LABORATORY EVALUATION of HEMOSTASIS

SIRRIDGE

SECOND EDITION

LABORATORY EVALUATION OF HEMOSTASIS

Second Edition

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Preface

Since the first edition of this book was published in 1967, I have continued to care for many patients with hemostatic problems, to teach many medical students and technologists about bleeding disorders, and to participate in several laboratories in the modification of some established tests and the evaluation and utilization of many new ones.

My primary interest continues to be in those studies that can be done in the routine laboratory and that relate to the diagnosis and care of patients. I appreciate the large role of the manufacturers of coagulation reagents in the progress in availability of new reagents and quality control. I have been increasingly impressed with the need for qualified medical technologists who really understand the process of hemostasis because of the many pitfalls that continue to plague the laboratory workers in this field.

Our understanding of the hemostatic process has increased greatly in the past six years, particularly with the wide recognition of the entity of disseminated intravascular coagulation and with beginning efforts to study hypercoagulability. The wide availability of factor concentrates has challenged the laboratory to develop more accurate methods for measuring levels of coagulation factors. New methods for the study of platelet function have added greatly to our understanding of some of the milder hemorrhagic syndromes.

Every year I see at least one patient with a combination of findings and

problems that I have not seen before, so I anticipate a future filled with continuing advancement and challenge.

A major effort has been made in this edition to standardize procedures and reagents, so that a laboratory can do complete and careful work with a minimum of equipment and reagents. This should be of particular importance to beginning technologists. Readily available reagents have been utilized whenever possible, and information about availability, stability, and sensitivity has been included.

I wish to thank Dr. James Davis for so adequately describing the study of platelet aggregation. His many years of careful study of platelets make him eminently qualified for this task. I also wish to thank Reaner Shannon and Henry Revels who have worked so diligently on the actual testing done in our coagulation laboratory and who have helped with the development and description of many procedures that appear in this book.

I am indebted to Dr. Larry Tretbar, Betty Steinmann, and the Audio-Visual Department of the UMKC School of Medicine for their invaluable help in the development of the new illustrations for this edition. Also for long hours of typing and proofreading, I wish to thank Sue Hodes, Verdis Moore, and Joan Rice.

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Medical Technology

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Mechanism of Hemostasis

Blood is normally fluid; in the body it circulates throughout the vascular system under pressure. The prevention of spontaneous bleeding and the control of traumatic hemorrhage are referred to as hemostasis—a complex mechanism which is dependent primarily upon:

1. Normal resistance and contractility of blood vessels and an adequate supportive framework for them.
2. Normal platelet activity.
3. An adequate coagulation system.
4. Stability of the clot.

In the prevention of spontaneous bleeding, the normality of the vascular system and the presence of adequate numbers of normally functioning platelets are of primary importance. It seems likely that bleeding due to minute traumatic stresses and injuries to small blood vessels is regularly controlled by the adherence of small numbers of platelets to injured areas. Perhaps the best indication of the effectiveness of such a protective mechanism is the rare occurrence of petechiae when the tourniquet test is performed on normal individuals. The mechanism is not sufficient, however, if there is an abnormality in the vessels themselves or if platelets are reduced in number or do not function normally. With increased vascular fragility, apparently even

normal platelets are inadequate, and in thrombocytopenia, normal vessels alone cannot prevent the appearance of petechial bleeding.

Vessels, platelets, the coagulation system, and clot stability are all important in hemorrhage control. The exact sequence of events is variable. Eventually, the openings in bleeding vessels must be closed so that the blood can no longer escape, and, except in very small venules and capillaries, this is usually accomplished by the formation of clots within the lumens of the vessels. The following factors are of variable importance in determining the pattern of hemostasis:

1. Location of the injured vessels and the chance of continuing trauma to them.
2. Size of the vessels and blood pressure within them.
3. Intrinsic abnormalities in the vessels.
4. Damage to surrounding tissue.
5. Accumulation of blood in tissues surrounding the vessels.
6. Application of external pressure or surgical intervention.

With large arterial vessels in which the blood pressure is high, usually there is no way for the flow of blood to be slowed sufficiently to allow clotting without the application of a tourniquet, the use of external pressure at the

bleeding site, or some type of surgical intervention. Occasionally there is enough tissue damage surrounding the vessel, with accumulation of blood in this tissue, to produce local tamponade of an artery and eventual cessation of bleeding. With repeated trauma, however, bleeding may be easily reactivated. In small venules and capillaries, hemostasis may be accomplished by the simple adhesion of endothelial surfaces and the local aggregation of platelets. In small arteries and arterioles, spontaneous control of bleeding is possible by a rather complex mechanism which is outlined in Figure 1-1.

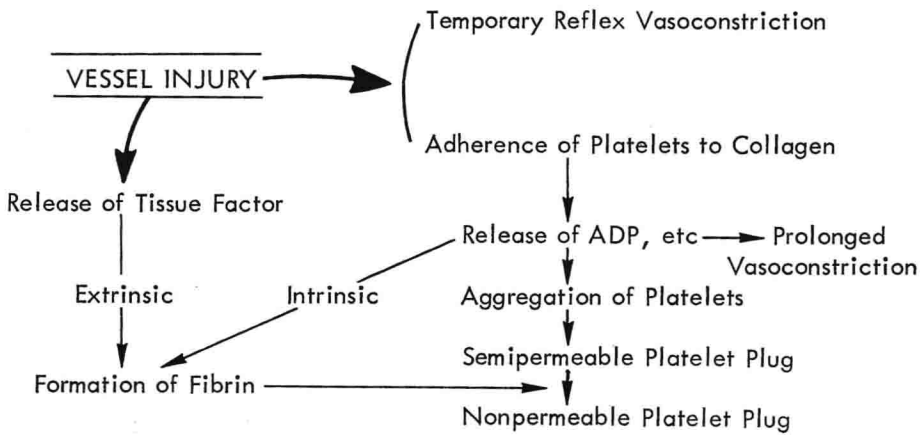

Figure 1-1. *Hemostasis in small arteries and arterioles.*

A more complete stepwise progression of changes occurring soon after injury to the wall of the blood vessel includes the following events and processes:

1. Adherence of platelets to the injured vessel wall and release of ADP, which occurs within 3 seconds, simultaneously with the beginning of activation of prothrombin to thrombin by tissue factor that was released by injury.
2. Formation of platelet aggregates with small fibrin loci within 30 seconds.
3. Disintegration of platelets and expansion of fibrin loci beginning in 1 minute.
4. Disintegration of platelet membranes and confluence of fibrin loci and platelet aggregates by 4 minutes.

BLOOD VESSELS

When blood vessels are normal, blood cells are retained within them except when actual injury occurs. However, defects in structure, permeability,

contractility, and resistance to trauma may interfere with adequate hemostasis. The recognition of such defects rests primarily on careful clinical observation rather than upon laboratory studies. Clinical evaluation is made not only upon observation of the bleeding area, but also upon inspection of the surrounding tissues and the remainder of the patient's body for evidence of generalized disease that might affect vascular function. The only laboratory procedures which aid in this problem are the tourniquet test and the determination of the bleeding time.

PLATELETS

Platelets have a variety of functions and, because of these, can be studied and tested in a number of ways. They aid in the maintenance of normal vascular integrity and plug holes in small vessels. They adhere to damaged vessels and aggregate at the sites of trauma, causing the slowing of blood flow, the prolonged constriction of surrounding vessels, and the actual beginning of clot formation. A number of separate platelet factors have been described. Platelet factor 1 is nothing more than adsorbed plasma factor V. Platelet factor 2 is located inside the platelet and accelerates the conversion of fibrinogen to fibrin. Platelet factor 3 is the lipoprotein which is necessary for the intrinsic formation of prothrombin converting complex. Platelet factor 4 neutralizes heparin. Fibrinogen seems to be partially adsorbed from plasma and partially located inside platelets. It has also been suggested that factor XIII is derived from platelets. Platelets are active in coagulation only after these factors are released.

Platelets may be counted directly, or their numbers may be estimated on blood smears. Estimation is much easier than actual counting because of the very small size of the platelets and is sufficiently accurate for the study of most hemostatic diseases, since numbers must be greatly altered (probably below 50,000 or over 1,000,000) to cause significant bleeding problems. In some situations, however, when rapid changes are occurring in platelet levels, it may be important to have sequential counts. Two such situations are the consumption coagulopathy associated with disseminated intravascular clotting and the patient with acute thrombocytopenic purpura. Estimation of the number of platelets on a stained smear is helpful also because it allows for observation of platelet morphology. Platelet function tests include the tourniquet test, the bleeding time, the prothrombin consumption test, the standard thromboplastin generation test, clot retraction, platelet adhesiveness, platelet aggregation, and the platelet factor 3 availability test.

COAGULATION SYSTEM

The coagulation system is by far the most complex part of the hemostatic mechanism and involves the interaction of ten or more different factors in

plasma and tissue, as well as the regulation of these factors by natural inhibitors. Our present concepts of the workings of the system are the result of some simple observations and the studies of many investigators. Some basic facts which are important in the understanding of coagulation include:

1. In a glass tube, blood clots in about 10 minutes.
2. In a plastic or siliconized tube, blood may take as long as 30 minutes to clot.
3. In a plastic or siliconized tube, if powdered glass or other activating substances are added, blood will clot in about 2 minutes.
4. Blood to which tissue extract has been added clots in a few seconds.
5. The clotting time of plasma is much shorter than that of whole blood and shows a variability which is at least partially dependent upon the speed and time of centrifugation.
6. The clotting time of plasma may be reduced by adding a platelet substitute, an activator substance, or tissue extract.

From the above and similar observations, the following conclusions can be drawn:

1. Blood contains all factors needed to form a clot.
2. Some factor or factors in blood must be activated by contact with a glass tube, and this appears to be a time-consuming process. Activation is less in plastic and siliconized tubes than in glass tubes.
3. The process of contact activation could not require calcium, since there is at least partial activation in blood or plasma to which a calcium-binding anticoagulant has been added.
4. Tissue factor contains a substance or substances that bypass contact activation.
5. Since platelets influence the speed of the clotting process to some extent, they must also be involved in the intrinsic coagulation system.

International Nomenclature of Coagulation Factors

An international nomenclature has been established for the coagulation factors. This should be mastered before trying to follow the discussion of the probable manner in which these factors interact. It is easier to refer to all factors by number, except for fibrinogen (I), prothrombin (II), thromboplastin (III), and calcium (IV) which, because of their key positions in the clotting mechanism and their early description, are regularly referred to by name. Table 1-1 designates the factors by number and by the various names used to refer to them and also includes some of the known facts that are important in studying and understanding them.