Ca^{2+} (96), so comparisons of systems with and without external Ca^{2+} cannot be made. The increase in cytosolic Ca^{2+} activates phospholipase A_2, which hydrolyzes arachidonic acid from membrane phospholipids. Arachidonic acid is oxidized by cyclooxygenase to prostaglandin G_2, which gives rise to prostaglandin H_2 and is then converted by thromboxane synthetase to thromboxane A_2 (TXA_2), a short-lived platelet-aggregating agent that quickly gives rise to thromboxane B_2 (TXB_2), which is inactive. Since cyclo-oxygenase is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs, TXA_2 formation can be prevented with such drugs. TXA_2 not only causes platelet aggregation, it also causes the release of the contents of platelet granules. On the surface of platelets that have undergone a release reaction, two steps of the intrinsic coagulation pathway are greatly accelerated (13), which may be of some importance since artificial surfaces may initiate this pathway by activating factor XII (22). If thrombin forms in and around the mass of aggregating platelets, it will have several effects. It will cause further release, TXA_2 formation, and aggregation; thrombin-induced aggregation can occur independently of TXA_2 or ADP. In addition, thrombin will convert fibrinogen to fibrin, which will stabilize the platelet aggregate on the surface (13).

Thrombus formation on a surface is unlikely to be extensive unless blood flow is disturbed with vortex formation so that the products from the stimulated platelets, and thrombin, can accumulate. Adams and Feuerstein (97) and Hubbell and McIntire (98) have investigated the concentration profiles of ADP, TXA_2, and thrombin in the vicinity of growing thrombi under several wall shear rates. According to the latter investigators, the local concentrations of ADP and TXA_2 were "marginally large enough" to stimulate platelets individually, whereas the concentrations of thrombin were much greater. These agonists can act synergistically with each other and with released serotonin to promote thrombus formation, so even low concentrations may be effective in causing the formation of thrombi.

Changes in the cytoskeleton that are associated with release of granule contents and aggregation involve polymerization of actin and hydrolysis of actin-binding protein. When platelets are activated, the GPIIb/IIIa complex on the platelet membrane assumes a configuration such that this complex can act as a receptor for fibrinogen, which is considered to be the main protein linking aggregated platelets. The GPIIb/IIIa complex becomes associated with the filaments of polymerized actin when platelets have aggregated, but there is no association upon fibrinogen binding to activated, but unaggregated, platelets (99). If extensive aggregation occurs, the Ca^{2+}-dependent protease (calpain) is activated and cleaves the actin-binding protein, disrupting the membrane skeleton. Fox (99) has suggested that this may be necessary before the bundles of actin filaments can associate with the GPIIb/IIIa complex. It is not known whether, or to what extent, any of these cytoskeletal changes occur during the initial adhesion of the platelet monolayer, although the fibrinogen receptor certainly becomes available on the adherent platelets since aggregates can form on them. Some of the proteins (e.g., throm-

bospondin) released from the α granules may stabilize the linkages between aggregated platelets (100) since the fibrinogen receptor does not remain available indefinitely. Alternatively, fibrinogen binding may, in time, become irreversible (101).

In addition to the metabolic changes that occur within platelets when they are stimulated by adhesion to an artificial surface, alterations or damage of receptors on their plasma membrane have been reported. During extracorporeal circulation through a membrane oxygenator, the number of fibrinogen receptors was reduced (102), partial loss of the glycoprotein IIb/IIIa complex was demonstrated with a monoclonal antibody to the complex (102), and glycoprotein IIIa was identified on the surface of the extracorporeal circuit (103). It seems likely that the bleeding diathesis induced by procedures such as cardiopulmonary bypass may be partly attributable to platelet dysfunction due to loss of a large proportion of the fibrinogen receptors. Loss of platelet α_2-adrenergic receptors during extracorporeal circulation has also been observed (104). The presence of PGE_1 largely prevented the losses of both the fibrinogen receptor and the α_2-adrenergic receptors. Loss of membrane glycoproteins (or of glycopeptides from them) may be at least in part responsible for the thrombocytopenia that follows procedures such as cardiopulmonary bypass, extracorporeal membrane oxygenation, and hemodialysis (3) since cleavage of glycopeptides from the platelet surface by treatment with enzymes such as chymotrypsin, plasmin, or trypsin also results in the removal of platelets from the circulation and shortens platelet survival (105).

Thrombocytopenia, Thrombosis, and Shortened Platelet Survival. In addition to the effect of dilution by administered fluids, the thrombocytopenia observed during cardiopulmonary bypass is probably attributable in part to adhesion and aggregation of platelets on the membrane (4). Severe defects in the ability of platelets to function normally also contribute to the problem of hemorrhage associated with this procedure (1), and platelet survival has been shown to be shortened following the extracorporeal perfusion during cardiopulmonary bypass (106). Thrombocytopenia may also accompany hemodialysis (107). Heparin administered with both cardiopulmonary bypass and hemodialysis may enhance the aggregability of platelets and contribute to the thrombocytopenia.

The major complication attributable to platelets in patients with artificial heart valves is thromboembolism (1, 4). In patients with prosthetic, small-diameter arterial grafts, thrombotic occlusion frequently occurs. In both these circumstances, platelet survival is shortened (108–110). Although Dacron grafts are very thrombogenic when first inserted, platelet survival is usually normalized by 1 year (111). Platelet survival in dogs with Dacron grafts returns to normal more rapidly if the grafts have been seeded with endothelial cells before insertion (34). However, platelet accumulation on Dacron grafts apparently persists beyond the period of shortened platelet survival (112). Savage *et al.* (113) have provided evidence that platelets in the circulation of patients with Dacron bifemoral aortic prostheses not only have shortened platelet survival, but also show a reduced content of dense granule ADP and ATP. As these authors point out, this could be due to (i) reversible platelet interactions with the foreign surface that involve the release of granule contents, but recirculation of the platelets; (ii) selective removal of the platelets with the greatest content of adenine nucleotides (possibly the most functional platelets); or (iii) increased platelet turnover, leading to the production of platelets with a lower content of adenine nucleotides. In keeping with these findings about adenine nucleotides, the buoyant density of platelets has been found to be reduced in patients who have undergone cardiopulmonary bypass (114); these observations support either of the first two theories listed above about changes in platelets when they interact with surfaces.

Inhibitors. A distinction must be made between agents that inhibit the initial adhesion of individual platelets to an artificial surface and agents that interfere with the formation of aggregates (thrombi) on these adherent platelets. The initial stages of adhesion and

spreading are of less consequence clinically than the subsequent thrombotic and thromboembolic events, and consequently, inhibition of the latter events has received the most attention. It should be emphasized that not all of the inhibitors of aggregation inhibit adhesion.

Inhibition of adhesion by pretreating surfaces with albumin or inhibitory prostaglandins (PGE_1, PGI_2 (prostacyclin), and its analogs) has been mentioned earlier. In recent studies, these materials have been covalently bonded to artificial surfaces (24, 27–30). The inhibitory prostaglandins raise the concentration of cyclic AMP in platelets and inhibit both adhesion and aggregation, although higher concentrations are required to inhibit adhesion. Their ability to prevent platelets from changing shape and hence from spreading on a surface may account for their inhibitory effects on adhesion. The spreading reaction is also inhibited by drugs that impair the contractile mechanism in platelets and by inhibitors of calmodulin (15).

In contrast, aspirin (which prevents the formation of the aggregating agent TXA_2 during platelet activation) appears to have no inhibitory effect on platelet adhesion (115, 116). Addonizio *et al.* (117) have concluded that platelet activation during simulated extracorporeal circulation occurs in at least two ways: an adhesion-mediated, aspirin-insensitive pathway, and a second pathway mediated by TXA_2 that is associated with aggregation and blocked by aspirin. Under the conditions of their experiments, release of platelet α granule proteins (assessed by measurement of release of low-affinity platelet factor 4) was decreased by 50% in the presence of aspirin.

There have been many investigations with experimental animals and a number of clinical studies of the effect of drugs that inhibit platelet functions on platelet accumulation on artificial surfaces *in vivo.* The drugs that have received attention include aspirin, dipyridamole, aspirin plus dipyridamole, ibuprofen, sulfinpyrazone, dazoxiben, and ticlopidine. For short-term applications such as during hemodialysis or cardiopulmonary bypass, prostacyclin and some of its analogs that have less pronounced hypotensive effects have been used. Some of the results have been reviewed (1, 4). The most suitable type of drug may depend on the particular application; for example, anticoagulants appear to be more effective than antiplatelet agents in controlling thromboembolic complications of prosthetic heart values (118), whereas thrombotic occlusion of prosthetic grafts is not well controlled with anticoagulants and is now most commonly treated with platelet-suppressive therapy, according to Bick (1); however, the evidence that the latter approach is clinically effective is not convincing.

Early studies showed that dipyridamole or sulfinpyrazone, but not aspirin, normalized shortened platelet survival in patients with prosthetic values (108–110). However, aspirin does reduce the thromboembolic complications of artificial heart valves (119). More recently, ^{111}In-labeled platelets were used to assess the effect of sulfinpyrazone on platelet deposition on Dacron vascular grafts in man, and the drug was found to be ineffective (120). Similar studies showed that ticlopidine also did not prevent platelet deposition on these grafts in man (121), and suloctidil had no effect in baboons (122). In studies in baboons of cannula-induced platelet consumption, aspirin by itself did not reduce platelet consumption, but it potentiated the reductions caused by dipyridamole or sulfinpyrazone (123).

Summary. Many conditions affect the interaction of platelets with foreign surfaces, including the type of surface, modifications of the surface, conditions of blood flow, the adsorbed layer of plasma proteins, changes in this protein layer with time, and the animal species in which experiments are done. Platelets probably never adhere directly to a foreign surface *in vivo,* because upon exposure of the surface to blood, plasma proteins, principally fibrinogen, are adsorbed almost immediately. When platelets adhere to such a surface and spread on it, they are activated in much the same way as when they are exposed to a strong aggregating and release-inducing agent, but in contrast to aggregation caused by some agonists, adhesion is not dependent on the formation of TXA_2 or the release of ADP. It does appear to depend on

external Ca^{2+}. Much less is known about the initial adhesion reaction than about platelet aggregation (thrombus formation) on the adherent platelets, although the morphological changes resulting from adhesion have been described. It is surmised that the metabolic and cytoskeletal changes upon adhesion are similar to those that are involved in the response of platelets to other activating agents. The consequences of adhesion include the formation of thrombi and thromboemboli, thrombocytopenia, reduced platelet survival, reduced platelet function in response to hemostatic stimuli, and the appearance in the circulation of products released or formed by activated platelets. Many efforts are being made to develop surfaces and to set up conditions that will minimize platelet adhesion, but it has not yet been possible to find a foreign surface that has and can maintain the nonthrombogenic characteristics of the normal endothelium.

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