THE Diagnosis OF Bleeding Disorders

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PREFACE

Concepts of blood coagulation and hemostasis are in a curious state of flux. Small facts are developed into large hypotheses which in turn become accepted as factual. A very personal vocabulary has evolved. Each of the terms *cascade, autoprothrombin, waterfall,* and *prothrombinogen* clearly characterizes a specific investigator. But patients bleed because of a variety of identifiable hemostatic abnormalities, so logical treatment depends on the accuracy of the identification, not on the terminology or hypothesis.

We have attempted a simple and logical review of the hemostatic abnormalities related to the blood vessel, the blood platelet, and the coagulation mechanism. As might be expected, this book is concerned mainly with blood platelets and blood coagulation because of the paucity of knowledge concerning the blood vessels. We are convinced that a number of bleeding disorders primarily affect the blood vessels. These disorders, however, remain poorly characterized, although in the future more research will undoubtedly be devoted to this aspect of hemostasis.

The chapters that review the bleeding diatheses are comprehensive, and each disease has a brief clinical description because we think that this is an area of medicine in which the clinical and laboratory features must be considered together in arriving at a diagnosis. The description of each bleeding disease includes a separate consideration of the tests actually used in making an exact diagnosis and, in many instances, case histories and laboratory results are detailed.

The chapter dealing with the basic tests of hemostasis includes only the tests we have used and consider helpful in clinical diagnosis. In nearly all cases, sufficient details are given to allow the tests to be instituted in a laboratory which has not previously performed them. Tables have been compiled to summarize the conditions in which the tests give abnormal results.

Bleeding disorders are the main subject of consideration but, because the hemostatic mechanism is involved in the formation of thrombi, a short consideration of thrombosis seemed appropriate. The interdependence of bleeding and thrombosis is best illustrated in the intravascular coagulation-fibrinolysis syndrome, and this syndrome is discussed in detail in a separate chapter.

Because we think that the subject of coagulation can be best understood in historical perspective, we have devoted a large chapter to the evolution of coagulation theories leading to the modern concepts including the Mayo Clinic, waterfall, cascade, Seegers', and Quick's schemata. The chapter contains the most complete summary available of platelet factors and other coagulation activities, whether or not they are accredited by the International Committee for the Nomenclature of Bleeding Clotting Factors. Rather than just mention the names of the outstanding research workers of the past, we have included photographs and biographical sketches to give more meaning to the historical review.

Anyone who has to deal with bleeding diseases will inevitably be asked about the inheritance pattern of the congenital disorders, so a chapter dealing with the basic genetic patterns has been included. Similarly, even though the book is primarily intended for the diagnostician and the diagnostic laboratory, a chapter on transfusion therapy has been included which deals primarily with stability of the various coagulation factors in vivo and in banked blood.

In summary, our book deals with the laboratory diagnosis of all the major bleeding disorders based on a clinical approach. A large proportion of patients studied because of suspected hemostatic problems have equivocal histories of bleeding; we have found the approach described herein particularly helpful in the evaluation of these patients. We have tried to be practical and have aimed our remarks at the clinical pathologist, the hematologist, and the medical technologist, although this book should also be of interest to the internist, the pediatrician, and the medical student.

We wish to thank our colleagues who kindly shared their clinical experience with us and especially Drs. Virgil F. Fairbanks, Albert B. Hagedorn, Francis J. Kazmier, James W. Linman, Jorge E. Maldonado, Sheldon G. Sheps, John A. Spittell, Jr., and Howard F. Taswell for valuable suggestions. We could not have written this book without the outstanding technical assistance of Mrs. Charlene Matthees, Miss Virginia Rauen, Mrs. Therese L. Cole, Mrs. Joan M. McGowan, Mrs. Joan A. Ayshford, and Miss Dorothy L. Bunting. The untiring efforts of Miss Isabelle Fitch and Mrs. Molly Genz, who devoted long hours to typing our manuscript and its many revisions, are gratefully acknowledged. Miss Sylvia H. Haabala and Miss Ruth J. Mann ingeniously tracked down obscure references and hard-to-find photographs. Our thanks are also due to Dr. Bernard K. Forscher, who edited the manuscript, and to those members of the Section of Publications who were responsible for the final preparation of the manuscript.

No book can be written without a certain amount of domestic turmoil (because husbands are lost in thought) and without a decrease in the time they devote to family pursuits. Quadruple authorship, furthermore, is in some ways more time-consuming because four people must

approve every step. We therefore are grateful for the devotion of our wives and families, who understood us enough to allow us to write this book with only intermittent and minor protesting.

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INTRODUCTION AND HISTORICAL PERSPECTIVE

Hemostasis is the mechanism by which animals with a vascular system are protected from death by bleeding after an injury. The hemostatic process depends upon a variety of phenomena including vascular integrity, vasoconstriction, platelet aggregation, and blood coagulation [258]. In general, more research has been devoted to aspects of coagulation, probably because the experiments are technically simpler and only a sample of blood is needed. However, in the past decade there has been renewed interest in the platelet and vascular aspects of hemostasis.

Vasoconstriction

Vasoconstriction is under the control of neural, local, and humoral mechanisms [296]. Neural control depends upon sympathetic vasoconstrictor fibers whose distribution and means of stimulation vary considerably in different tissues and organs. Local factors include temperature, blood pH and P_{CO_2}, and effects of exercise. Humoral agents include epinephrine, norepinephrine, serotonin, adenosinetriphosphate (ATP), adenosinediphosphate (ADP), acetylcholine, histamine, vasopressin, oxytocin, bradykinin, and the fibrinopeptides released at the onset of fibrin formation [60]. What may cause constriction in a vessel under one set of circumstances may cause dilatation under a different set. Thus, serotonin is both a vasoconstrictor and a vasodilator. Although an artery constricts when irritated or cut, a capillary does not necessarily respond similarly. Since capillaries lack muscle cells, they are apparently not under neurogenic control but may be subject to humoral stimulation. The dominant structures in regulating blood flow are the precapillary sphincter, the arteriole, and the venule [353]. The older evidence that vasoconstriction is of primary importance in hemostasis, well summarized by Quick [254], may correctly state the case, but considerably more documentation is needed.

The hemostatic importance of the blood vessel has been emphasized by Cruz. He showed the significance of plasma lipids and vessel innervation in the hemostatic process in experiments using the isolated hind limb of the dog [64]. In subsequent studies he used an improved

rat-tail bleeding-time test to produce further evidence of the part played by the contraction of arteriolar smooth muscle [63]. Cruz suggested that the arteriolar events are primary in effecting hemostasis and that the formation of the platelet plug and the fibrin clot are after-effects.

Platelets

Only in recent years has the concept of platelet plugging of damaged vessels been placed on a firm footing. However, the basic principle is not new. Platelet aggregation after vascular injury, independent of and preceding clot formation, was described in 1888 by Eberth and Schimmelbusch [84]. Morawitz , in his monumental review [204] published in 1905, stated, "In the event of damage to the vascular wall . . . there occurs in this locale an agglutination of platelets and leukocytes which need not necessarily lead to true coagulation if the lesion is minor [205]." The translation is by Hartmann and Guenther.

Even earlier, in 1820, Thackrah [310] recognized the importance of the "vitality" of intact blood vessels in maintaining the fluidity of blood.

The first reasonably clear description of platelets was published in 1842 by Donné [79], who thought they might be derived from chyle and be the precursors of leukocytes. It was not until forty years later that Bizzozero identified them clearly as cells distinct from erythrocytes and leukocytes [35]. He, as well as Schimmelbusch [281], showed that these cells could adhere to each other and to foreign surfaces. Finally, Wright recognized the origin of platelets, or "plates" as he called them, from megakaryocytes [345].

Various hemostatic functions have been ascribed to the platelet, and these have even been formalized into numbered factors [290b]. How many of these platelet factors have relevance in hemostasis is far from clear. For example, platelet factor 1 seems to be synonymous with factor V (labile factor) of plasma, and in parahemophilia this activity seems to be absent from the platelet [121]. Perhaps other activities in the platelet also simply represent absorption of plasmatic substances

such as serotonin, fibrinogen, antifibrinolysin, and fibrin-stabilizing factor. On the other hand, three platelet functions—platelet factor 3 activity, aggregation, and clot retraction—are apparently unique, and at least two of them are unquestionably related to hemostasis.

Platelet factors

PLATELET FACTOR 1. This is probably identical to plasmatic factor V [122, 184].

PLATELET FACTOR 2. This is a fibrinoplastic substance—that is, it shortens the clotting time of a mixture of purified thrombin plus purified fibrinogen [332].

PLATELET FACTOR 3. This is the phospholipid procoagulant activity arising from platelet lipoproteins. It was originally described as also having antiheparin activity, but the two activities have been differentiated [74] and the antiheparin activity [320, 321] has been called platelet factor 4.

By the term "platelet factor 3" is meant a clot-promoting activity which is "partial" or "incomplete" when compared with tissue juices ("thromboplastin") [184]. Platelet factor 3 activity may be reduced to such a low level that the patient has a bleeding tendency (deficit thrombopathy) or it may be present in but ineffectively released from the platelet (functional thrombopathy) [42]. Because certain phospholipids can simulate platelet factor 3 activity, it appears likely that such compounds in the platelet, perhaps in the form of phospholipoproteins, are responsible for this clotting activity. The platelet factor 3 activity seems to reside in phosphatidyl serine in some coagulation tests and in phosphatidyl ethanolamine in others [185]. Other phospholipids, such as phosphatidyl inositol, may be active, but the plasmalogens apparently are not. Whatever the exact chemical nature, White and Krivit [343] have shown that, in the platelet clump, phospholipid globules develop within the platelet and move steadily toward and through the platelet-plasma membrane to be discharged into the periplatelet environment (*milieu périplaquettaire*). Although Marcus and associates [186, 187] think that platelet factor 3 activity is physiologically limited to the platelet membrane, many regard it as being derived from the granules [96, 142, 289, 343], perhaps the alpha granules [288]. At least part of the platelet factor 3 activity may be dissolved in the protoplasm, constituting "nonsedimentable" [124] platelet factor 3.

PLATELET FACTOR 4. This is an antiheparin factor. When heparin is added to blood, its anticoagulant activity is inversely proportional to the number of platelets present [61].

PLATELET FACTOR 5. This is platelet fibrinogen. It has the same immunologic properties as plasma fibrinogen and is absent from platelets in patients with afibrinogenemia [279]. One curious feature of the platelet fibrinogen is its partial incoagulability in the presence of thrombin [210]. In Glanzmann's thrombasthenia the amount of fibrinogen in platelets is less than one third of normal [209].

PLATELET FACTOR 6. This is an antifibrinolysin. The more platelets in a clot, the more resistant the clot is to fibrinolysis. In addition, the fibrinogen of platelets is resistant to fibrinolysis [141].

PLATELET FACTOR 7. This is described as being co-thromboplastic—that is, it activates thromboplastin. Effective clotting of plasma with Russell's viper venom requires prothrombin, fibrinogen, calcium,

factor V, and factor X, as well as platelet factor 7 [167]. The evidence that this factor differs from platelet factor 3 is incomplete.

MISCELLANEOUS. Other activities of the platelet, not numbered as factors, include its high concentration of serotonin (5-hydroxytryptamine) [86] and other vasoactive amines, procbagulant inhibitor (excess of platelets slows down clotting) [146], thrombosthenin [29], and fibrin-stabilizing factor [174]. More than 30 enzymes have been identified, so the platelet apparently is metabolically very active.

PLATELET SUBSTITUTES. A number of partial or incomplete thromboplastins are commonly used as substitutes for platelets in the coagulation laboratory because platelets are not easy to obtain free of other cellular elements and their stickiness makes them difficult to work with. Of the partial thromboplastins, the oldest is probably "cephalin" by which is usually meant a petroleum ether-soluble fraction of acetone-dried brain. An extract of whole brain, the thromboplastin of the Quick test, is a combination of lipids constituting "complete thromboplastin" [136] whereas Bell and Alton [28] utilized the chloroform-extractable material from acetone-dried brain. Quick and associates [259], following Morawitz' lead [203], have promoted the use of erythrocytin which is an acetone-dried preparation from hemolyzed erythrocytes. Because of its ready availability and uniform activity, a soybean preparation, Inosithin, is our favorite [231]. It is readily emulsified in saline and can be used in the partial thromboplastin test, the prothrombin consumption test, and the thromboplastin generation test, as well as in specific assays of the prothromboplastic factors.

PLATELET AGGREGATION

The second property of platelets which seems to be clearly related to hemostasis is their tendency to adhere to one another and to damaged vessel surfaces. ADP apparently is the key to this reaction, as first shown by Hellem and associates [103, 118]. However, washed platelets will undergo no change when ADP is added unless calcium and an unidentified constituent of plasma are also present. Rodman and colleagues [275] think the plasma factor may be fibrinogen, and Inceman and co-workers [135] have shown that "platelet spreading adhesion" and ADP aggregation are reduced in afibrinogenemia. The observation that collagen is an active agent for inducing platelet aggregation [40, 127, 131, 132], mediated by platelet ADP [128], suggests that it may be the physiologic tropistic factor for platelet plugging at the site of vascular damage. ADP not only induces clumping of platelets but it also accelerates coagulation by several mechanisms including increased platelet factor 3 availability and activation of a factor in plasma [241, 242].

According to Jackson and associates [137], of all the cells of the

body, only the platelet is susceptible to the effects of thrombin which is rapidly generated at sites of injury [140]. After platelets are exposed to thrombin, they release serotonin [266], adenine nucleotides [39], and other chemicals [113, 351], and they begin to fuse together or aggregate. Fonio , who devoted much of his life to the study of platelets, first [94, 95] recognized that thrombin initiated morphologic changes in the platelet [93].

Several names have been applied to the phenomenon of clumping of platelets. Since none has a self-evident meaning, their use becomes one of tradition. The term "platelet agglutination" should be confined to the clumping of platelets on an immunologic basis, comparable to erythrocyte agglutination when incompatible bloods are mixed. "Platelet aggregation" refers to the nonimmunologic attachment of platelets to each other. When a solution of calcium chloride is added to platelet-rich oxalated plasma, platelet aggregation precedes the actual clotting. "Platelet adhesiveness" is the term used to describe the cementing of

platelets to nonplatelet surfaces, such as damaged vessel walls in vivo or glass surfaces in vitro. By "viscous metamorphosis" was originally meant [35, 83, 346] the morphologic change in a single platelet before aggregation had occurred. Essentially this phenomenon consists of the loss of the rounded form of the platelet and the development of dendrites. Viscous metamorphosis rarely occurs in Glanzmann's thrombasthenia, a fact perhaps related to the inability of clots to retract in this disease. Currently the concept of viscous metamorphosis is confused and is often described as the changes which occur in vitro when platelets are exposed to thrombin, collagen, and so on [176]. Many think that the term should be abandoned.

Clot retraction

The third property of platelets is not so clearly a feature of the hemostatic mechanism. It has long been known that, in the absence of platelets, blood clots fail to retract [117]. When Glanzmann found a clinical bleeding state characterized by an absence of clot

retraction despite the presence of adequate numbers of platelets [105], it seemed that some platelet constituent probably was necessary for the retraction. This constituent has been called "thrombosthenin" and has been described as a protein of the actomyosin class [29]. How thrombosthenin actually makes a clot retract is not clear. Tocantins [312, 313] explained it by the shortening of fibrin strands to which the platelets were adherent. But even though retraction occurs normally, there is no convincing evidence that the mechanism is of hemostatic significance. The extreme views are those of Frank [99, 100], who thought that as clots retracted in vivo the walls of the injured vessels were drawn together, and those of Budtz-Olsen [50], who questioned the existence of any physiologic role for clot retraction.

The studies by Gross and co-workers [108] have shown that some patients with Glanzmann's thrombasthenia have a low content of ATP in their platelets. They related this deficiency to decreased amounts of the enzymes glyceraldehyde phosphate dehydrogenase and pyruvic kinase. Other patients, however, have normal platelet glycolysis. Thrombasthenic platelets do not aggregate on the addition of ADP, a finding which may be related to the glycolytic abnormalities [103]. Salzman [280] finds that thrombosthenin has adenosinetriphosphatase (ATPase) activity, confirming the work of Bettex-Galland and Lüscher [29].

Although a prolonged bleeding time and absence of clot retraction are now considered necessary for the diagnosis of thrombasthenia, Glanzmann's original patients had normal bleeding times and some were thrombocytopenic.

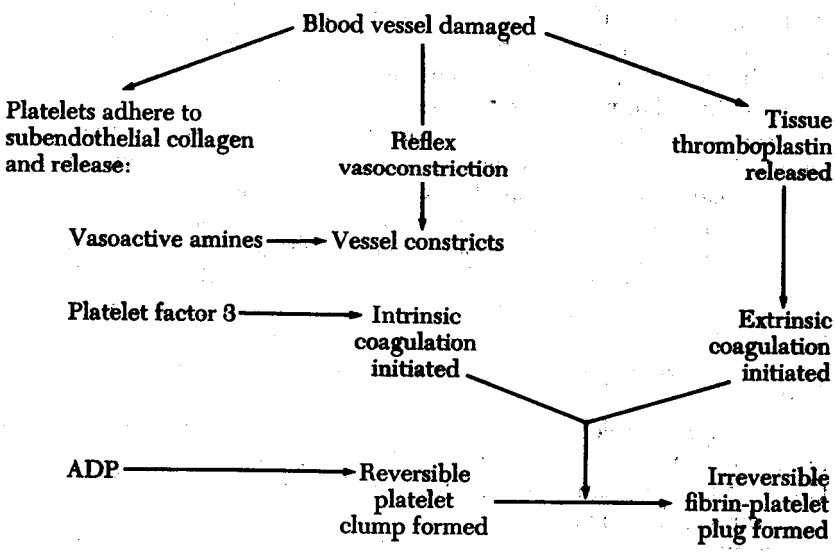
Despite the facts that the platelet actively concentrates serotonin from the plasma [86, 352] and contains some 90% of all the serotonin in the blood, it is not altogether certain that the release of serotonin at the site of a platelet plug is important in hemostasis. That serotonin at times is an active vasoconstrictor is unquestioned, and it has been shown [350] that thrombin releases serotonin from platelets quantitatively. Although Shore and colleagues [299] found that patients with serotonin-depleted platelets did not have prolonged bleeding times, there is some evidence [138, 149] that the serotonin in platelets has a hemostatic effect.

Summary of platelet functions

From these various observations one may develop a three-stage concept of the role of platelets in hemostasis, as reviewed by Lüscher [175]. Initially there is adhesion of platelets to denuded subendothelial collagen at the site of injury. As platelets progressively adhere to the collagen and to each other, they undergo fusion, and a platelet plug develops. Finally, there is fibrin consolidation of the plug, initiated by release of platelet factor 3 and local clotting (Table 1-1). Perhaps there are two additional steps: initial local vasoconstriction from release of

platelet serotonin and other vasoconstrictive amines, and subsequent vasoconstriction from retraction of the clot.

TABLE 1–1
SCHEME OF PLATELET CONTRIBUTION TO HEMOSTASIS



Classic Theory of Coagulation

Morawitz is given the credit for assembling the work of a number of investigators into a comprehensive theory of clotting. This theory [204], published in 1905, acknowledged the work of Hammarsten on the purification of fibrinogen and its clottability by thrombin [109–111]. Buchanan , whose contributions [49] have been reviewed by Verso [323], and subsequently Schmidt [285] were primarily responsible for the concept of an inactive precursor of thrombin or "fibrin ferment" in plasma—namely, prothrombin. The role of calcium was first clearly defined by Arthus and Pages [18, 19]. "Thromboplastin" is the current term for the zymoplastic substance in tissues which Schmidt [284] described as necessary for the conversion of prothrombin to thrombin. It remained for Morawitz [204] and Fuld and Spiro [102] to show convincingly that tissue juices did not contain thrombin or prothrombin as their active constituent.