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Platelet Function

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HEMOSTASIS is the process by which the body seals off leaks of the circulatory system to prevent loss of contents. There are three components equally important in the maintenance of hemostasis: vascular integrity, platelets, and coagulation factors. Bleeding results from defects in one or more of these elements. Of the three components of hemostasis, platelet function is perhaps the least understood, and therapy is thus often the least well managed. It is the purpose of this review to discuss current knowledge of platelet function. Basic physiology of platelet function, the interaction of platelet activity with coagulation factors, and tests of platelet function are considered. In addition, topics with immediate clinical implications in the practice of anesthesia are covered: interaction of platelets with drugs and anesthetics, platelet storage and transfusion, and effects of extracorporeal techniques on platelet function.

Early investigators independently devised methods of studying platelet function and created new terms with which to describe their results. Unfortunately, little communication seems to have occurred among research groups, and both terminology and methodology proliferated into a mass of literature very difficult to interpret. Progress in understanding platelet function was slow, and many clinicians attempting to follow developments in this field were confused. As laboratory techniques became standardized and a generally accepted platelet "language" evolved, one of the reasons for the early confusion became clear. Platelet physiology was found to be much more complex than originally thought. It also became apparent that platelets are very sensitive structures. A series of distinct but related events must occur in sequence before a circulating platelet can accomplish its hemostatic mission. These sequential events can be inhibited or activated by minor alterations in experimental conditions and by many substances both *in vivo* and *in vitro* (fig. 1). Difficulties still exist in separating actual platelet characteristics from experimentally-induced artifacts.[1]

Platelet Physiology

Table 1 lists the generally accepted sequence of steps that occur during activation. The initial event in platelet activation is exposure of the platelet to an appropriate stimulus.[2] *In vivo*, the stimulus is usually platelet contact with collagen-containing subendothelial basement membrane following damage to a vessel. The platelet reacts by undergoing a shape change, from its natural disc form to a "spiny" sphere.[3]

Having completed the shape change, the activated platelets become "sticky" and form primary aggregates. "Stickiness," more formally called "platelet adhesion," and primary aggregation appear to develop simultaneously. Platelet adhesion is the affinity of platelets for non-platelet surfaces. Primary aggregation is a reversible process in which platelets develop an affinity for each other. One theory suggests that adhesion is initiated by platelet contact with collagen, and primary aggregation is stimulated by low levels of adenosine diphosphate (ADP) released during the initial injury to the vessel.[4] It is important to note that calcium and fibrinogen must be present for ADP-induced aggregation to occur.[5]

This phase of platelet activation culminates with the release reaction, wherein the contents of cytoplasmic granules are released extracellularly.[6] The released substances include ADP, serotonin, platelet factor 4 (PF4), catechols, and factors that modify vascular permeability and integrity.[1,4] ADP is the most potent aggregating agent, and its escape from platelets undergoing the release reaction causes more and more platelets to become activated. When the stimulus to platelet activation is minor and few platelets extrude their intragranular contents, insufficient ADP will be released to cause self-sustaining aggregation. Platelets already aggregated will resume their native shape and return to the circulation.[7] When, however, a threshold concentration of ADP is achieved, large numbers of platelets undergo the release reaction and aggregate irreversibly with each other. This is called "secondary" aggregation.

Interaction of Platelets with Clotting Factors

Platelets not only form a plug as an initial step in securing hemostasis, they also actively participate in several steps of the coagulation cascade[8] (fig. 2). Platelet factor 3 (PF3) is essential for activation of factor X by the complex of factor IXa, factor VIII and calcium ion, and for conversion of prothrombin to thrombin by the complex of factor Xa, factor V and calcium ion. PF3 has many characteristics of a phospholipid, but Marcus has presented convincing evidence that PF3 is a property of lipoproteins within, and inseparable from, the platelet membrane.[1]

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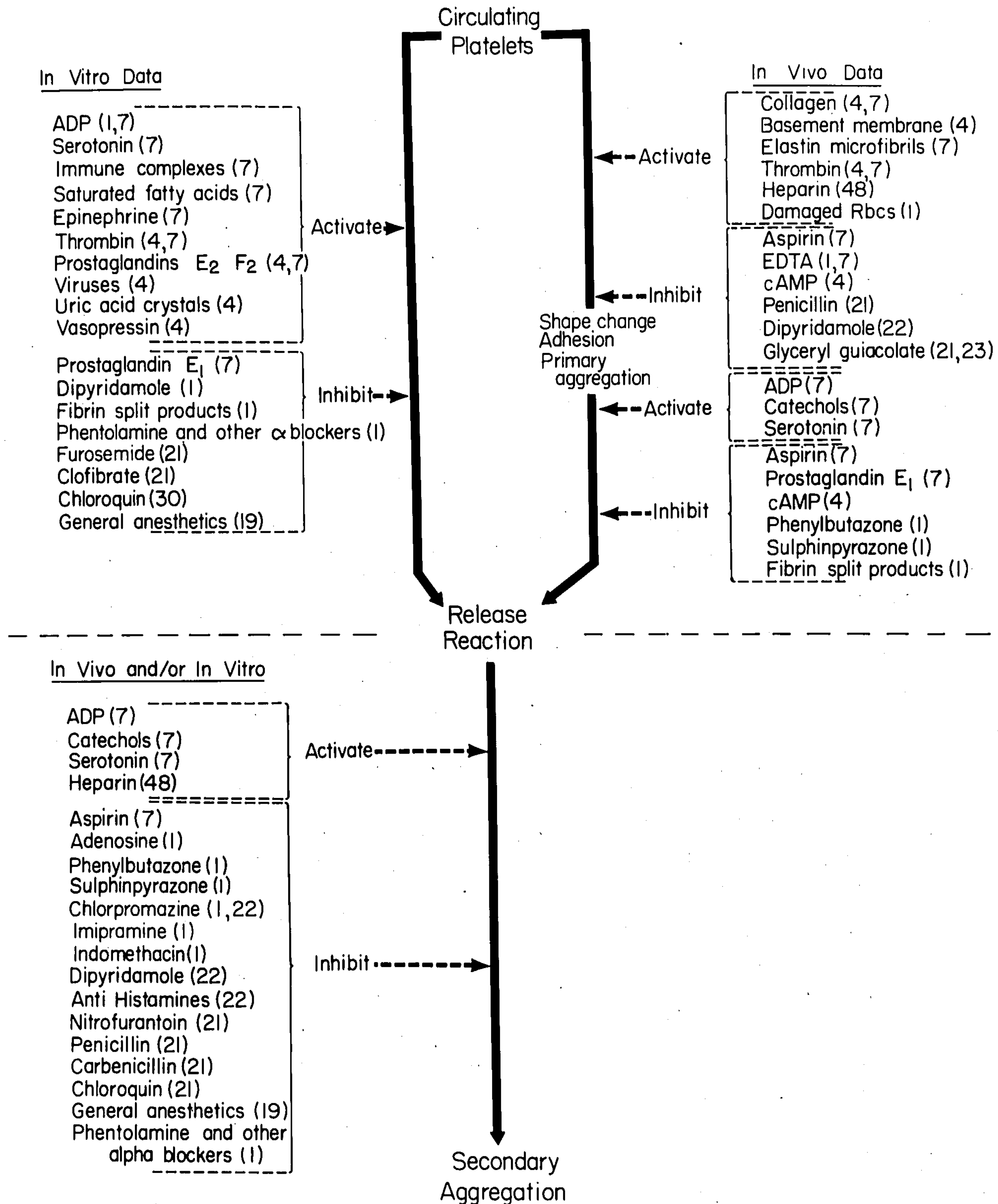

FIG. 1. Substances proven or theorized to affect platelet function. A precise categorization of substance or site of interaction with platelet function is not possible, because study techniques varied widely and results are not strictly comparable. Substances affecting primary aggregation *in vivo* are separated into activators and inhibitors in the right-hand column. Substances affecting primary aggregation *in vitro* are similarly separated in the left-hand column. *In-vitro* testing does not permit the isolation of the several steps in platelet aggregation prior to the release reaction and thus, these steps are indicated *in vivo* only. Substances influencing secondary aggregation either *in vivo* or *in vitro* are listed in the lower left column. Several substances, such as ADP, are shown to influence platelets at more than one site. This occurs when a substance is studied under various experimental conditions such as different concentrations or temperatures.

Numbers in parentheses refer to references.

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TABLE 1. Steps in Platelet Activation

1. Shape change
2. Platelet adhesion
3. Primary aggregation
4. Release reaction
5. Secondary aggregation
6. Fibrin formation
7. Retraction

In contrast, platelet factor 4 (PF4) can be recovered from plasma after platelet aggregation. PF4 is a protein present in the granular fraction of platelet homogenates, and it neutralizes heparin. The exact role of PF4 is not definitely established, but the inactivation of factor Xa, which requires only minute amounts of heparin in circulating blood, might be prevented by PF4.[4]

Platelets have also been shown to play a role in the activation of factor XII in the presence of ADP, and possibly to provide an alternative route bypassing factor XIIIa by collagen-induced activation of factor XI. This may explain why patients deficient in factor XII, who have markedly abnormal coagulation profiles, do not bleed excessively.[9]

Fibrinogen, originating from both plasma and stimulated platelets, is activated by thrombin, and a network of fibrin surrounds and permeates the platelet mass. *In vitro*, thrombin has also been shown to release the contents of platelet lysosomes. The significance of this interaction *in vivo* has not been determined. The final step in the sequence, clot retraction, is probably due to a contractile mechanism found in platelets.[10]

Tests of Platelet Function

Bleeding disorders on the basis of thrombocytopenia are more common than those based on a defect in platelet function. Therefore, a *platelet count* is the first step in evaluating platelets. Normal platelet counts are 240,000 ± 100,000/mm^3.[11] Thrombocytopenia due to decreased production can be differentiated from that due to increased destruction by a bone marrow examination, with the former showing hypoplasia of megakaryocytes and the latter, increased thrombocytopoiesis. A platelet count greater than 100,000/mm^3 is necessary to provide a normal Ivy bleeding time of less than 6 minutes,[12] and more platelets than 25,000/mm^3 are necessary to avoid spontaneous hemorrhage.[13]

The *template bleeding time* method of Ivy is now a well-accepted measure of platelet function. In fact, Levine has recently described this test as "the only test of platelet function capable of predicting excessive bleeding."[14] A blood-pressure cuff on the arm to be tested is inflated to a pressure of 40 torr and an incision 9.0 mm long and 1.0 mm deep is made with a scalpel on the volar surface of the midforearm. The incision is touched at exactly 30-second intervals with a piece of absorbant paper, and the time interval until blood no longer moistens the paper is determined. Normal time is less than 6 minutes. Earlier criticisms of the test were due to failure to perform the test in a uniform manner. Strict adherence to precise depth and length of incision has been facilitated by a special blade holder and has greatly improved the reliability of this test. The bleeding time method of Duke is rarely used today since the Ivy method is more sensitive and reproducible.

The tourniquet (Rumpel-Leede) test and the *capillary fragility test (Gofflin index)* are performed in the same way. A tourniquet is inflated to the point midway between systolic and disastolic pressure for 5 minutes. The number of petechiae in a circle 3.0 cm in diameter on the volar surface of the midforearm is counted 5 minutes after release of the tourniquet. A normal value is fewer than ten petechiae within the circle. However, when the test is positive, the number of petechiae often need not be counted, as the forearm is covered with showers of petechiae. In the presence of an adequate number of normally functioning platelets, a positive test is attributed to alterations in capillary pressure and fragility. Positive test results are also obtained in the presence of thrombocytopenia and certain primary disorders of platelet function, such as von Willebrand's disease and Glanzmann's thrombasthenia. This test is nonspecific, yields many false-positive results and is infrequently performed today.

Clot retraction is another function of platelets that can be grossly measured. When maintained at 37 C, a clot should begin to retract within two to four hours. While efforts to quantitate the extent of retraction have been attempted (*e.g.*, measuring the amount of serum expressed by the clot after four hours), the test remains a qualitative "positive" or "negative" test, *i.e.*, the presence of any clot retraction is accepted as satisfactory. Clot retraction is a function of hematocrit, fibrinogen, platelets, and possibly viscosity. Retraction may be delayed or incomplete in thrombocytopenia or primary platelet defects.

Platelet adhesiveness has been measured by many different techniques. Bowie's method is the most widely used.[15] Platelet counts are determined before and after a 5.0-ml blood sample is infused over 2.6-g glass beads (Superbrite type 070, Minnesota Mining and Manufacturing Company) in a polycarbonate plastic tube at a rate of 5.1 ml/min, which gives contact time of blood with glass beads of 12 seconds. The difference between the two counts divided by the original count is expressed as a percentage. The normal range is 37 to 84 per cent. Rossi and Green have suggested using the term "platelet retention test" to describe

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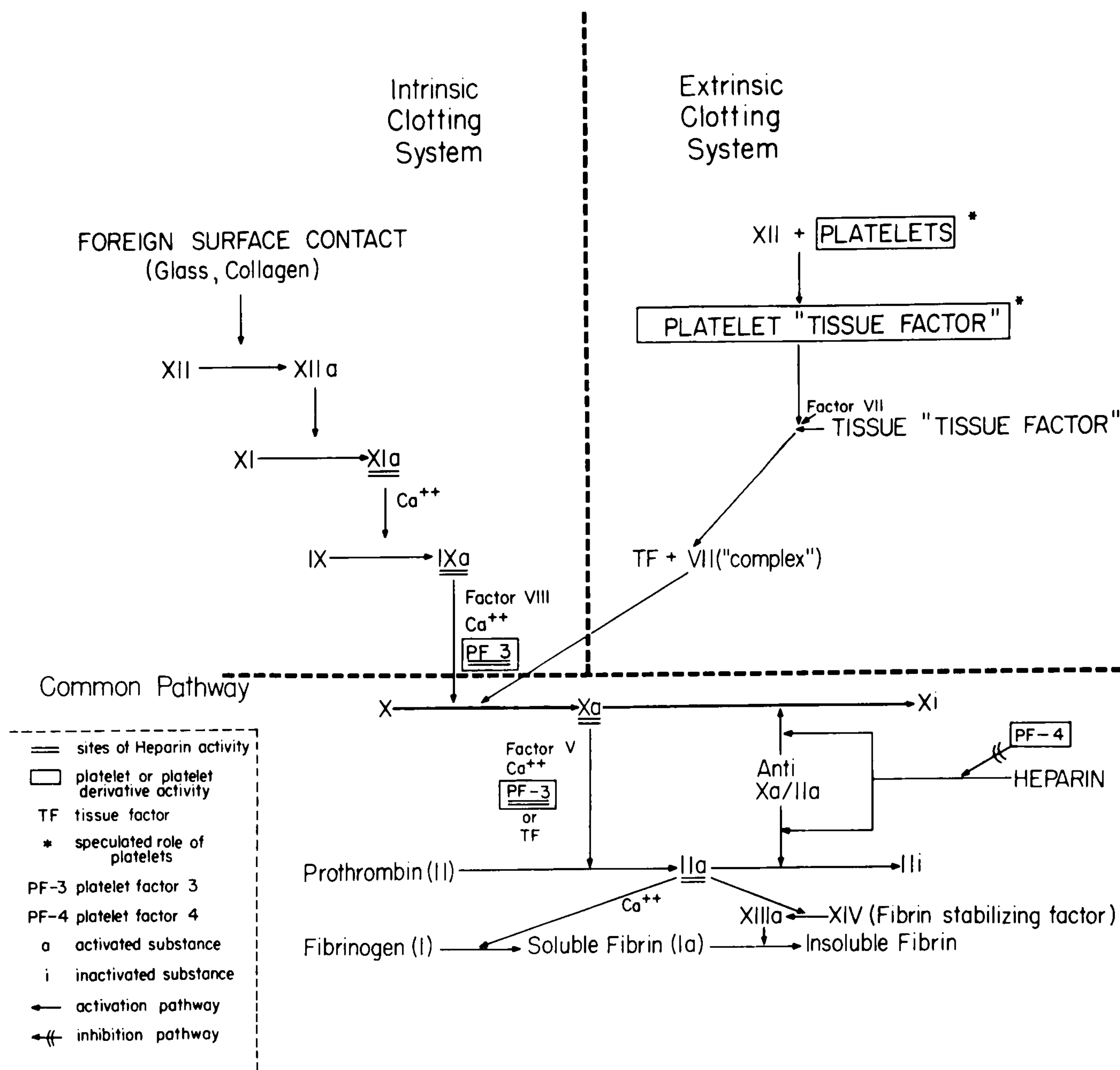

FIG. 2. Sites of platelet action in the coagulation cascade. The generally accepted scheme of the coagulation cascade is modified to emphasize the sites of platelet and heparin activity.[9,10] The upper left section contains the intrinsic clotting system; the upper right section contains the extrinsic clotting system and the lower section contains the final common pathway. Substances enclosed in boxes represent platelet or platelet derivative activity. Underlined factors illustrate sites of heparin activity. The right-hand portion of the final common pathway illustrates the theoretical interaction of heparin with PF4 and their effect on the inactivation of factors Xa and IIa. Heparin, in combination with circulating substances termed anti-Xa/IIa, facilitates inactivation of Xa and IIa. PF4 antagonizes this heparin activity.

this test, since aggregation of platelets to each other as well as adhesion of platelets to the glass beads determines the percentage reduction.[16] In addition to the decrease in platelet retention seen in primary platelet function defects such as von Willebrand's disease, platelet function defects secondary to myelofibrosis or uremia will decrease retention.

An *in-vivo* variation of this test has been described. Serial platelet counts are performed on blood exuding from an incision made in the performance of a template bleeding time. Serial platelet counts are made at 2-minute intervals beginning at one minute and continuing until bleeding stops or the ninth minute. Platelet counts will decrease

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to very low levels as the bleeding diminishes due to the adherence of most of the platelets to the surfaces of the cut tissues.[11]

In addition to the above-listed tests, which are available for clinical use, *platelet aggregation* in response to various stimuli is being measured more commonly. In 1962, Born described a method for quantifying the measurement of platelet aggregation based on an increase in light transmission through platelet-rich plasma as the platelets aggregate at different rates in response to various stimuli.[17] Much of the knowledge of platelet physiology has been determined with the use of the platelet aggregometer, a machine designed to permit recording and quantifying the change in light transmission produced by platelet aggregation.

Clinical Applications of Platelet Physiology

Interactions of Platelets with Drugs and Anesthetics

The chance observation that anesthetic agents affected platelet function occurred early in the development of platelet function testing and has been repeated frequently with other drugs. This effect is not to be confused with the well-known thrombocytopenia produced by some drugs through either bone-marrow depression or platelet destruction. While determining the period of time following mechanical injury to cerebral vessels during which a platelet thrombus could produce emboli, Born and Philip observed that different anesthetics significantly altered this period. Urethane produced a four- to fivefold increase in the duration of this period compared with ether and pentobarbital, all other variables being constant. They speculated that the known local irritant and sclerosing properties of urethane injured the vascular endothelium sufficiently to prolong the effects of mechanical trauma.[18] More recently, Ueda nicely demonstrated that ADP-induced platelet aggregation was inhibited *in vitro* in a dose-related manner by methoxyflurane, halothane, ether, cyclopropane, and nitrous oxide. The concentrations of anesthetic agents that produced 50 per cent inhibition of platelet aggregation showed a close correlation with concentrations used clinically. Ueda suggests that anesthetics may change the surface characteristics of platelet cell membranes, thereby interfering with their cohesion.[19] The clinical importance of this observation remains unclear.

Drugs may affect platelet activation at any step.[20] Aspirin and non-steroidal anti-inflammatory drugs such as indomethacin and phenylbutazone are most frequently cited. These drugs impair the release of endogenous ADP in response to an appropriate stimulus, thereby inhibiting the release reaction and secondary aggregation. Dipyridamole inhibits primary aggregation by an unknown mechanism. Many other drugs have important *in-vitro* effects on platelet function, and there is a need for further tests of their *in-vivo* effects on platelets. The list of drugs that affect platelet function continues to grow (fig. 1).[21,22,23]

High-dose aspirin therapy (5.0–6.0 g per day for seven days) has long been known to prolong the prothombin time in a manner similar to that of coumarin. More recently it has been appreciated that much smaller doses will prolong the bleeding time. In fact, platelet concentrates from donors taking just two aspirin (600 mg) have been shown to be less effective in shortening the bleeding times of patients with thrombocytopenia than platelet concentrates from non-aspirin-consuming donors.[24]

While the original interest in this area was related to drug effects on hemostasis *per se*, the concept of pharmacologic inhibition of platelet reactivity as an approach to antithrombotic therapy has gained considerable support with the recognition of the important role of platelets in thrombus formation.[20] Dipyridamole, a coronary dilator introduced for treatment of angina pectoris in the early 1960's, was associated with increased bleeding when patients taking this drug underwent surgical procedures. Subsequent investigation suggested that dipyridamole in high doses (50 μg/ml) inhibited platelet aggregation, at least to collagen. Becker *et al.* tested the effects of dipyridamole on porcine platelets during cardiopulmonary bypass and found platelet counts after bypass to be higher than those in control animals, suggesting that the drug may have inhibited aggregation of platelets and thus prevented loss of these platelet aggregates during bypass. Post-bypass platelet aggregation was also found to be better in study than in control animals, leading to the conclusion the dipyridamole's effect on platelets was transitory. Thus, more platelets were able to survive bypass and contribute toward hemostasis in the post-bypass period.[25] Further work is needed in this area to produce a very short-acting inhibitor of platelet function for use in the preoperative period. Alternatively, a combination of an inhibitor and its pharmacologic antagonist, analogous to *d*-tubocurarine and prostigmine, may be used. This need for a very short-acting inhibitor of platelet function in the surgical patient is in contrast to the goal of a sustained-action drug for use in antithrombotic therapy of medical patients. For example, the effect of aspirin on platelet function may last as long as 72 hours. This duration of action is undesirable in a patient who may need surgery during that period. It has even been suggested that if a specific antiaggregant not affecting adhesion could be developed, it might be used in conjunction with coumarin or heparin to prevent thrombosis without significant impairment of hemostasis.[26]

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The "white" arterial thrombus consists of a plug of platelet aggregates, while the "red" venous thrombus, the result of activation of the intrinsic clotting system, consists of erythrocytes incorporated into a fibrin network. While platelets may play a role in initiating the clotting process, and reduced platelet survival in patients who have recurrent venous thrombosis does argue for such a role, it is generally agreed that the drugs of choice for treating thromboembolic disease are anticoagulants rather than platelet function inhibitors.[27,28] These act at various places in the coagulation cascade and have been shown to have a significant effect in reducing morbidity and mortality from thromboembolic phenomena in patients with venous disease. In contrast, the results of anticoagulant therapy alone in arterial disease have been equivocal at best, despite an abundance of research in this area.

Pharmacologic inhibition of platelet function is receiving increasing attention since the arterial "white" thrombus is known to be composed primarily of platelets.[29] There are several disease processes where platelet inhibition may be potentially beneficial: cerebral, peripheral and cardiovascular arterial disorders, geriatrics in general, and shock-lung syndromes. Dipyridamole, in conjunction with coumarin, was significantly more effective in preventing emboli in patients who had prosthetic heart valves than was coumarin alone. Several investigations have suggested that anti-platelet therapy is beneficial in coronary artery disease, and large, multicenter, well-controlled clinical studies are presently under way to determine the value of platelet-inhibiting drugs in this population.[20] It is difficult to study cerebrovascular disease since its clinical course is so variable and the incidences of the two end points, stroke and death, are so low. Nevertheless, evidence suggests that both aspirin and sulphinpyrazone, a uricosuric agent that has an *in-vitro* effect on platelets similar to that of aspirin but no demonstrable *in-vivo* effect, may prevent ischemic attacks. Evidence that platelet inhibition is helpful has also been found in a geriatric population. Sulphinpyrazone produced a significant reduction in mortality, especially due to vascular disorders, in an institutionalized geriatric male population.[29] Chloroquine, which inhibits primary, collagen-induced platelet aggregation, has been shown to improve pulmonary hemodynamics in an animal shock-lung model.[30]

Platelet Storage and Transfusion

Platelet concentrates (PC) and platelet-rich plasma (PRP) have been prepared for years, and have been infused into patients in an attempt to control hemorrhage due to thrombocytopenia. Widely divergent results have been reported concerning the ability of such transfusions to increase platelet counts, alter platelet function tests, or diminish clinical bleeding. A close look at these studies reveals that conditions during collection, handling, and storage of platelets vary markedly.[31] Type of anticoagulant, temperature, *p*H, mechanical trauma, and duration of storage have been shown to affect the ability of platelet concentrates and platelet-rich plasma to achieve hemostasis.

A recent symposium reviewed the available data on platelet storage.[32] Optimal conditions for platelet storage include storage volume of 50 to 70 ml, maintenance of *p*H greater than 6, continuous gentle agitation, and selection of appropriate containers. The optimal storage temperature remains controversial. Prior to 1969 most investigations of platelet function were accomplished with platelets stored at 4 C. Gardner and Murphy accumulated evidence from the literature and then produced data that showed *in-vivo* platelet life span to be significantly diminished by storage at 4 C. They found that normal platelet life span could be demonstrated upon reinfusion following storage at room temperature (22 C) for as long as 96 hours.[33] In 1973, Becker *et al.* again showed longer *in-vivo* platelet survival in platelets stored at 22 C than at 4 C for 72 hours. However, they demonstrated that the 22 C platelets were unable to correct the bleeding disorder in thrombocytopenic patients or healthy volunteers treated with aspirin, and that platelets stored at 4 C for 72 hours were capable of shortening bleeding time and improving hemostasis. They concluded that when platelet storage is to exceed 24 hours, refrigeration at 4 C should be utilized.[34] Valeri has reported similar conclusions.[35]

Today the majority opinion holds that storage of platelets at room temperature (22–24 C) causes a reversible "storage lesion" that prevents effective hemostasis during the first 8–24 hours following infusion into a bleeding patient. After this 8–24-hour interval, however, these platelets are able to improve the bleeding time and to survive longer than platelets stored at 4 C. It seems advisable that platelets stored at 4 C be utilized to treat active bleeding secondary to platelet deficiencies, and platelets stored at 22 C be used for prophylaxis in thrombocytopenic patients needing platelet transfusions. When storage is for periods shorter than 12–24 hours, platelet function appears to be preserved regardless of storage temperature.[32]

Prostaglandin E_1 is a potent inhibitor of both adhesion and aggregation. Platelet storage life in whole blood can be prolonged by the addition of prostaglandin E_1 (e μg/unit stored whole blood) after collection of blood from donors. Higher platelet yields and improved platelet function are attributed to this technique even though the time between blood collection and platelet fractionation is prolonged three days.[36,37]

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Those benefiting from platelet transfusions include leukemic patients who become thrombocytopenic spontaneously, or secondary to chemotherapy; patients who have severe aplastic anemia; patients made thrombocytopenic by massive blood transfusion; and some patients undergoing surgical procedures that necessitate cardiopulmonary bypass.[38]

The first two categories of patients rarely need the attention of an anesthesiologist. When operations on these patients are necessary, management includes preoperative platelet transfusions with frequent monitoring of platelet count and bleeding time to insure an uneventful course. Ideally, such transfusions should be administered immediately prior to operation rather than several hours beforehand or the preceding evening, and the bleeding time and platelet count should be determined immediately before as well as 15–60 minutes, 4–8 hours, and 24 hours after transfusion to assess the efficacy of transfusion.[32,38] While it is impossible to fix an absolute minimum platelet count below which the risk of hemorrhage contraindicates surgical procedures, a range of 50,000 to 75,000 is generally accepted,[39] with an upper minimum level being 100,000.[38]

Massively bleeding patients, however, present more complex problems with little time available for preoperative evaluation. Many etiologic factors have been cited in the coagulopathies associated with massive transfusions. Miller *et al.*[39] showed that war casualties requiring more than 20 units of transfused blood developed bleeding problems that could not be corrected by fresh-frozen plasma. The reductions in platelet count were found to vary directly with the amounts of blood transfused in these patients, and abnormal bleeding was very likely to occur when the platelet count fell below 65,000/mm^3. Normal euglobulin lysis times and absence of fibrin split products suggested that disseminated intravascular coagulation and primary fibrinolysis were not important etiologic factors in these cases. Transfusion of fresh whole blood corrected the bleeding disorders and increased platelet counts in all patients. It seems clear from Miller's data that dilutional thrombocytopenia occurred and was the major factor contributing to the abnormal bleeding in their patients. Since fresh whole blood was used in this study, and since fresh-frozen plasma does not contain platelets, it is highly probable that the platelets present in the fresh whole blood were responsible for securing hemostasis. As major centers devote more efforts to preparation and administration of individual blood components, platelet concentrates will be more readily available.

Platelet transfusions are not usually indicated in management of patients who are thrombocytopenic secondary to increased destruction of platelets, since the survival of infused platelets may be measured in minutes to hours. Platelet transfusions may even be harmful in some conditions characterized by rapid platelet destruction, such as disseminated intravascular coagulation (DIC), where it is possible that platelet transfusion will stimulate further coagulation, with deleterious effects. In cases of idiopathic thrombocytopenic purpura (ITP) the use of steroid therapy is much more effective than platelet transfusion in increasing the platelet count.[38]

It is very difficult to predict the ability of a platelet transfusion to increase a patient's platelet count because so many variables are involved. However, given the ideal situation of a healthy unmedicated donor and recipient, an increase of about 5,000–10,000 platelets/mm^3 for each unit of platelet concentrate infused is a reasonable estimate.[13,40] Such platelets stored at 22 C and meticulously handled, can be expected to circulate for approximately eight days, in contrast to a normal life span of 9–11 days. Storage at 4 C reduces survival to 2 to 3 days.[32,35,38]

Platelets, Extracorporeal Circulation, and Hemodilution

A reduction in platelet count is a consistent finding following cardiopulmonary bypass (CPB). In addition, the remaining platelets may not function normally.[41] The severity of damage to the platelets and clotting factors is directly related to duration of cardiopulmonary bypass. This potential qualitative as well as quantitative defect in platelets has led many centers to seek methods of minimizing the problem. One solution has been the administration of platelet concentrates routinely after bypass. However, such a practice is wasteful of a preparation still in short supply, and has been shown not to be necessary on a routine basis.[42,43] Since it is impossible to predict which patient will have defective platelets following bypass, use of platelet concentrates should be reserved for those patients who have demonstrated a need for platelets based on evaluation of platelet count and function.

A more practical technique to aid in avoiding thrombocytopenia is isovolemic hemodilution, which involves immediate pre-bypass removal of a portion of the patient's blood volume and replacement with either crystalloid or colloid volume expanders. Following cardiopulmonary bypass, the patient's own, fresh, undamaged, whole blood can be reinfused while the volume substitutes are removed via natural or drug-induced diuresis. Although reports are conflicting and none demonstrated either a significant increase in platelet count or shortening of the partial thromboplastin or prothrombin time, significant savings in the amounts of stored blood needed for patients undergoing cardiopulmonary bypass have usually been demonstrated.[44,45] Any technique that permits a reduction in the blood requirement for these patients also

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reduces the risks of serum hepatitis, transfusion reactions, and isoimmunization. These advantages, as well as the financial savings, have led many centers to use some form of hemodilution.

The major differences among hemodilution techniques lie in the methods of collection and storage, replacement solution compositions, and types of anticoagulants chosen for the sequestered blood. Regardless of technique, collection must include careful mixing of blood with anticoagulant, maintenance of sterile collection pathways, and minimal disruption of cardiovascular stability. Short-term storage of blood with careful handling is unlikely to diminish platelet function. Replacement fluids are either balanced salt solution or 5 per cent protein solution or combinations of the two. Proper choice must take into account the patient's volume status, disease processes, and type of operation planned.

Choice of anticoagulant has long been debated. Blood may be collected from patients into citrate solutions or directly into empty containers following systemic heparinization. We originally advocated the former technique, since heparin may adversely affect platelets and thereby defeat one of the aims of hemodilution, *i.e.*, the preservation of viable platelets. However, there are several disadvantages to this technique. Phlebotomy utilizes relatively small arterial or venous lines, which prolong the collection time and may delay operation. Withdrawal through these lines, prior to heparinization, is slower, and is accompanied by simultaneous, but prolonged, replacement. As a result, the blood withdrawn is itself progressively hemodiluted. When the monitoring arterial cannula is utilized for collection, vital blood pressure monitoring must be temporarily suspended, and if blood samples are needed for analysis, potential breaks in the system increase the risk of contamination of the arteriotomized blood.

Because of these disadvantages, techniques of hemodilution utilizing heparinized blood are becoming more popular. One technique involves sequestering the calculated volume of heparinized blood directly from the right atrial or vena caval cannula into reservoirs or blood bags just prior to initiation of bypass and simultaneously replacing intravascular volume with pump prime via the aortic cannula.[44] This technique of rapid (1–2 minutes) withdrawal and replacement permits blood with a higher hematocrit and a higher platelet count to be preserved. Since the systemic heparinization technique offers some distinct clinical and technical advantages, the effects of heparin on platelets must be carefully evaluated.

It is now known that heparin adversely affects several steps in platelet activation *in vitro*, in addition to its long-recognized inhibition of certain clotting factors.[46,47] The sites at which heparin interacts with platelet function are indicated in figure 1, and interactions with the clotting mechanism are shown in figure 2. Many investigations into the platelet–heparin interaction produced conflicting data because different aspects of platelet activation were being observed and control of experimental conditions was not uniform.[48,49]

Recently, Zucker has shown that, with careful handling of stored blood, platelet counts in heparinized and citrate-phosphate-dextrose (CPD) blood are similar. After reviewing the conflicting laboratory data on this subject, she concludes that heparin enhances primary aggregation, induces secondary aggregation by increasing the release reaction, and may reduce the release reaction subsequently provoked by other stimulants such as epinephrine.[49]

The significance of these *in-vitro* findings to clinical practice may be minor. Recent clinical data suggest that heparin has minimal deleterious effects on platelets or clinical bleeding. Studies conducted during open-heart surgery have measured pre- and post-bypass bleeding. No difference was found in platelet counts of patients following reinfusion of sequestered blood after bypass regardless of the anticoagulant utilized during storage.[50,51] Although no significant change in platelet count or post-bypass bleeding was found, there are no data to prove that platelet function is indeed normal following these procedures. Radioactive tagging of platelets, survival studies, and platelet function testing are needed to document the actual effects of storage and anticoagulants on reinfused platelets. In the absence of such studies, our present system of hemodilution is based on the fact that ionized calcium is necessary for platelet adhesion and aggregation to occur.[5,47] We collect blood via the vena caval cannula after systemic heparinization because of the many technical advantages listed above. However, we collect it in bags containing either ACD or CPD solution. The duration of storage is so short that it is not crucial whether CPD or ACD be used. Theoretically, it may be possible to avoid any deleterious effect of heparin on platelets by collecting heparinized blood from the patient prior to bypass directly into citrate-containing reservoirs, thus complexing calcium, removing it from the stored blood, and preventing platelet aggregation.

There has only been one study of the effect of protamine on platelet function. In this *in-vitro* study protamine adversely affected platelet aggregation in response to ADP and epinephrine; the doses of protamine used were these that would be used clinically. ‡This adverse effect of protamine may explain why it has been reported to produce ex-

‡ Ellison N, Colman, RW, Edmunds, LH Hr, et al: The effect of heparin and protamine on platelet function. Abstracts of Scientific Papers, 1976 Meeting of the American Society of Anesthesiologists, pp 439–440.

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cessive bleeding postoperatively.[52] despite the lack of effect of protamine on the coagulation mechanism.[53]

Summary

Basic knowledge regarding platelet physiology has been reviewed and related to clinical situations where platelet function is critically important. Platelet function tests such as aggregation and survival studies, which are research tools now, may soon be available to aid in the diagnosis and management of clinical bleeding problems. A growing understanding of drug–platelet interactions is allowing manipulation of platelets and the clotting system to improve the proganoses for patients who have thromboembolic diseases. Advances in platelet storage and transfusion techniques can save patients who previously might have bled to death. Proper application of the new developments in cardiopulmonary bypass and hemodilution, with attention to platelet and coagulation factor preservation, will contribute to securing hemostasis and conserving blood products.

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