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Medical Progress

Disorders of Platelet Function

Mechanisms, Diagnosis and Management

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Platelets play an important role in hemostasis, and alterations in platelet function may be the cause of abnormal bleeding in a wide variety of congenital and acquired clinical disorders. Platelet dysfunction may be classified as disorders of (1) substrate connective tissue, (2) adhesion, (3) aggregation and (4) platelet-release reaction. The congenital defects of platelet function, although uncommon, have provided important insights into platelet physiology and pathophysiology and, as a group, are less common, better characterized and more readily classified than the acquired defects.

The severity of bleeding resulting from platelet dysfunction varies greatly and is substantially increased when another defect of hemostasis coexists. A disorder of platelet function is suspected on the basis of the history and physical examination and is confirmed by the finding of a prolonged bleeding time in the presence of an adequate number of platelets. A specific diagnosis often requires measurements of the factor VIII and von Willebrand factor complex and other tests of platelet function. Some of these tests may be available only in specialized laboratories.

Therapy for bleeding episodes resulting from platelet dysfunction is directed at (1) removing or treating the underlying cause of the platelet disorder; (2) replacing the missing plasma cofactors needed to support normal platelet function (such as by the transfusion of cryoprecipitate in patients with von Willebrand disease, and (3) transfusing functional platelets in the form of platelet concentrates in patients with disorders of intrinsic platelet dysfunction.

THE PLATELET REACTIONS of adhesion, aggregation and release are initiated by vascular injury and each has an important role in the mechanisms of hemostasis, arterial thrombus formation, thromboembolism and atherogenesis.[1-4] Disorders of hemostasis may arise from a variety of congenital and acquired abnormalities of platelet function.[5-8] In this review, we discuss clinically significant defects in platelet function, their pathophysiology, diagnosis and management.

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Platelet Physiology

Platelets normally circulate in blood for about ten days as cytoplasmic discs of approximately a

ABBREVIATIONS USED IN TEXT

ADP = adenosine diphosphate
ATP = adenosine triphosphate
β-TG = β-thromboglobulin
cAMP = cyclic adenosine monophosphate
FVIII/VWF = factor VIII/von Willebrand factor
GP = glycoprotein
PG = prostaglandin
PDGF = platelet-derived growth factor
SLE = systemic lupus erythematosus
SPD = storage-pool deficiency
VWD = von Willebrand disease
VWF = von Willebrand factor

10-fl average volume at a concentration of 250,000 ± 50,000 platelets per μl. Under normal circumstances, platelets are nonadherent to all blood elements and to intact vascular endothelium. Vascular disruption exposes blood to subendothelial connective tissue structures of the vessel wall, which initiates the formation of a hemostatic plug.[1,2]

Platelet Structure

Platelets[9] are limited by a typical trilaminar membrane with an outer glycoprotein coat 10 to 20 nm thick that mediates platelet activation reactions (Figure 1). The platelet membrane forms a sponge-like network of tortuous surface connecting channels that expand the surface area of platelets and serves as a conduit for the extrusion of released secretory products. The dense tubular system that is derived from the endoplasmic reticulum interdigitates with the surface connecting system.

The platelet cytoplasm contains three types of granules.[10] Dense α-granules contain storage-pool calcium, serotonin, adenosine triphosphate (ATP) and adenosine diphosphate (ADP). These α-granules contain the following platelet-specific proteins: platelet factor 4 (PF4), β-thromboglobulin (β-TG) and platelet-derived growth factor (PDGF).[11] Coagulation proteins such as fibrinogen, factor VIII, factor V, as well as albumin and fibronectin, are also contained in the α-granules. The lysosomal granules contain hydrolytic enzymes.

Platelets also contain a circumferential band of microtubules that appears to be anchored to the surface membrane and which provides a cytoskeletal structure that maintains the platelet's discoid shape and contractile orientation. The process of platelet activation results in the centripetal movement of these microtubules and the platelet granules. Platelets also contain the contractile proteins actin and myosin, which together

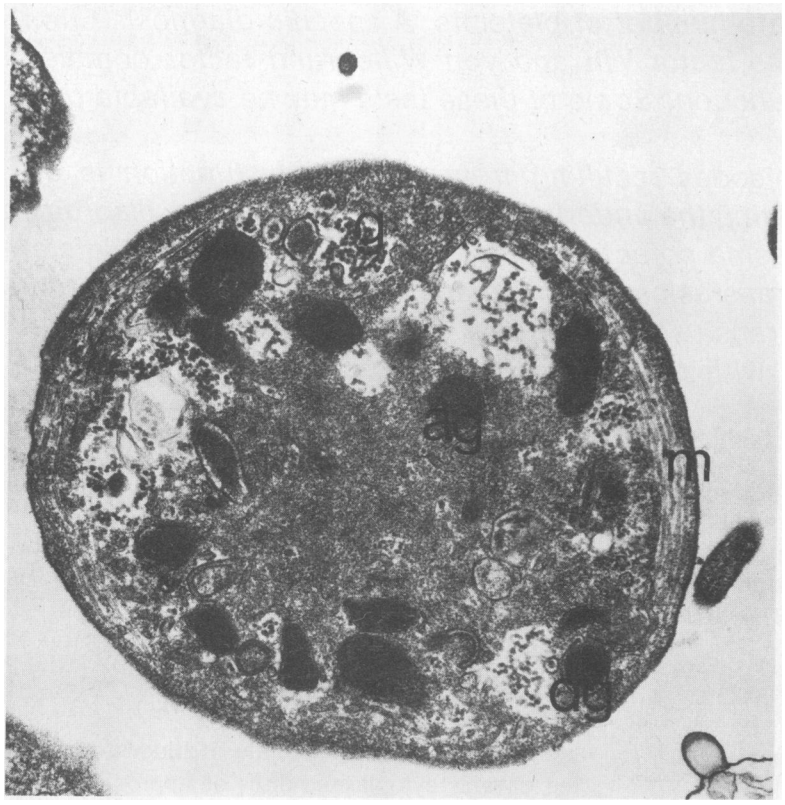

Figure 1.—Platelet ultrastructure. The outer glycoprotein coating mediates platelet reactions. Microtubules (m) form a circumferential cytoskeleton that maintains the platelet discoid. Two types of granules are visible in this electron micrograph, the abundant α-granules (ag) and the less common dense granules (dg). The third type of lysomal granules cannot be differentiated by electron microscopy. The focal collection of smaller glycogen granules (g), microfilaments and tubular systems are also present. (Electron micrograph courtesy of Dr. Russell Ross.)

	1. Surface	2. Adhesion	3. Aggregation	4. Release
Required Factors	collagen microfibrils	surface receptors plasma cofactors e.g., FVIII/vWF	surface receptors plasma cofactors e.g., fibrinogen	surface receptors messengers granules contractile system
Hereditary Defects	Ehlers-Danlos	Bernard-Soulier von Willebrand Disease	thrombasthenia afibrinogenemia	storage-pool deficiency cyclo-oxygenase deficien thromboxane synthetase deficiency
Acquired Defects	scurvy	acquired vWD dipyridamole	fibrin(ogen) degradation products macromolecules penicillins	cardiopulmonary bypass collagen-vascular disease aspirin, etc.

Figure 2.—Platelet hemostatic plug formation. This process includes the sequential events of platelet adhesion to subendothelial structures, platelet aggregation and platelet release reaction. (Electron micrograph courtesy of Dr. Hans Baumgartner.)

constitute about 20 percent of the total platelet protein. Formation of microfilaments reflects the interaction of actin and myosin in the process of platelet spreading, pseudopod formation, secretion of granule constituents and clot retraction.[12] Mitochondria are few in number and structurally simple, and abundant glycogen granules are present in the cytoplasm. Platelets have no DNA, and ribosomes are seen only rarely; a small amount of RNA is present, and protein synthesis has been noted. The cytosol also contains important enzymes involved in platelet metabolism.

Platelet Function[13,14]

Platelet Adhesion

Under normal circumstances, platelets are nonreactive to all blood elements and normal vascular endothelium. A breach in vascular integrity exposes circulating blood to subendothelial structures (Figure 2). The process of platelet adhesion involves the interaction of platelet surface glycoproteins with connective tissue elements of the subendothelium, especially collagen and microfibrils, and requires the von Willebrand* factor (VWF)[1,15] and, possibly, fibronectin as plasma cofactors.

The von Willebrand factor may be synthesized by endothelial cells and circulates in plasma as a multimeric glycoprotein. It is also absorbed onto platelets. When subendothelium is exposed by vessel injury, von Willebrand factor is also absorbed onto exposed collagen.[16] Similarly, fibronectin, a ubiquitous glycoprotein present in platelets, is important in attachment processes of cells. Fibronectin has been postulated to be necessary for the normal interaction of von Willebrand factor with its platelet receptors and with collagen.[17] Platelet adhesion requires a platelet surface

*THE WESTERN JOURNAL's style regarding eponyms is that they are not written in the possessive form; therefore Graves disease, Ewing sarcoma and Paget disease. An explanation may be found on page 78 of the July 1978 issue.

TABLE 1.—*Disorders of Platelet Function*

	Hereditary	*Acquired*
Disorders of substrate connective tissue	Ehlers-Danlos syndrome	Scurvy Amyloidosis
Disorders of adhesion	Bernard-Soulier syndrome von Willebrand disease	Acquired von Willebrand disease Uremia Drugs (eg, dipyridamole)
Disorders of platelet interaction	Thrombasthenia Afibrinogenemia	Fibrin(ogen) degradation products (eg, DIC), liver disease, fibrinolytic liver disease, fibrinolytic therapy Macromolecules (eg, paraproteins and dextran) Drugs (eg, semisynthetic penicillins)
Disorders of release		
Storage granule deficiency		
α-Granule deficiency	Gray platelet syndrome Autosomal recessive disorders	Cardiopulmonary bypass operation
δ-Granule deficiency	Albinism Familial disorders	Immune-mediated release (eg, ITP, collagen vascular diseases) Drugs (eg, reserpine, methysergide, tricyclics, phenothiazines)
Defective release	Cyclooxygenase deficiency Thromboxane synthetase deficiency	Platelet dyspoiesis (eg, leukemia, myeloproliferative syndrome) Drugs (eg, aspirin and other nonsteroidal anti-inflammatory drugs, furosemide, nitrofurantoin) Ethanol Diet
Altered nucleotide metabolism	Glycogen storage disease Fructose-1, 6-diphosphate deficiency	Drugs (eg, phosphodiesterase inhibitors), stimulators of adenyl cyclase (eg, PGI_2, PGE_1)
Others	Platelet factor 3 deficiency	Drugs (eg, heparin, sympathetic blockers, clofibrate, hydroxychloroquine) Diet Viral Infections Hypothyroidism

DIC = disseminated intravascular coagulation; ITP = idiopathic thrombocytopenic purpura; PG = prostaglandin

glycoprotein (GP) complex designated GPIb and GPIs.[18] This complex may act as the platelet receptor for the von Willebrand factor, or, alternatively, the complex may facilitate proper interaction of the von Willebrand factor with its platelet receptor. Absence of this glycoprotein complex causes a serious defect of platelet adhesion.[18,19]

A variety of congenital and acquired abnormalities of platelet function have been attributed to defects in one or more of these factors of platelet adhesion (Table 1).

Platelet Aggregation

Vessel disruption not only induces platelet adhesion but also produces (1) the release of dense-granule ADP from adherent platelets, (2) the formation of small amounts of thrombin and (3) the generation of phospholipase activity to cleave arachidonic acid from membrane phospholipids to synthesize thromboxane A_2.[4,13-15,20,21] These factors—ADP, thrombin and thromboxane A_2—together with exposed collagen may act independently but generally act synergistically to promote the accumulation of additional platelets to the already adherent platelets at the site of vascular damage. Through this process of platelet aggregation the growing platelet mass forms the hemostatic plug (Figure 2).

Platelet aggregation in vitro has been widely used to test platelet to platelet interaction. When platelet agonists such as ADP, epinephrine, collagen or thrombin are added to an opalescent stirred suspension of platelets, the platelets respond by forming aggregates, a process which is recorded as an increase in light transmittance through the platelet suspension. Most platelet agonists induce a biphasic wave of platelet aggregation—primary aggregation, which is the direct result of the agonist-platelet interaction, and secondary aggregation, which is the result of endogenous ADP released during the primary phase of aggregation. Platelet aggregation in vitro is abnormal in a variety of platelet function disorders.

ADP-induced aggregation. Locally formed ADP promotes platelet aggregation by its binding to specific platelet receptors to induce a change in platelet shape from discs to spiny spheres and to decrease adenyl cyclase activity. The interaction of ADP with its surface receptor mobilizes fibrinogen-binding sites on the platelet surface. Fibrinogen binding is essential for platelet-to-platelet interaction.[22] The fibrinogen-binding site contains the glycoprotein complex designated GPIIb and GPIIIa.[18,19] The GPIIIa component of this complex may also serve as the surface membrane anchor for the contractile protein actin, thereby providing a direct link between the membrane structures involved in aggregation and the contractile-secretory apparatus of platelets. Lack of fibrinogen or absence of the putative fibrinogen receptor GPIIb and GPIIIa substantially impairs platelet aggregation.

Epinephrine-induced aggregation. Epinephrine induces platelet aggregation. Primary interaction of epinephrine with its platelet α-receptor induces reversible aggregate formation and inhibition of adenyl cyclase activity. Second-wave aggregation is mediated through the release of ADP from the δ-granules and through the synthesis of thromboxane A_2. Although the concentration of catecholamines usually found in the circulation may not be sufficient to cause aggregation, they may, however, sensitize the platelets to the effects of other aggregatory factors. The secondary phase of aggregation induced by epinephrine is blocked by inhibition of cyclo-oxygenase as well as by deficiency of δ-granule ADP.

Collagen-induced platelet aggregation. In vitro, collagen induces primary platelet aggregation.[23] Low concentrations of collagen cause a few platelets to adhere to the collagen, and these adherent platelets then release ADP and thromboxane A_2. In vitro, this response to small concentrations of collagen is partially inhibited by inactivation of platelet cyclo-oxygenase or by the removal of released ADP. However, in vivo the collagen platelet interaction provides a strong stimulus for the release of granule contents. Adherent platelets undergo the release reaction even in the presence of drugs (such as aspirin) that inhibit platelet cyclo-oxygenase.

Thrombin-induced aggregation. Thrombin plays a central role in the growth and stability of the platelet hemostatic plug.[24,25] Vessel injury leads to rapid local production of thrombin in small amounts, which then interacts with platelet receptors. Receptor stimulation may lead to platelet aggregation directly. Thrombin also stimulates thromboxane A_2 synthesis and causes the release of platelet δ-granule ADP. Thus, thrombin magnifies its effect on platelet aggregation and release by activating several additional pathways. Thrombin also causes the appearance of receptors for factors

Va and Xa, which enhances the conversion of prothrombin to thrombin on the platelet surface, in turn allowing for fibrinogen to be converted to fibrin.[24] Polymerized fibrin adheres to the surface of platelets and imparts stability to the platelet aggregate. Fibrin may be formed from platelet fibrinogen independent of plasma fibrinogen.

Platelet Release Reaction

The release reaction is the secretory process whereby substances stored in platelet granules are extruded from the platelet.[10] ADP, epinephrine, collagen and thrombin are the physiologically important agents that induce such release. The α-granule contents are the most readily released; δ-granule release requires a somewhat greater platelet stimulus, and lysosomal granule contents are released only with potent stimuli such as concentrated collagen or thrombin.[10]

The exact biochemical reactions induced by ADP, collagen and thrombin that lead to granular release remain unclear. However, the release reaction, like platelet adhesion and aggregation, is a membrane-mediated process involving specific glycoprotein receptors located on the platelet surface. Receptors are present for ADP, epinephrine, collagen and thrombin, the physiologically important agents that induce platelet release. The binding of these agonists initiates the formation of intermediaries that relay the signal to the contractile-secretory apparatus.[25] The system of independent but interactive second messengers is thought to trigger a transfer of free, ionized calcium as the final mediator in the formation of actomyosin microfilaments, which then execute the release reaction.[26]

The platelet arachidonic acid pathway illustrates this sequence. It has been worked out in some detail,[27,28] whereas the intermediate pathways specific for collagen, ADP and thrombin remain to be characterized. Arachidonic acid is stored in platelet phospholipids, especially monophosphotidylinositol. Arachidonic acid is cleaved from these phospholipids either by phospholipase C and diacylglycerol lipase or by phospholipase A_2.[29] Free arachidonic acid is subsequently converted to the prostaglandin endoperoxides PGG_2 and PGH_2 by platelet cyclo-oxygenase. The unstable endoperoxides are converted to thromboxane A_2, a labile but potent substance capable of inducing platelet aggregation and release, which is also a potent arterial vasoconstrictor. The short-lived thromboxane A_2 spontaneously breaks down to thromboxane B_2, a stable inactive metabolite measurable by radioimmunoassay. ADP, thrombin and collagen can all induce platelet aggregation and the platelet release reaction directly, but usually these agents also activate the platelet arachidonic acid pathway. It is not clear how membrane stimulation by collagen, thrombin or ADP initiates thromboxane A_2 synthesis. However, these agents may, in part, act by their ability to raise free, ionized, intracellular calcium levels, which in turn activates calcium-sensitive phospholipases.

Thromboxane A_2 synthesis is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs that act by inhibiting platelet cyclo-oxygenase.[30] The fact that aspirin can induce a complete block of this pathway, and in so doing only induces a mild defect in hemostasis, suggests that the important physiological agents that induce platelet release and aggregation can act on platelets independent of activation of the arachidonic acid pathway. This suggests that activation of the platelet arachidonic acid pathway acts only as a mechanism of potentiation or amplification of physiological stimuli leading to aggregation and release but is not critical to platelet function.

Agents that inhibit the platelet release reaction generally increase adenyl cyclase activity and elevate platelet cAMP (cyclic adenosine monophosphate) levels, causing free calcium levels to drop and, thereby, inhibiting the interaction of actin and myosin. Inhibitory effects exerted on platelets by such prostaglandins as PGD_2, PGI_2 and PGE_1 are initiated by the binding of these platelet antagonists to specific surface membrane receptors.[31]

A wide spectrum of acquired and congenital abnormalities of the platelet release reaction have been identified and, on the whole, result in mild bleeding disorders (Table 1).

Disorders of Platelet Function

Disorders of Substrate Connective Tissue

Ehlers-Danlos Syndrome

Some inherited disorders of collagen biosynthesis such as the Ehlers-Danlos syndrome are characterized in part by extremely fragile skin and easy bruising. Such patients may sustain extensive ecchymoses from minimal trauma. In Ehlers-

Danlos syndrome type IV, there is a pronounced propensity for easy bruising and a predisposition to sudden death from spontaneous hemorrhage as a result of arterial rupture. Abnormal bleeding is thought to be the result of defective connective tissues (Table 1). For example, Ehlers-Danlos syndrome type IV is characterized by a deficiency of type III collagen in the skin and vessel wall.[32] Poor connective tissue support leads to a propensity for vessel wall disruption, poor vessel wall retraction after disruption and extension of the hematoma along fragile tissue planes. Intrinsic platelet function is usually normal in this syndrome, but impaired platelet adhesion to the disrupted defective vessel wall impairs platelet plug formation and leads to a bleeding disorder simulating qualitative platelet defects. The diagnosis is suspected on clinical grounds together with normal findings on in vitro tests of hemostasis. Confirmation requires documentation of the abnormal collagen in the subcutaneous tissue. There is no known specific therapy for the bleeding tendency in these patients.

Scurvy

Scurvy, an acquired disorder now rarely seen, may be associated with a significant bleeding tendency, the result of a connective tissue defect caused by vitamin C deficiency.[33,34] The deficiency impairs the synthesis of hydroxyproline, an important building block of normal collagen. The resultant defective collagen weakens the connective tissue, producing hemorrhagic manifestations such as gingival bleeding, and subcutaneous and muscle hemorrhages. Finding perifollicular petechiae on physical examination is characteristic. The bleeding time is slightly prolonged and in vitro platelet function is normal. The disorder responds within one to three weeks following oral administration of vitamin C.

Amyloidosis

Purpura may be acquired from the diffuse vascular and mucosal infiltration that occurs in systemic amyloidosis.[35,36] Periorbital purpura is typical. The risk of hemorrhage is high after minor surgical procedures such as skin, gingival or liver biopsy. When amyloidosis occurs in association with plasma cell myeloma, hemostatic defects resulting from the latter condition may further aggravate the bleeding tendency. In a few patients with amyloidosis, an acquired factor X deficiency has also developed.[37] In vitro platelet function is normal. Management of such patients includes the avoidance of liver biopsy because this procedure may lead to serious bleeding after biopsy. Primary treatment is directed at the underlying disorder.

Disorders of Platelet Adhesion

Bernard-Soulier Syndrome

The Bernard-Soulier syndrome is a rare, familial bleeding disorder of moderate severity characterized clinically by epistaxis, menorrhagia, and cutaneous and visceral hemorrhages.[7] The platelet count is variable, and on blood film the platelets appear enlarged because of their flat disc shape. The bleeding time is substantially prolonged—that is, more than 20 minutes. Ristocetin-induced platelet aggregation is absent and is not correctible by the addition of exogenous von Willebrand factor.[6,13] Platelet aggregation to such other aggregating agents as ADP, collagen, epinephrine and thrombin is normal.

The impairment of hemostasis in this syndrome is secondary to an intrinsic defect of platelet adhesion to subendothelium.[38] The membrane glycoprotein of platelets from these patients shows a deficiency of membrane glycoproteins designated GPIb and GPIs (glycocalicin). There is evidence to support the hypothesis that the absence of these membrane glycoproteins is responsible for the observed functional defect. It has been postulated that this glycoprotein I complex may normally act as the platelet receptor through which the von Willebrand factor mediates not only the platelet subendothelium interaction but also the in vitro ristocetin-induced aggregation of platelets.[18]

The diagnosis is based on the patient's history and on the presence of the morphological, functional and biochemical characteristics outlined above. Therapy is limited to the transfusion of platelet concentrates for control of threatening hemorrhage. The condition appears to improve with age, but fatal bleeding episodes have been reported.

Von Willebrand Disease

Von Willebrand disease (VWD) is a relatively common heterogeneous group of congenital bleeding disorders,[39-42] in which a prolonged bleeding time and impaired hemostasis are the result of

TABLE 2.—*Nomenclature of Factor VIII/von Willebrand Factor Used in This Review**

Factor VIII procoagulant activity (FVIII:Co). Clot-promoting activity that is absent in severe hemophilia A, but normal or decreased in vWD.

Ristocetin cofactor (FVIII:RCo). Activity that supports ristocetin-induced platelet aggregation; it is normal in hemophilia A, but reduced or absent in most (but not all) cases of vWD.

Factor VIII-related antigen (FVIII:Ag). Antigen of FVIII/vWF, detected by heterologous antibodies. It is normal in hemophilia A, but may be normal, decreased or absent in vWD.

Factor VIII/von Willebrand factor (FVIII/vWF). Complex of FVIII:Co, FVIII:Ag and FVIII:RCo. It is present in normal plasma as high multimers.

vWD = von Willebrand disease

*Source: Zimmerman et al.[44]

quantitative and qualitative abnormalities of the factor VIII/von Willebrand factor (FVIII/VWF) (Table 2).

The classic form of von Willebrand disease is inherited as an autosomal dominant trait and is characterized clinically by a mild to moderate bleeding tendency that frequently involves epistaxis, gingival bleeding, menorrhagia or postoperative bleeding. Gastrointestinal, muscle and joint bleeding are much less common. The template bleeding time is prolonged. Biochemically, two main variants can be defined on the basis of laboratory findings. In type 1 there is a concordant decrease in FVIII:Co, FVIII:Ag and FVIII:RCo (Table 2). In type 2, a qualitative abnormality of FVIII/VWF complex can be shown on radiocrossed immunoelectrophoresis, which shows the absence of the larger molecular forms of the FVIII/VWF complex.[43]

A less common but more severe form of von Willebrand disease is transmitted as an autosomal recessive trait and is clinically similar to hemophilia. Muscle, gastrointestinal and joint bleeding, as well as mucosal bleeding are the most common clinical manifestations. The disease is inherited from both parents, although the heterozygote parents are usually clinically normal or have only a mild bleeding tendency with modest abnormalities of the FVIII/VWF complex. The template bleeding time in affected patients is greatly prolonged. Both qualitative and quantitative abnormalities of the FVIII/VWF can be observed on crossed immunoelectrophoresis. Such studies have shown that the FVIII:Ag level is severely depressed and that the very small amount of antigen that is present is structurally abnormal.[44]

The quantitative and qualitative abnormalities of FVIII/VWF impair hemostasis by virtue of the defect of platelet adhesion that results from decreased platelet interaction with subendothelium, a process that is normally supported by high molecular weight multimers of FVIII/VWF. This abnormality is best observed by failure of platelets to adhere to segments of de-endothelialized artery in patients with VWD.[45] This defect of adhesion to subendothelium is most pronounced at sites of high-shear flow as found in the microcirculation. The defect of adhesion is corrected by the addition of normal plasma that contains FVIII/VWF, such as is present in cryoprecipitate.[46] The second factor that contributes to bleeding in some patients appears to be the FVIII:Co that is seen in the autosomol recessive variety of von Willebrand disease. In some such cases the FVIII procoagulant activity may be depressed to the same degree as in hemophilia, and this may be an important additional mechanism for abnormal hemostasis in this disease.

The diagnosis in von Willebrand disease is usually based on the finding of a combination of an inherited bleeding disorder characterized by a prolonged bleeding time and a combination of biochemical abnormalities of FVIII/VWF—typically, concordantly low FVIII:Co, low FVIII:Ag and decreased or absent ristocetin-induced platelet aggregation.[39,47] However, it is becoming increasingly apparent that von Willebrand disease represents a heterogeneous group of diseases both clinically and biochemically. While no single test is diagnostic, the measurement of FVIII:RCo has been found to be the most consistent biochemical abnormality. Even this test may give false-negative results as shown in a recent report of a subgroup of autosomal dominant VWD with accelerated ristocetin-induced platelet aggregation.[48]

The preferred treatment for bleeding problems in patients with von Willebrand disease is administration of cryoprecipitate and, occasionally, concomitant epsilon aminocaproic acid. The aim of therapy with cryoprecipate is to achieve hemostatic levels of FVIII procoagulant activity rather than the normalization of the bleeding time, which is achieved only transiently. Using the principles of transfusion therapy developed for hemophilia A, surgical procedures can be safely carried out in these patients, and most bleeding episodes can be well controlled.[47,49]

Acquired von Willebrand disease.[50-53] An in-

creasing number of patients are being described who have a von Willebrand-like defect as an acquired phenomenon secondary to the development of an autoimmune disease (such as systemic lupus erythematosus [SLE]) or a lymphoproliferative syndrome (such as lymphoma or multiple myeloma). The resultant acquired bleeding disorder has been termed von Willebrand syndrome. Patients may present with a bleeding diathesis similar to that seen in the inherited types of von Willebrand disease. The molecular defect is the absence of adequate amounts of functionally active VWF, which leads to a defect in platelet adhesion. In several patients this has been due to an antibody directed against the von Willebrand factor, with either rapid clearance of this complex or interference with its normal function. In other patients, such as those with lymphoproliferative disorders, the malignant cells may avidly absorb active VWF, leaving the patients depleted of this factor. The end result is a defect in platelet adhesion detectable by a prolonged bleeding time and, in some patients, a clinically significant bleeding disorder. This bleeding tendency may remit if the primary disease is successfully treated.

Uremia

Uremia produces an extrinsic platelet defect as a result of the progressive accumulation of metabolic products in the blood.[54] In untreated patients with uremia the template bleeding time is usually greater than 30 minutes and, predictably, shortens to near normal in patients treated with peritoneal dialysis.[55] Hemodialysis is less effective in this regard.

The mechanism of the platelet defect in uremia remains unclear, but appears to be due in part to the accumulation of uncleared metabolites such as guanidinosuccinic acid, phenol and phenolic acids.[56] These metabolic end products can inhibit platelet procoagulant availability and aggregation of platelets to ADP, collagen and epinephrine in vitro.

Therapy for the platelet disorder is adequate dialysis, which will tend to restore the prolonged bleeding time towards normal. Occasionally, platelet concentrates are necessary for control of bleeding during surgical procedures.

Drugs

Dipyridamole is an antiplatelet agent used in the prevention of arterial and venous thrombosis. Dipyridamole diminishes platelet adherence to collagen, the subendothelium and artificial surfaces in vivo.[57-60] Increased platelet consumption in humans is normalized by the administration of dipyridamole, a phosphodiesterase inhibitor that increases platelet cAMP levels and possibly potentiates the antiaggregatory activity of endogenous prostacyclin.[61] Despite these antithrombotic actions, the administration of dipyridamole does not prolong bleeding time and has no detectable influence on hemostatic function in vivo at the doses usually given. Dipyridamole has been shown to decrease the thromboembolic complications of prosthetic heart valves,[62] and aspirin potentiates the antithrombotic effects of the drug. In a recent major trial using dipyridamole and aspirin, a reduced incidence of coronary disease (coronary deaths and nonfatal infarction) was reported.[63]

Under physiological conditions of flow and hematocrit, drugs such as sulfinpyrazone and aspirin, both of which inhibit cyclo-oxygenase, do not inhibit the adherence of the initial layer of platelets to the subendothelium or prevent the adherent platelets from releasing their constituents. These drugs do, however, inhibit subsequent platelet aggregation, probably by preventing thromboxane A_2 formation. Sulfinpyrazone, in contrast to aspirin, does not prolong the bleeding time and does not impair hemostatic function when given in pharmacological doses.[57]

Disorders of Platelet Interaction

Thrombasthenia

Thrombasthenia is a rare congenital bleeding disorder transmitted as an autosomal recessive trait.[64,65] The bleeding tendency starts early in life, is of variable severity and is characterized by cutaneous, mucosal and postoperative bleeding. The prolonged bleeding time in the presence of a normal platelet count seen in this disorder is due to an abnormality of platelet aggregation, which is manifested as an absence of in vitro platelet aggregation following the addition of ADP, epinephrine, collagen or thrombin.

The defect in thrombasthenia is an abnormality of platelet to platelet interaction which results in an inability to form a hemostatically effective cohesive platelet aggregate. Platelet adherence to subendothelial connective tissue structures is normal. Although thrombasthenic platelets fail to aggregate in vitro to the usual aggregating agents, these same agents do produce normal changes in platelet shape and a near normal release reaction.

This defect in platelet to platelet interaction is associated with an inability of the thrombasthenic platelets to bind fibrinogen to their surface following ADP activation.[22] This failure of fibrinogen binding in thrombasthenia may be related to a deficiency in the platelet surface glycoproteins designated GPIIb and GPIIIa.[18,19] It has not been actually shown that this glycoprotein complex is the fibrinogen receptor. Alternatively, it has also been postulated that the glycoprotein IIb and IIIa complex serves as the membrane anchor for the actin component of the cytoskeleton. A deficiency of this glycoprotein complex may then lead to a failure in the contractile process involved in platelet orientation during the formation of a cohesive platelet aggregate.

The recent development of a monoclonal antibody to the GPIIb and GPIIIa complex may clarify not only the nature of the defect in thrombasthenia, but may also provide greater insight into normal platelet aggregation.[66] In addition, the use of such an antibody has allowed detection of the heterozygote state of this condition by measuring the moderate decrease in the amount of GPIIb and GPIIIa on the platelet surface.

The diagnosis is made on the basis of the history, prolonged bleeding time and platelet aggregation abnormalities described above. The transfusion of platelet concentrates for bleeding episodes is effective therapy for this condition. Even with this mode of treatment, however, the development of antibodies to glycoproteins of normal platelets in some patients may make platelet concentrates only transiently beneficial.

Afibrinogenemia

The bleeding time is prolonged in this rare autosomal recessive condition, probably because there is insufficient binding of fibrinogen to platelet surfaces for significant platelet to platelet interaction.[67] ADP-induced platelet aggregation is reduced, a defect that can be corrected in vitro by the addition of fibrinogen. This condition demonstrates the crucial mediating role played by fibrinogen in platelet aggregation. Bleeding episodes are treated with the transfusion of fibrinogen as cryoprecipitate.

Proteolytic Products of Fibrin(ogen)

Proteolytic degradation products of fibrin and fibrinogen may inhibit platelet plug formation.[68] These complexes are increased in a number of clinical conditions, such as disseminated intravascular coagulation, fibrinolytic therapy with urokinase or streptokinase, and liver disease. Platelet function may be impaired by fragments X, Y and D, all products of plasmin digestion of fibrin(ogen). These products bind to fibrin monomers and interfere with fibrin polymerization and platelet-fibrin interaction.[69]

The relative importance of platelet dysfunction in some of these clinical situations is not entirely clear because other aspects of hemostasis are often profoundly impaired. For example, patients with liver disease may also have a deficiency of vitamin-K-dependent coagulation proteins, increased fibrinolytic activity, and quantitative and qualitative platelet defects.[70]

Treatment is directed at the underlying disease process. Administration of platelet concentrates may be of significant benefit in those patients with combined qualitative and quantitative platelet defects.

Dysproteinemia

The monoclonal immunoglobulins in the dysproteinemia syndromes, such as multiple myeloma, sometimes induce a disorder of platelet function.[71] In such patients the bleeding time is prolonged. Platelet adhesion and aggregation are impaired and platelet procoagulant activity is also reduced. The platelet defect is thought to be the result of interference by immunoglobulins, especially IgA, of platelet receptor-mediated reactions, presumably as a result of coating of the platelet surface by the paraprotein.

Like other acquired defects of platelet function, the hemostatic defect in these disorders is complex, with the frequent coexistence of thrombocytopenia, impaired fibrin formation, and hyperviscosity as possible additional contributing factors. The severity of the bleeding disorder correlates with the prolongation in bleeding time and the level of paraprotein. An improvement is usually seen when therapy of the underlying plasma cell dyscrasia successfully lowers the protein level. However, with high levels of monoclonal protein, therapy with platelet concentrates may be ineffective. Plasmaphoresis may lower the paraprotein level suddenly and ameliorate the bleeding tendency in some patients.

Dextran

Dextran, a high molecular weight polysaccharide derived from sucrose, has been used in the

prophylaxis of postoperative deep venous thrombosis and pulmonary thromboembolism.[72] Therapeutic doses of dextran prolong the bleeding time and decrease platelet aggregation and procoagulant activity.[73] The exact mechanisms of platelet dysfunction remain uncertain, although coating of the platelet surface has been assumed. Bleeding as a complication of administration of dextran requires its discontinuation and, occasionally, platelet transfusions.

Drugs

A number of antibiotic drugs have significant effects on platelet function. These effects are of particular importance in patients receiving intensive chemotherapy. Carbenicillin is often used in pancytopenic patients for suspected sepsis in doses that cause significant platelet dysfunction.[74] Ticarcillin, penicillin G, ampicillin and cephalosporins, although less well studied than carbenicillin, appear to induce a similar defect when given in high enough doses.

Carbenicillin prolongs the bleeding time as a result of a defect in platelet to platelet interaction. Decreased sensitivity to the aggregating effects of ADP, with absent primary and secondary aggregation has been seen. The mechanism is unclear, but it has been postulated that the antibiotic drug or its metabolites inhibit interaction of platelet agonists (such as ADP and epinephrine) with von Willebrand factor and their respective platelet surface glycoprotein receptors.[75]

Patients with severe thrombocytopenia who are receiving carbenicillin treatment may have a bleeding tendency at an absolute platelet level that is considerably higher than might be expected in patients not receiving these antibiotic drugs.[76] The use of platelet concentrates in such patients is then recommended at a higher platelet count than the 5,000 platelets per μl generally recommended for those patients who do not have a qualitative defect in addition to severe thrombocytopenia.[76] It is unclear whether ticarcillin may be effectively substituted for carbenicillin at doses that do not affect platelets to the same degree.

Disorders of Platelet Release Reaction

Abnormalities of platelet release can be divided into deficiencies of one or more of the platelet granules, disorders of thromboxane A_2 synthesis from membrane arachidonic acid, or conditions in which altered cAMP metabolism changes the reactivity to platelet agonists.

Granule Storage-Pool Deficiency

α-Granule depletion. A small number of patients have an inheritable deficiency of platelet α-granules (α-SPD), a condition termed the gray platelet syndrome.[77] The platelets appear enlarged and gray on a Wright-stained blood film. The platelet function defect, which is characterized by a deficiency of α-granules and their contents, including PF4, β-TG, PDGF, platelet fibrinogen and fibronectin.[78] These platelets have normal dense granules and lysosomal granules. Clinically, these patients present with a syndrome of easy bruisability. The bleeding time and platelet aggregation induced by ADP, thrombin, and collagen are minimally abnormal. Mild to moderate thrombocytopenia is also present in some, and may improve with splenectomy. The fact that an α-granule deficiency induces only a mild disorder of platelet function is consistent with the notion that α-granules play a minor role in normal hemostasis.

An acquired α-granule depletion has been observed in patients during open-heart and pump-oxygenator bypass operations.[79] These patients are at risk for serious bleeding as a result of the procedures and the accompanying acquired defects in hemostasis. There are a number of potential contributing factors in the hemostatic defect during cardiopulmonary bypass operations, including a reduction in the levels of coagulation factors, heparinization or its inadequate reversal, increased fibrinolytic activity and thrombocytopenia; however, the most important is a transient defect in platelet function.[80] With the onset of the cardiopulmonary bypass operation, the bleeding time becomes greatly prolonged, in parallel with platelet α-granule release as indicated by substantial elevations in plasma PF4 and β-TG. At the end of a bypass operation, the remaining circulating platelets are partially depleted of their α-granules. This deficiency persists despite the bleeding time returning to normal within one to two hours after completion of bypass oxygenation. The dense granules do not undergo release in this setting. However, in some patients the bleeding time after a bypass operation remains prolonged and may be the cause of excessive postoperative bleeding. Bleeding from a persistent platelet function defect after a bypass operation

should be treated by administering platelet concentrates even when the platelet count is adequate.

δ-Granule deficiency. Congenital dense-granule storage-pool deficiency (δ-SPD)[81,82] is a term used to designate a group of lifelong platelet function disorders clinically manifested by easy bruisability, menorrhagia and excessive postoperative bleeding. In some patients oculocutaneous albinism is also present, in which case the disorder is referred to as the Hermansky-Pudlak syndrome. The bleeding time is moderately prolonged and in vitro platelet aggregation to collagen is impaired as is the second phase of aggregation to ADP and epinephrine. The platelet count is normal or slightly decreased, and platelet morphology under light microscopy is normal. Electron microscopy shows a deficiency of δ-granules and, usually, a normal number of α-granules. Biochemically, platelet ATP and ADP are below normal and the ATP to ADP ratio is increased (because more ADP than ATP is contained in the δ-granules). The congenital δ-granule deficiencies can be distinguished from the aspirin-like platelet defects by the normal ATP to ADP ratio and the normal number of δ-granules seen on electron microscopy in the latter syndromes.

The dense-granule deficiencies are not a homogeneous group of disorders and can be separated into several subgroups on the basis of associated biochemical abnormalities.[82] Some patients may have an associated partial or complete deficiency of α-granules. Management involves avoidance of aspirin therapy as well as judicious use of platelet concentrates for bleeding episodes when clinically indicated. On the whole, these disorders are benign, although occasionally easy bruising can be troublesome.

Chronic immune thrombocytopenic purpura,[83] autoimmune hemolytic anemia,[84] and systemic lupus erythematosus (SLE)[85] occasionally have been associated with an acquired platelet defect characterized by a prolonged bleeding time and decreased aggregation to collagen, thrombin and ADP. In such circumstances, an acquired deficiency of dense granules and dense granular contents is seen. Antiplatelet antibodies or antigen-antibody complexes associated with these underlying diseases may act on circulating platelets by inducing the release reaction and causing the depletion of dense-granule ADP and serotonin.[86] It appears then that human antiplatelet antibodies are capable of inducing a wide spectrum of platelet defects, ranging from profound thrombocytopenia to subtle qualitative defects of platelet function. Specific mechanisms whereby the platelets in such conditions become depleted of dense-granule contents are not known, although activation of the complement system appears to be associated in some instances. The diagnosis should be suspected in a patient who has an inappropriately long bleeding time or bleeding for the degree of thrombocytopenia in a setting of any of the immune-mediated diseases mentioned above. Therapy is generally directed at the underlying condition.

In both congenital and acquired dense-granule depletion, it appears that the deficiency of ADP underlies the bleeding tendency seen in these patients. Serotonin, on the other hand, although also low in such conditions, appears to have no well-established role in human platelet function. Selective depletion of platelet serotonin by such drugs as reserpine and the phenothiazines causes no detectable disorder of platelet function in vivo.

Abnormalities of Platelet Release Reaction

A heterogeneous group of mild bleeding disorders involving defective platelet release mechanisms has been observed. These disorders are characterized by mild to moderate prolongation of the bleeding time with impaired in vitro platelet aggregation induced by ADP, collagen and epinephrine, but with normal platelet δ- and α-granule content.

A group of patients have been described with (1) a history of intermittent excessive bleeding in response to trauma, in association with a normal platelet count and a normal or minimally prolonged bleeding time, (2) an exaggerated prolongation of the bleeding time in response to aspirin therapy and (3) abnormal platelet aggregation suggestive of a defect in the platelet release mechanism. This has been termed the "intermediate syndrome" of platelet dysfunction.[87] The underlying defect is not known.

Recently, in some of these patients a specific platelet defect has been identified.

Cyclo-oxygenase deficiency. In a number of patients a deficiency in platelet cyclo-oxygenase has been confirmed.[88] This condition is characterized by defective platelet aggregation to collagen as well as absent second-phase aggregation to ADP and epinephrine. The platelets fail to aggregate to arachidonic acid, but do aggregate with

addition of the endoperoxides, such as PGH_2.[89] The patients have a normal ATP to ADP ratio and their platelets appear normal by microscopy. Clinically, these patients have a mild impairment of hemostasis with easy bruisability, menorrhagia and excessive postoperative bleeding. The impairment is the result of an inability of platelets to generate thromboxane A_2, a potent inducer of the platelet release reaction and of platelet aggregation. Platelet adhesion is normal. From a diagnostic point of view, it may be difficult to exclude aspirin ingestion as the basis for the observed abnormalities. It is imperative that a patient not take aspirin for two weeks before doing any platelet function tests for this and other disorders.

Thromboxane synthetase deficiency. Several patients with a bleeding disorder of moderate severity due to presumed thromboxane synthetase deficiency have been described.[90,91] The bleeding time is prolonged and in vitro platelet aggregation to ADP, collagen and epinephrine is impaired. Furthermore, platelets in this disorder fail to aggregate to arachidonic acid or the endoperoxide PGH_2. Failure to aggregate in response to the endoperoxides distinguishes this syndrome from platelet cyclo-oxygenase deficiency, where PGH_2-induced aggregation is normal. In one case of congenital thromboxane synthetase deficiency, the patient's bleeding responded to platelet transfusion.

Diet. Thromboxane A_2 is generated from membrane arachidonic acid. Diets rich in another fatty acid, eicosapentaenoic acid, as consumed by Eskimos, for example, leads to replacement of phospholipid arachidonate by phospholipids rich in eicosapentaenoic acid. This latter fatty acid is a poor substrate and in effect leads to decreased thromboxane A_2 synthesis and a slight prolongation of the bleeding time. It has been postulated that such dietary manipulations, by impairing platelet function and possibly enhancing synthesis of antiaggregatory prostaglandins by the vessel wall, may retard the development of arterial thrombosis and atherosclerosis.[92,93]

Platelet dysfunction due to abnormalities of hematopoiesis. Acute nonlymphocytic and acute lymphocytic leukemia as well as the preleukemic states that sometimes precede them, are often associated with quantitative as well as qualitative abnormalities of platelet function.[94,95] The functional defects are variable and often complex, but include (1) giant platelets, (2) abnormal cytoplasmic granules, (3) defective granule migration, (4) deficient storage-pool content and (5) impaired thromboxane A_2 synthesis. It is important to recognize this qualitative defect as it may be superimposed on the thrombocytopenia in these disorders.[96]

The myeloproliferative syndromes—polycythemia vera, agnogenic myeloid metaplasia and idiopathic thrombocythemia—as well as chronic myelogenous leukemia are clonal disorders of the hematopoietic stem cells. Not surprisingly, both morphological and functional abnormalities of megakaryocytes and platelets are common in these disorders.[97,98] These abnormalities may explain the increased risk for bleeding or thrombosis seen in these conditions.

In essential thrombocythemia elevated platelet counts (often above a million) are seen, sometimes with prolonged bleeding times. Typically, a diagnostically useful absence of platelet aggregation to epinephrine secondary to a deficiency of platelet α-adrenergic receptors can be observed.[99] In vitro aggregation to ADP and collagen are more variable. To a lesser degree polycythemia vera and agnogenic myeloid metaplasia may show a similar abnormality, whereas platelet dysfunction in chronic myelogenous leukemia is rarer.[100] Interestingly, clinically significant episodes of thrombosis or hemorrhage in these disorders do not correlate well with platelet counts, bleeding times or in vitro platelet aggregation tests.[101] The actual biochemical abnormalities in some of these disorders include deficiency in surface-related platelet receptors, in the surface connecting system, the dense tubular system and in platelet storage-pool granules. Abnormalities in arachidonic acid metabolism with decreased activity of the lipoxygenase and cyclo-oxygenase enzymes have also been reported.

Drugs.[57,58] In a dose of 200 mg, aspirin consistently and predictably doubles the bleeding time[102] by irreversibly inhibiting platelet cyclo-oxygenase.[103-105] The result is impaired thromboxane A_2 formation and a mild bleeding tendency. The platelet defect lasts for four to ten days after a single dose and is the result of irreversible acetylation of cyclo-oxygenase. As in congenital cyclo-oxygenase deficiency, the diagnosis is based on findings of impaired collagen-induced aggregation and the absence of second-phase aggregation to ADP and epinephrine in conjunction with a normal ATP to ADP ratio. If aspirin is given to

a patient with an independent hemostatic defect, such as thrombocytopenia or hemophilia, severe bleeding may result. In such cases the drug should be discontinued. In some instances (such as during surgical procedures), excessive bleeding resulting from dysfunctional platelets that were produced by earlier aspirin therapy may require platelet transfusion.

Other nonsteroidal anti-inflammatory drugs such as phenylbutazone, indomethacin, fenoprofen and ibuprofen are also capable of reversibly inhibiting platelet cyclo-oxygenase and impairing the mechanism of thromboxane A_2 generation.[104] Similar to the defect induced by aspirin therapy, the inability to generate thromboxane A_2 is the cause for impaired platelet release and aggregation. The bleeding time is slightly prolonged. Aspirin ingestion may cause easy bruising, menorrhagia and gastrointestinal bleeding.

The drug sulfinpyrazone, used in the secondary prevention of myocardial infarction, is becoming more widespread since the results of the Anturane Reinfarction Trial Group's[106] study have become available. Sulfinpyrazone and its metabolites inhibit cyclo-oxygenase and decrease platelet aggregation.[107] The drug has no effect on platelet adhesion and shortened platelet survival may be returned to normal. The mechanism of action of sulfinpyrazone in vivo, however, remains unknown, but it is important to note that ingestion of sulfinpyrazone does not impair platelet function in vivo, and no bleeding tendency is seen in patients takjng this drug.

Nitrofurantoin prolongs the bleeding time by one to three minutes by inhibiting ADP-induced platelet aggregation and collagen-induced platelet release. The clinical significance of this effect is uncertain.[108]

Alcohol. Alcoholism is associated with significant abnormalities of platelets and of blood clotting.[109,110] In alcoholic patients without liver disease, high doses of alcohol induce a quantitative and qualitative defect of platelets. The qualitative abnormalities are multiple and include decreased platelet aggregation and release, decreased storage-pool ADP as well as decreased platelet procoagulant activity. Intake of large amounts of ethanol inhibits the rate of synthesis of prostaglandin endoperoxides and thus impairs thromboxane A_2 formation.[111] These changes are associated with abnormalities in platelet morphology at the ultrastructural level. The qualitative defect of platelet function superimposed on a variable degree of thrombocytopenia places the patient at additional risk of bleeding, which is even greater when the patient also has coagulation abnormalities associated with significant liver disease. The effect of alcohol on platelets is reversible.[109]

Evaluation of platelet function in a patient with alcoholism is but one aspect of the complete evaluation of hemostasis needed in such patients because of the complexity of the hemostatic defect so frequently seen. Thrombocytopenia with or without an associated qualitative platelet defect is treated with platelet transfusion of concentrates if the patient has significant bleeding.

Altered cAMP Metabolism

A number of drugs, especially the phosphodiesterase inhibitors and a number of prostaglandins are able to inhibit platelet function by elevating cAMP levels within platelets. Dipyridamole is a phosphodiesterase inhibitor that prevents the breakdown of cAMP[57,58,112] and thereby elevates the levels of this second messenger within platelets, making the platelets less likely to undergo aggregation and release when stimulated. It has been suggested that this is the mechanism of action of dipyridamole. It has also been suggested that prostaglandins such as prostacyclin (PGI_2) may potentiate the effect of dipyridamole on platelets because prostacyclin itself is also able to elevate cAMP levels in platelets. Pharmacologic doses of PGE_1 and PGD_2, acting through a common receptor also, elevate cAMP levels of platelets and thereby inhibit platelet aggregation and release both in vitro and in vivo. In the case of the prostaglandins, such platelet inhibitory activity may prolong the bleeding time and induce a mild hemostatic defect. It is postulated that the effects of these agents are mediated through specific membrane receptors, and that the resultant elevated cAMP level inhibits the ATP-dependent translocation of intracellular calcium; low levels of free calcium inhibit interaction of actin and myosin and, hence, impair platelet secretory activity.

Other Disorders

Platelet Procoagulant Activity

Platelets contribute significantly to the rapid early generation of fibrin by providing platelet surface phospholipid receptor sites for activated clotting factors Va and Xa, which bind to the

platelet surface and rapidly catalyse the conversion of prothrombin to thrombin. Fibrin formed by thrombin stabilizes the platelet aggregate and allows for the formation of a stable hemostatic plug. The complex interaction of platelet surface receptors and activated clotting factors has in the past been called platelet factor 3 activity. A patient with a congenital deficiency of receptors for factor Va has been described.[113,114] The patient had a normal bleeding time but had a significant history of menorrhagia and postoperative bleeding as well as easy bruising.

Heparin

Heparin is used in the treatment of venous thrombosis because of its inhibitory effect on coagulation.[115] It does not generally affect platelet function significantly as shown by the fact that the bleeding time remains normal following bolus injection of heparin. However, occasionally it may have a significant effect on platelets. In some patients heparin-induced thrombocytopenia develops, either as a result of an immunologic mechanism[116] or because of activation of platelets by a contaminating fraction in certain types of heparin preparation.[117]

Clinical Approach

The congenital disorders of platelet function have been studied extensively and have provided important insights into platelet physiology and pathophysiology; however, these defects are uncommon in clinical medicine. On the other hand, acquired syndromes of platelet dysfunction are more complex, less well studied, more difficult to classify, but occur frequently in a wide variety of clinical settings.

The severity of a bleeding disorder caused by platelet dysfunction depends not only on the nature of the platelet defect itself, but also on the severity of the hemostatic challenge. Disorders of platelet function vary in severity from the mild syndromes of easy bruisability seen, for example, in the storage-pool deficiencies, to the severe and sometimes fatal hemorrhages seen in such conditions as autosomal recessive von Willebrand disease. The bleeding tendency in any of the platelet function disorders may be aggravated by the introduction of additional hemostatic defects such as chemotherapy-induced thrombocytopenia or the administration of aspirin or anticoagulant drugs. Gastrointestinal or genitourinary bleeding may be a manifestation of defective platelet function; such bleeding may be precipitated by an underlying structural abnormality such as an ulceration or polyps of the colon or bladder.

The diagnostic approach to patients with platelet dysfunction should depend somewhat on the urgency with which the provisional diagnosis must be made in order to manage the bleeding diathesis. For example, the diagnostic workup of a patient with an acute bleeding episode may be limited to a screening test evaluation of hemostasis followed by a decision as to the possible role that a platelet defect may play in causing the bleeding. Because acute disorders are usually transient, comprehensive evaluation may not be possible. In contrast, the diagnostic workup in a patient with chronic or recurrent bleeding should be definitive. Most often the diagnosis can be suggested on the basis of information obtained in the history, physical examination and simple laboratory assessment; however, confirmation frequently requires diagnostic tests that are available only in specialized laboratories. In the case of a patient with a mild congenital bleeding disorder in whom von Willebrand disease has been excluded and for which there is not a specific therapy, there is greater flexibility in the time permitted for laboratory confirmation.

Approach to Diagnosis

The diagnosis of platelet dysfunction in most instances is made by the systematic analysis of information obtained in the history and physical examination and by laboratory evaluation of platelet function as well as of other aspects of hemostasis.[118]

Diagnostically, the most valuable data from a patient's history include (1) the onset, site, extent, severity and time course of bleeding episodes and the severity of the trauma that elicits such episodes; (2) a positive drug history; (3) a positive family history for bleeding tendencies and the pattern of bleeding in such family members, and (4) the presence of other illnesses that may be contributing factors in the disorder of platelet function.

On physical examination, the documentation of petechiae or purpura is suggestive of a defect in platelet plug formation. Because spontaneous gastrointestinal or genitourinary bleeding may in-

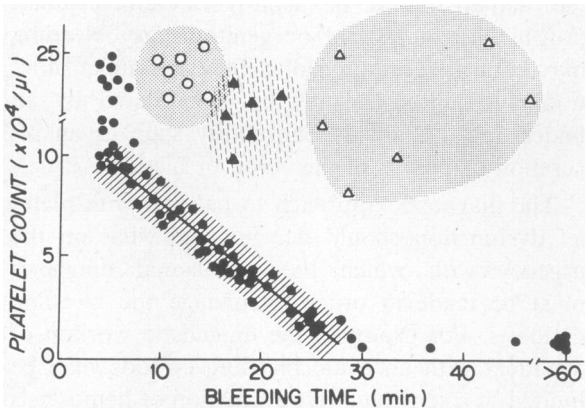

Figure 3.—Relationship of bleeding time to circulating platelet count in normal subjects and in patients with thrombocytopenia resulting from decreased production of platelets (closed circles). Platelet functional defects are also represented in subjects taking aspirin (open circles), in those with uremia (closed triangles) and in those with inherited von Willebrand disease (open triangles). (Reproduced with permission from Seminars in Hematology.)

dicate platelet dysfunction, the findings of guaiac-positive stools or hematuria may be important. Hemarthrosis and muscle hematomata are unusual manifestations of a qualitative defect of platelet function, except in severe von Willebrand disease, and generally suggest an alternative diagnosis such as hemophilia.[47] In patients in hospital with serious systemic illnesses, the presence of petechiae as well as oozing from surgical wounds, sites of trauma or venipuncture are important as evidence of defective platelet plug formation.

The screening laboratory evaluation of platelet function begins with a platelet count and a template bleeding time (Figure 3). The bleeding time is a simple, reproducible test of overall platelet function and has a normal range of 5.5 ± 1.5 minutes.[102,119] The bleeding time is significantly but variably prolonged in patients with a serious platelet function defect but adequate platelet counts. A qualitative abnormality superimposed on thrombocytopenia manifests as an inappropriately prolonged bleeding time for the degree of thrombocytopenia (Figure 3).

Light microscopic examination of a stained blood film may be useful. Large-appearing platelets may suggest the Bernard-Soulier syndrome, gray platelets are present in the gray platelet syndrome and pronounced platelet poikilocytosis may be seen in the myeloproliferative syndromes. Examination of the other formed elements of the blood may also be helpful.

In most situations of suspected platelet dysfunction a screening evaluation of the coagulation cascade is required. This consists of determining prothrombin time, partial thromboplastin time, thrombin time and amount of fibrinogen present. Many acquired disease states have an accompanying disorder of platelet function; therefore, the easiest way to make a diagnosis of a platelet dysfunction under such circumstances is to find a disproportionately prolonged bleeding time in a clinical situation in which platelet dysfunction is known to be relatively common.

Any patient with a history of abnormal bleeding and a prolonged bleeding time should be evaluated for the presence of VWD. The diagnosis is best established by the simultaneous measurements of FVIII:Co and FVIII:Ag. These measurements combined with a prolonged bleeding time and decreased platelet aggregation to ristocetin allow a diagnosis to be made in most patients with von Willebrand disease.[39]

Platelet aggregation is widely available for the evaluation of platelet function and may provide useful diagnostic information, especially in the congenital disorders of platelet function. For example, in vitro aggregation to ADP, epinephrine and collagen is absent with thrombasthenia and may be decreased in aspirin-like defects and storage-pool deficiencies. Ristocetin-induced aggregation is decreased to absent in most patients with von Willebrand disease or the Bernard-Soulier syndrome. Epinephrine fails to aggregate platelets in most patients with idiopathic thrombocythemia, a fact of some diagnostic use. Although in vitro testing of platelet aggregation may provide important diagnostic clues, the technique is difficult to reproduce and to standardize, and the abnormalities seen with such tests may have little or no relationship to the actual mechanism of the hemostatic defect in vivo.

Electron microscopy has been used in the enumeration of platelet δ-granules and α-granules to confirm storage-pool deficiencies.[120] Direct measurements of δ-granule ADP and ATP are required to differentiate storage-pool deficiencies from those like aspirin-caused defects. Platelet α-granule contents may be evaluated by measuring releasable pools of PF4 and β-TG by radioimmunoassay, tests that are now available commercially. Platelet arachidonic acid metabolism may be evaluated by measuring its stable end

product thromboxane B_2 using radioimmunoassay or by noting an absence of in vitro platelet aggregation to added arachidonic acid.

Management

Proper treatment of disorders of platelet function depends on an accurate diagnosis, and it varies from patient to patient and from disease to disease. Generally, treatment is directed at removing, if possible, the cause of the platelet dysfunction, such as treating systemic diseases like uremia or multiple myeloma and removing, if possible, drugs that may be causally implicated in platelet dysfunction syndromes. Immunosuppressive therapy may be indicated in immune-mediated dysfunction. In patients with platelet dysfunction resulting from deficiency of a required plasma cofactor such as von Willebrand disease, replacement therapy (for example, cryoprecipitate) is needed. If, on the other hand, an extrinsic factor acts as an inhibitor, as with plasma dysproteinemia, then replacement using platelet concentrates may be ineffective and removal of the inhibitor is necessary (for example, removal of a paraprotein by plasmapheresis). If the hemostatic disorder is due to an intrinsic platelet defect and the patient is bleeding, administration of platelet concentrates is indicated.

If both qualitative and quantitative platelet defects are present, the guidelines for use of platelet concentrates remain unclear. Patients have been shown to lose little blood if they have more than 5,000 per μl of normally functioning platelets and no underlying structural pathological condition (such as a colonic polyp or peptic ulcer).[76] Under such circumstances, prophylactic platelet concentrates are given only when the platelet count falls below 5,000 per μl. If, however, a qualitative defect is superimposed on a profound thrombocytopenia (as for example, carbenicillin therapy in a patient receiving pancytopenic chemotherapy), the risk of bleeding increases considerably and the recommendations of prophylactic platelet transfusions may be made at a platelet count of 20,000 per μl or more. If a patient is bleeding and this is due in whole or in part to a functional platelet defect, then platelet concentrates are indicated irrespective of the platelet count. Platelet concentrates should be used judiciously, however, because alloimmunization to platelets will develop, resulting in refractoriness to subsequently transfused platelet concentrates.[76] In such situations human leukocyte antigen (HLA)-typing and platelet cross-matching procedures are helpful in identifying compatible donors.[76,121]

REFERENCES

1. Baumgartner HR, Muggli R: Adhesion and aggregation: Morphological demonstration and quantitation in vivo and in vitro, *In* Gordon JL (Ed): Platelets in Biology and Pathology. Amsterdam, North-Holland, 1976, pp 23-60
2. Zucker MB: The functioning of blood platelets. Sci Am 242: 86-103, 1980
3. Harker LA, Ross R: Pathogenesis of arterial vascular disease. Sem Thrombos Hemostas 4:274-292, 1979
4. Mitchell JRA: Clinical events resulting from thrombus formation. Br Med Bull 34:103-106, 1978
5. Hardisty RM: Disorders of platelet function. Br Med Bull 33:207-212, 1977
6. Weiss HJ: Congenital disorders of platelet function. Sem Hematol 17:228-241, 1980
7. Caen JP, Cronsberg S, Cubisz P: Platelets: Physiology and Pathology. New York, Stratton International Medical Book Corp, 1977
8. Malpass TW, Harker LA: Acquired disorders of platelet function. Sem Hematol 17:242-258, 1980
9. White JG: Platelet morphology and function, *In* Williams WJ, Beutler E, Erslev AJ, et al (Eds): Hematology. New York, McGraw-Hill, 1977, pp 1159-1175
10. Holmsen H, Weiss HJ: Secretable storage pools in platelets. Ann Rev Med 30:119-134, 1979
11. Kaplan KL, Broekman MJ, Chernoff A, et al: Platelet alpha-granule proteins: Studies on release and subcellular localization. Blood 53:604-618, 1979
12. Crawford N, Taylor DG: Biochemical aspects of platelet behaviour associated with surface membrane reactivity. Br Med Bull 33:199-206, 1977
13. Weiss HJ: Platelet physiology and abnormalities of platelet function. N Engl J Med 293:531-541 and 580-588, 1975
14. Mustard JF, Packham MA: Normal and abnormal hemostasis. Br Med Bull 33:187-192, 1977
15. Baumgartner HR, Muggli R, Tschopp TB, et al: Platelet adhesion, release and aggregation in flowing blood: Effects of surface properties and platelet function. Thromb Hemostas 35: 124-138, 1976
16. Sakariassen KS, Bolhuis PA, Sixma JJ: Human blood platelet adhesion to artery subendothelium is mediated by factor VIII —von Willebrand factor bound to the subendothelium. Nature 279:636-638, 1979
17. Santoro SA, Cunningham LW: Fibronectin and the multiple interaction model for platelet-collagen adhesion. Proc Natl Acad Sci USA 76:2644-2648, 1979
18. Nurden AT, Caen JP: The different glycoprotein abnormalities in thrombasthenia and Bernard-Soulier platelets. Sem Hematol 16:224-250, 1979
19. Phillips DR: Membrane glycoproteins mediating platelet aggregation, *In* Mann KG, Taylor FB (Eds): The Regulation of Coagulation. New York, Elsevier, 1980, pp 259-270
20. Burch JW, Majerus PW: The role of prostaglandins in platelet function. Sem Hematol 16:196-207, 1979
21. Shick PK: The role of platelet membrane lipids in platelet hemostatic activities. Sem Hematol 16:221-233, 1979
22. Coller BS: Interaction of normal thrombasthenic, and Bernard-Soulier platelets with immobilized fibrinogen: Defective platelet--fibrinogen interaction in thrombasthenia. Blood 55:169-177, 1980
23. Kinlough-Rathbone RL, Packham MA, Reimers H-J, et al: Mechanisms of platelet shape change, aggregation, and release by collagen, thrombin or A23, 187. J Lab Clin Med 90:707-719, 1977
24. Majerus PW, Millotich JP, Kane WH, et al: The formation of thrombin on platelet surface, *In* Mann KG, Taylor FB (Eds): The Regulation of Coagulation. New York, Elsevier, 1980, pp 215-224
25. White JG, Rao GHR, Gerrard JM: Platelet stimulus activation contraction secretion coupling: A frequently fractured chain of events, *In* Mann KG, Taylor FB (Eds): The Regulation of Coagulation. New York, Elsevier, 1980, pp 363-376
26. Hathaway DR, Eaton CR, Adelstein RS: Regulation of human platelet myosin kinase by calcium—calmodulin and cyclic AMP. *In* Mann KG, Taylor FB (Eds): The Regulation of Coagulation. New York, Elsevier, 1980, pp 271-276
27. Moncada S, Vane JR: Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. N Engl J Med 300:1142-1147, 1979
28. Gerrard JM, White JG: Prostaglandins and thromboxanes: "Middlemen" modulating platelet function in hemostasis and thrombosis. Prog Hemost Thromb 4:87-125, 1978
29. Bell RL, Majerus PW: Thrombin-induced hydrolysis of phosphatidylinositol in human platelets. J Biol Chem 255:1790-1792, 1980

30. Burch JW, Stanford N, Majerus PW: Inhibition of platelet prostaglandin synthetase by oral aspirin. J Clin Invest 61:314-319, 1978

31. DiMinno G, Silver MJ, DeGaetano G: Prostaglandins as inhibitors of human platelet aggregation. Br J Hematol 43:632-647, 1979

32. Pinnell SR: Disorders of collagen, *In* Stanbury JB, Wyngaarden JB, Fredrickson DS (Eds): The Metabolic Basis of Inherited Disease. New York, McGraw-Hill, 1978, pp 1366-1394

33. Weary PE, Wheeler CE, Canley EP: Adult scurvy. Arch Dermatol 83:657-659, 1961

34. Crandon JH, Lund CC, Dill DB: Experimental human scurvy. N Engl J Med 223:353-369, 1940

35. Glenner GG: Amyloid deposits and amyloidosis. The β-fibrilloses. N Engl J Med 302:1283-1292 and 1333-1343, 1980

36. Brownstein MH, Helwig EB: The cutaneous amyloidosis. Arch Dermatol 102:20-28, 1970

37. Greipp PR, Kyle RA, Bowie EJW: Factor X deficiency in primary amyloidosis: Modulation after splenectomy. N Engl J Med 301:1050-1051, 1979

38. Weiss HJ, Tschopp TB, Baumgartner HR, et al: Decreased adhesion of giant (Bernard-Soulier) platelets to subendothelium: Further implications on the role of the von Willebrand factor in hemostasis. Am J Med 57:920-925, 1974

39. Hoyer LW: Von Willebrand's disease. Prog Hemost Thromb 3:231-287, 1976

40. Miller CH, Graham JB, Goldin LR, et al: Genetics of classic von Willebrand's disease—I. Phenotypic variation within families. Blood 54:117-136, 1979

41. Italian Working Group: Spectrum of von Willebrand's disease: A study of 100 cases. Br J Haematol 35:101-112, 1977

42. Salzman EW: Measurement of platelet adhesiveness: A simple in vitro technique demonstration on abnormality in von Willebrand's disease. J Lab Clin Med 62:724-735, 1963

43. Gralnick HR, Sultan Y, Coller BS: Von Willebrand's disease: Combined qualitative and quantitative abnormalities. N Engl J Med 296:1024-1025, 1977

44. Zimmerman TS, Abildgaard CF, Meyer D: The factor VIII abnormality in severe von Willebrand's disease. N Engl J Med 301:1307-1310, 1979

45. Tschopp TB, Weiss HJ, Baumgartner HR: Decreased adhesion of platelets to subendothelium in von Willebrand's disease. J Lab Clin Med 83:296-300, 1974

46. Weiss HJ, Baumgartner HR, Tschopp TC, et al: Correction by factor VIII of the impaired platelet adhesion to subendothelium in von Willebrand's disease. Blood 51:267-279, 1978

47. Rizza CR: Von Willebrand's disease, *In* Biggs R (Ed): The Treatment of Haemophilia A and B and von Willebrand's Disease. Oxford, Blackwell Scientific Publications, 1978, pp 172-180

48. Ruggeri FM, Pareti FI, Mannucci PM, et al: Heightened interaction between platelets and factor VIII/von Willebrand factor in a new subtype of von Willebrand's disease. N Engl J Med 302:1047-1051, 1980

49. Nilsson IM, Bergentz S-E, Larsson SA: Surgery in von Willebrand's disease. Ann Surg 190:746-752, 1979

50. Ingram GIC, Prentice CRM, Forbes CD, et al: Low factor VIII-like antigen in acquired von Willebrand's syndrome and response to treatment. Br J Haematol 25:137-140, 1973

51. Handin RI, Martin V, Moloney WC: Antibody-induced von Willebrand's disease: A newly defined inhibitor syndrome. Blood 48:393-405, 1976

52. Joist JH, Cowan JF, Zimmerman TS: Acquired von Willebrand's disease. N Engl J Med 298:988-991, 1978

53. Meyer D, Frammel D, Larrien MJ, et al: Selective absence of large forms of factor VIII/von Willebrand factor in acquired von Willebrand's syndrome—Response to transfusion. Blood 54: 600-606, 1979

54. Castaldi PA, Rozenberg MC, Stewart JA: The bleeding disorder of uraemia—A qualitative platelet defect. Lancet 2:66-69, 1966

55. Steiner RW, Coggins C, Carvallho ACA: Bleeding time in uremia: A useful test to assess clinical bleeding. Am J Hematol 7:107-117, 1979

56. Horowitz HI, Stein IM, Cohen BD: Further studies on the platelet-inhibiting effect of guanidino succinic acid and its role in uremic bleeding. Am J Med 49:336-345, 1970

57. Packham MA, Mustard JF: Clinical pharmacology of platelets. Blood 51:555-573, 1977

58. Wautier JL, Caen JP: Pharmacology of platelet-suppressive agents. Sem Thromb Hemost 5:293-315, 1979

59. Kinlough-Rathbone RL, Groves HM, Cazenave J-P, et al: Effect of dipyridamole and aspirin in platelet adherence to damaged rabbit aortas in vitro and in vivo. Fed Proc 37:260, 1978

60. Harker LA, Hanson SR: Experimental arterial thromboembolism in baboons—Mechanism, quantitation and pharmacologic prevention. J Clin Invest 64:559-569, 1979

61. Moncada S, Korbut R: Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. Lancet 1:1286-1289, 1978

62. Sullivan JM, Harken DE, Gorlin R: Pharmacologic control of thromboembolic complications of cardiac-valve replacement. N Engl J Med 284:1391-1394, 1971

63. The Persantine Aspirin Reinfarction Study Research Group: Persantine and aspirin in coronary heart disease. Circulation 62: 449-461, 1980

64. Caen J, Castaldi PA, Leclerc JC, et al: Congenital bleeding time and normal platelet count—I. Glanzmann's thrombasthenia (report of 15 patients). Am J Med 41:4-26, 1966

65. Caen JP: Glanzmann's thrombasthenia. Clin Hematol 1: 383-392, 1972

66. McEver RP, Baenziger NL, Majerus PW: Isolation and quantitation of the platelet protein lacking in thrombasthenia using a monoclonal hybridoma antibody. Clin Res 28:495A, 1980

67. Weiss HJ, Rogers J: Fibrinogen and platelets in the primary arrest of bleeding: Studies in 2 patients with congenital afibrinogenemia. N Engl J Med 285:369-374, 1971

68. Sharp AA: Diagnosis and management of disseminated intravascular coagulation. Br Med Bull 33:265-272, 1977

69. Orloff KG, Michaeli D: Inhibition of fibrin-platelet interactions by fibrinogen-degradation fragment D. Am J Physiol 233: H305-H311, 1977

70. Deutsch E: The liver and blood clotting. Sem Thromb Hemost 4:1-82, 1977

71. Perkins HA, MacKenzie MR, Fudenberg HH: Hemostatic defects in dysproteinemias. Blood 35:695-707, 1970

72. Clagett GP, Salzman EW: Prevention of venous thromboembolism. Prog Cardiovasc Dis 17:345-366, 1975

73. Weiss HJ: The effect of clinical dextran on platelet aggregation, adhesion and ADP release in man: in vitro and in vivo studies. J Lab Clin Med 69:37-46, 1967

74. Brown III CH, Natelson EA, Bradshaw, MW, et al: The hemostatic defect produced by carbenicillin. N Engl J Med 291: 265-270, 1974

75. Shattil SJ, Bennett JS, McDonough M, et al: Carbenicillin and penicillin G inhibit platelet function in vitro by impairing the interaction of agonists with the platelet surface. J Clin Invest 65: 329-337, 1980

76. Slichter SJ, Harker LA: Thrombocytopenia: mechanisms and management of defects in platelet production. Clin Hematol 7:523-539, 1978

77. Raccuglia G: Gray platelet syndrome: a variety of qualitative platelet disorders. Am J Med 51:818-828, 1971

78. Gerrard JM, Philips DR, Rao GH, et al: Biochemical studies of two patients with the gray platelet syndrome—Selective deficiency of platelet alpha granules. J Clin Invest 66:102-109, 1980

79. Harker LA, Malpass TW, Branson HE, et al: Mechanism of abnormal bleeding in patients undergoing cardiopulmonary bypass: Acquired transient platelet dysfunction associated with selective α-granule release. Blood 56:824-834, 1980

80. Bick, RL: Alteration of hemostasis associated with cardiopulmonary bypass: Pathophysiology, prevention, diagnosis and management. Sem Thromb Hemost 3:59-82, 1976

81. Holmsen H, Weiss HJ: Further evidence for a deficient storage pool of adenine nucleotides in platelets from some patients with thrombocytopathia—"Storage pool disease." Blood 39:197-209, 1972

82. Weiss HJ, Witte LD, Kaplan KL, et al: Heterogeneity in storage pool deficiency: Studies on granule bound substances in 18 patients including variants deficient in α-granules, platelet factor 4, β-thromboglobulin, and platelet-derived factor. Blood 54:1296-1319, 1979

83. Clancy RL, Jenkins E, Firkin B: Qualitative platelet abnormalities in idiopathic thrombocytopenic purpura. N Engl J Med 286:622-626, 1972

84. Russel NH, Keenan JP: Thrombocytopathy associated with autoimmune hemolytic anemia. Br Med J 2:604, 1978

85. Karpatkin S, Lackner HL: Association of antiplatelet antibody with functional platelet disorders. Autoimmune thrombocytopenic purpura systemic lupus erythematosus and thrombopathia. Am J Med 59:599-604, 1975

86. Zahavi J: Acquired "storage pool disease" of platelets. Thromb Hemost (Stugg) 35:501-507, 1976

87. Czapek EE, Deykin D, Salzman E, et al: Intermediate syndrome of platelet dysfunction. Blood 52:103-113, 1978

88. Malmsen C, Hamberg M, Svensson J, et al: Physiological role of an endoperoxide in human platelets: Hemostatic defect due to platelet cyclooxygenase deficiency. Proc Natl Acad Sci USA 72:1446-1448, 1975

89. Lagarde M, Byron PA, Vargaftig BB, et al: Impairment of platelet thromboxane A_2 generation and of the platelet release reaction in two patients with congenital deficiency of platelet cyclooxygenase. Br J Hematol 38:251-266, 1978

90. Weiss HJ, Lages BA: Possible congenital defect in platelet thromboxane synthetase. Lancet 2:760-761, 1977

91. Mestel F, Oetliker O, Beck E, et al: Severe bleeding association with thromboxane synthetase. Lancet 1:157, 1980

92. Dyerberg J, Bang HO: Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 2:433-435, 1979

93. Siess W, Scherer B, Bohlig B, et al: Platelet-membrane fatty acids, platelet aggregation and thromboxane formation during a mackerel diet. Lancet 1:441-444, 1980

94. Cowan DH, Graham RC Jr, Baunach D: The platelet defect in leukemia—Platelet ultrastructure, adenine nucleotide metabolism, and the release reaction. J Clin Invest 56:188-200, 1975

95. Russell NH, Kennan JP, Bellingham AJ: Thrombocytopathy in preleukemia: Association with a defect of thromboxane A_2 activity. Br J Haematol 41:417-425, 1979

96. Lisiewicz J: Mechanisms of hemorrhage in leukemia. Sem Thromb Hemostasis 4:241-267, 1978

97. Cardamone JM, Edson JR, McArthur JR, et al: Abnormalities of platelet function in the myeloproliferative disorders. JAMA 221:270-273, 1972

98. Murphy S, Davis JL, Walsh PN, et al: Template bleeding time and clinical hemorrhage in myeloproliferative disease. Arch Intern Med 138:1251-1253, 1978

99. Kaywin P, McDonough M, Insel PA, et al: Platelet function in essential thrombocythemia. N Engl J Med 299:505-509, 1978

100. Gerrard JM, Stoddard SF, Shapiro RS, et al: Platelet storage pool deficiency and prostaglandin synthesis in chronic granulocytic leukemia. Br J Haematol 40:597-607, 1978

101. Boughton BJ, Corbett WEN, Ginsberg AD, et al: Myeloproliferative disorders: Paradox of in vivo and in vitro platelet function. J Clin Pathol 30:228-234, 1977

102. Mielke Jr CH, Kanehiro MM, Maher IA, et al: The standardized normal Ivy bleeding time and its prolongation by aspirin. Blood 34:204-215, 1969

103. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (New Biol) 231: 232-235, 1971

104. Moncada S, Vane JR: Mode of action of aspirin-like drugs. Adv Intern Med 24:1-22, 1979

105. Roth GJ, Stanford N, Majerus PW: Acetylation of prostaglandin synthetase by aspirin. Proc Natl Acad Sci USA, 72:3073-3076, 1975

106. The Anturane Reinfarction Trial Research Group: Sulfinpyrazone in the prevention of sudden death after myocardial infarction. N Engl J Med 302:250-255, 1980

107. Ali M, McDonald JWD: Effects of sulfinpyrazone on platelet prostaglandin synthesis and platelet release of serotonin. J Lab Clin Med 89:868-875, 1977

108. Rossi EC, Levin NW: Inhibition of primary ADP-induced platelet aggregation in normal subjects after administration of nitrofurantoin (Furadantin). J Clin Invest 52:2457-2467, 1973

109. Cowan DH: Effect of alcoholism on hemostasis. Sem Hematol 17:137-147, 1980

110: Haut MJ, Cowan DH: The effect of ethanol in hemostatic properties of human blood platelets. Am J Med 56:22-33, 1974

111. Stuart MJ: Ethanol-inhibited platelet prostaglandin synthesis in vitro. J Studies Alcohol 40:1-6, 1979

112. Michel H, Caen JP, Burn GUR, et al: Relation between the inhibition of aggregation and the concentration of cAMP in human and rat platelets. Br J Hematol 33:27-38, 1976

113. Miletich JP, Kane WH, Hofmann SL, et al: Deficiency of factor X_a-factor V_a binding sites on the platelets of a patient with a bleeding disorder. Blood 54:1015-1022, 1979

114. Weiss HJ, Vicic WJ, Lages BA, et al: Isolated deficiency of platelet procoagulant activity. Am J Med 67:206-213, 1979

115. Rosenberg RD: Biologic actions of heparin. Sem Hematol 14:427-440, 1977

116. Babcock RB, Dumper CW, Scharfman WB: Heparin-induced immune thrombocytopenia. N Engl J Med 295:237-241, 1976

117. Salzman EW, Rosenberg RD, Smith MH, et al: Effect of heparin fractions on platelet aggregation. J Clin Invest 65:64-73, 1980

118. Ingram GIC: Investigation of a longstanding bleeding tendency. Br Med Bull 33:261-264, 1977

119. Harker LA, Slichter SJ: The bleeding time as a screening test for evaluation of platelet function. N Engl J Med 287:155-159, 1972

120. Weiss HJ, Ames RP: Ultrastructural findings in storage pool disease and aspirin-like defects of platelets. Am J Pathol 71: 447-458, 1973

121. Slichter SJ: Controversies in platelet transfusion therapy. Ann Rev Med 31:509-540, 1980