UPDATE, EDIT AND REVISION OF THE MEDICAL LABORATORY TECHNICIAN PROGRAM CLASS NOTES TO REFLECT CURRENT PRACTICES IN THE STUDY OF COAGULATION

Approved by Patricia Bromley on September 14, 2012

UPDATE, EDIT AND REVISION OF THE MEDICAL LABORATORY TECHNICIAN PROGRAM CLASS NOTES TO REFLECT CURRENT PRACTICES IN THE STUDY OF COAGULATION: An Educational Project

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by

Susan M. Bergs

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Abstract

UPDATE, EDITING AND REVISION OF THE MEDICAL LABORATORY TECHNICIAN PROGRAM CLASS NOTES TO REFLECT CURRENT PRACTICES IN THE STUDY OF COAGULATION

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This educational project was an endeavor to effectively update, edit and revise the *Class Notes* for the *Coagulation* class in Madison Area Technical College's Medical Laboratory Technician program. The *Class Notes* were written at least eight years ago by a former instructor in the Medical Laboratory Technician program as a learning tool to replace reading a lengthy textbook due to the accelerated format of this program. Classes in the accelerated Medical Laboratory Technician program are completed within a two to six week period necessitating a condensed version of necessary information. The information found in the original version of *Coagulation Class Notes* was verified for accuracy. Through a review of literature, it was determined that there was much new information regarding the subject of coagulation, anticoagulant usage and testing. The project then entailed rewriting, editing and updating the material in this document so that it reflects current clinical and laboratory practices in coagulation.

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Chapter One: Introduction

The Medical Laboratory Technician (hereafter referred to as MLT) program at Madison Area Technical College is available in two formats: the five semester "in-person" format and the accelerated one year "hybrid" format. At least eight years ago, a former instructor wrote what is named *Class Notes* for many of the program courses. They could be compared to "Cliff Notes" for a novel, as they are composed of a condensed version of important information for the class. They were developed as a tool for the accelerated MLT program since often they are completing a course within a two to four week span and do not have time to read an entire textbook.

For one course in particular, *Coagulation*, the current edition of *Class Notes* contained many clerical errors and was not up to date. Additionally, the document contains a number of excellent images and charts. Unfortunately, the document is in portable document format (pdf) rendering it unable to be edited or to copy most of the images. This project entailed rewriting, editing and updating the material in this document so that it reflects current clinical and laboratory practices in coagulation. In addition, the existing information was studied to verify its accuracy. The information was compiled in an easy to read and navigable format.

Statement of the Problem

The problem to be addressed was: Since the *Coagulation Class Notes* were written at least eight years ago, what new research, clinical practices and laboratory testing have arisen on the topic of coagulation that are relevant to teaching in the Madison Area Technical College Medical Laboratory Technician program? What updates of *Coagulation Class Notes* needed to take place in order for the *Class Notes* to be current, accurate and easy to navigate for students in the Medical Laboratory technician program at Madison Area Technical College? The goal was to equip the MLT students with information based upon the most current evidence based clinical practice and to meet the course objectives as outlined by WIDS, WTCS and NAACLS.

Definition of Terms

Coagulation: Also known as *hemostasis,* the balanced, controlled process by which the body maintains blood in a fluid state within vessels and prevents excessive loss of blood from vessels following injury (McKenzie, 2010).

Anticoagulant: Chemical substance added to whole blood to prevent blood from coagulating or clotting (McKenzie, 2010).

Class Notes: Condensed version of subject matter content provided for Medical Laboratory Technician students studying at Madison Area Technical College. This is comparable to “Cliff Notes” in literature (Nelson, n.d.).

MLT: Medical Laboratory Technician, associate degree in applied science offered at Madison Area Technical College which prepares students to work in a medical laboratory setting ("Medical laboratory technician," n.d.).

MATC: Madison Area Technical College, a college which offers technical and college transfer programs in the form of associate degrees, technical diplomas or certificates ("About us -,").

accelerated MLT program: Madison Area Technical College offers its medical laboratory training program in two formats, the regular two-year, five-semester program and an accelerated Medical Laboratory Technician program. The accelerated program begins early August and concludes by the end of the following June. Successful students have all the required general education support classes completed prior to beginning the accelerated core classes. The accelerated curriculum didactic instruction is offered online. Students come to campus Thursday evenings and every other Saturday to complete the labs for this Medical Laboratory Technician program ("Medical laboratory technician," n.d.).

WIDS: Worldwide Instructional Design System is the software that is used to document official curriculum Outlines of Instruction at Madison College (Rettler, 2011).

WTCS: Wisconsin Technical College System is a group of sixteen technical colleges in Wisconsin ("Wisconsin Technical College," n.d.).

NAACLS: National Accrediting Agency for Clinical Laboratory Science is an agency which accreditates and approves educational programs in the clinical laboratory sciences. ("NAACLS," n.d.).

Delimitations of Research

The references used for the review of literature were collected over a period of 180 days using the resources of the Karmann Library at the University of Wisconsin – Platteville and Madison Area Technical College library and Madison Area Technical College Medical Laboratory Technician department resources. Several search engines provided by MEDLINE were used. The key search terms were “new anticoagulants,” “laboratory monitoring of new anticoagulants,” “ideal anticoagulants” and “coagulation instrumentation”.

Method of Approach

A brief literature review regarding current coagulation theory, testing and instrumentation was conducted. A review of literature relating to applications of new anticoagulants and laboratory monitoring was conducted. The findings were summarized and incorporated into the *Coagulation Class Notes* tool for accelerated learning in the *Coagulation* class at Madison College in the MLT program.

Chapter Two: Review of Related Literature

Assurance that *Class Notes* contents are accurate and relevant

The *Coagulation Class Notes* was written by one of the instructors in the MLT program from Madison Area Technical College at least eight years ago. The contents are without references, so needed to be verified for accuracy. This was accomplished by reviewing the coagulation-related chapters in two commonly used hematology textbooks. Also, being at least eight years old, there was concern that some of the contents may be outdated or obsolete. Determination of whether any of the contents was obsolete was made by examining coagulation chapters in recent hematology textbooks, reviewing course objectives and referring to the most recent certification review book that students use to prepare for their certification exam upon completion of the program. If there were still questions in the certification review book concerning a topic in question, the contents were retained even if the content was termed obsolete in a reference.

The first task at hand involved editing out misspellings, duplicate information and typographical errors in the existing version of *Coagulation Class Notes*. The document contained several pictures which were unclear or blurry which were omitted in the edited version. Formatting inconsistencies were realigned to assure consistent formatting throughout. Proceeding on to verification of information revealed largely correct contents when compared to content in recent hematology textbooks (Rodak, 2011; McKenzie, 2010). There were found only two cases of erroneous information. In the “Laboratory Evaluation of Coagulation Disorders – Part 2” section, it had stated that therapeutic international normalized ratio (INR) values in patients with mechanical prosthetic heart valves or recurrent systemic emboli being treated with warfarin should be 3.0 to 4.5, when in fact it should be in the 2.5 to 3.5 range (Fritsma &

Marques, 2009). In the same section, the reference range for fibrinogen was listed as 150 to 350 mg/dL which should have been 200 to 400 mg/dL (McKenzie, 2010). These were the only erroneous pieces of information identified.

New anticoagulants

Anticoagulant therapy is given to patients as prevention or treatment of thrombosis (Weitz, 2012). The ideal anticoagulant should be administered orally, be effective, be safe, have a rapid onset of action, be readily and rapidly reversible and have few interactions with food and drugs. Reversibility is essential due to potential overdose or the need for a surgical procedure in a patient being treated with anticoagulants (Trujillo, 2010).

Eight years ago, when the *Class Notes* was written, anticoagulants were limited to three agents, each effective, but each having characteristics making them less than ideal for use. As the only available agents, they were effective and widely used. The benefits of the traditional anticoagulants are that their side effects and usage are well known among clinicians (DeLoughery, 2011). Current research has uncovered many new anticoagulants, a few of which have jumped the hurdles of Federal Drug Administration (FDA) approval and made their way into accepted clinical practice. In the *Class Notes* revision, emphasis is placed upon the agents which have been FDA approved and accepted into clinical practice in the United States. These new anticoagulants offer benefits over traditional agents in use since the 1940's (Garcia, Libby & Crowther, 2010). These agents are discussed in the new version of *Class Notes* in regards to mode of action, benefits and limitations and laboratory monitoring. They are grouped according to their mechanism of action.

Until recently, warfarin was the only available oral anticoagulant. Dabigatran (trade name Pradaxa) is an oral direct thrombin inhibitor recently approved by the FDA. Unlike warfarin, due

to the predictable nature of pharmokinetics, dabigatran does not require laboratory monitoring. Of great benefit is that it provides almost immediate anticoagulation at two to three hours as compared with warfarin's two to three days. It also has few interactions with food or drugs, so it appears that it should be a perfect replacement for warfarin which requires weekly monitoring by laboratory testing and has many food or drug interactions. The potential detriments to using dabigatran include renal toxicity, the lack of an antidote and increased gastrointestinal bleeding side effects (DeLoughery, 2011). If an accidental overdose occurs, there is no way to reverse or negate its effects (Trujillo, 2010). For these reasons, the manufacturer has added a "black box" warning to the package which is used when there are serious risks or side effects ("An FDA guide," 2011). Applications of this drug may thus be restricted to use in patients for whom weekly monitoring of warfarin is unavailable (Soff, 2012).

Another thrombin inhibitor, desirudin (trade name Iprivask) offers an option for patients being treated for prevention of clots following hip replacement surgery. It provides a good option for patients requiring a non-heparin regimen. It has a very short half-life making it a desirable option for acutely ill patients (Trujillo, 2010) who may need to stop anticoagulation. It can be monitored using the activated partial thromboplastin time, a common coagulation laboratory test.

A derivative of the well-known intravenous heparin anticoagulant, rivaroxaban (trade name Xarelto) is a newly developed factor Xa inhibitor. It is preferred for use over intravenous or subcutaneous injection anticoagulants since it can be taken orally. This increases compliance due to ease of administration. It also has few interactions with foods or other drugs. Outcomes of studies comparing it to enoxaparin (a well-known heparin derivative) are favorable with similar efficacy and risk of bleeding (Trujillo, 2010). Unfortunately, there is no standardized laboratory test to monitor use of this anticoagulant which poses a detriment to its usage.

There are many more anticoagulants under investigation. The cost of the newer drugs appears to be a factor influencing their acceptance (Trujillo, 2010). Additional updates in the coagulation study material will be necessary periodically if and when additional drugs are developed and prove to be beneficial for use and are accepted into clinical practice.

New methods of laboratory methods and testing in coagulation

Traditionally, methods of coagulation testing involved electromechanical clot detection or photo-optical detection using nephelometric or turbidimetric principles. Current commonly used methods include the use of magnetic force. A small metal ball is included in the test cuvette. The electromagnetic field created alternately on either side of the cuvette maintains a swinging motion. After the sample and reagents are added, the constant pendular swings of the ball are observed. Variation of the ball's oscillation amplitude corresponds to an increase of the medium viscosity indicating coagulation (*STA Satellite Reference*, 2008). This method has become widely accepted since it is fully automated and not affected by lipemic, icteric or hemolyzed samples.

Summary

Including the preceding findings in the new version of *Class Notes* will provide MLT students at Madison Area Technical College information that is current, thorough and accurate. Additionally, the format is organized and easy to navigate for students.

Chapter Three: Project

The process of document revision began with a preliminary review searching for clerical and formatting errors and was followed by a detailed analysis of content for verification. Then, a literature review was done to update the material in this document so that it reflects current clinical and laboratory practices in coagulation.

The revised *Coagulation Class Notes* contains condensed, yet detailed, information about all aspects of coagulation. It begins with an overview and introduction which includes the history of the study of coagulation, basic terms and theory. Next, it proceeds into discussion of the three phases of coagulation: primary hemostasis, secondary hemostasis and fibrinolysis. Once an understanding of the normal function of the coagulation system is presented, the document explains potential defects in the three stages of coagulation in the following sections: bleeding disorders due to defects in primary hemostasis, bleeding disorders due to defects in secondary hemostasis and thrombosis. Following this comes the laboratory aspect of diagnosing and monitoring these conditions. The laboratory evaluation section includes an introduction to coagulation testing, which details the accurate method of specimen preparation and processing for testing and outlines methods of testing. It then proceeds to examine the various coagulation tests and their purposes in the three "Laboratory Evaluation of Coagulation Disorders" sections. It concludes with discussion of anticoagulant therapy and thrombolytic therapy and their relation to coagulation testing. Many of these tests are performed in the laboratory segment of the coagulation class providing students with not only the background knowledge, but hands on experience in coagulation testing.

Appendix A includes the entire revised version of *Coagulation Class Notes.*

Chapter Four: Conclusions and Recommendations

The idea of updating the *Class Notes* was spurred by a television commercial for Pradaxa, a new anticoagulant, and the realization that Madison Area Technical College students were not learning about it. A hospital pharmacist was consulted to verify that these newer anticoagulants were actually being used. Upon learning that they were being used, although limited to specific purposes, it was determined that a *Class Notes* update was necessary. The main findings after conducting research include several new anticoagulants and one new major laboratory testing method which have been included into the updated version.

Madison Area Technical College MLT instructors remain updated in their content areas by attending educational seminars, reading trade journals and talking to hospital and clinic technologists while doing clinical site visits for students. The MLT program has demonstrated success by passing the most recent NAACLS accreditation with no deficiencies and consistently having student scores in the top five percent in the nation on their MLT certification exam. Plans for ensuring continuous updating of curriculum by instructors include periodic literature reviews and inquiring of the national laboratory educators group as to what other programs have instituted to ensure updated curriculum. Reporting updates at MLT department meetings would ensure that all instructors in the program are aware of curriculum updates.

In summary, this project successfully provides a concise, yet thorough, updated and organized version of *Coagulation Class Notes* for the MLT students at Madison Area Technical College. As suspected, a review of current literature revealed new anticoagulants and coagulation laboratory methods not included in the original version. These were then incorporated into the updated and revised *Class Notes* in order to provide a more complete and updated preparation for

MLT students at Madison Area Technical College. The *Class Notes* for *Coagulation* class are printed and bound by Madison Area Technical College's Duplicating Service for purchase by students in the bookstore in lieu of a textbook.

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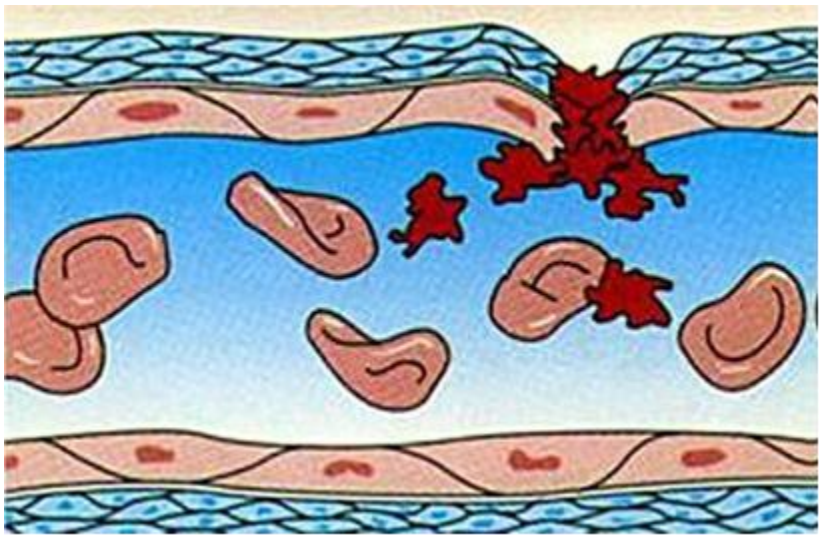
APPENDIX A: Coagulation Class Notes

Coagulation

Class Notes



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revised by Sue Bergs MT (ASCP) July 2012

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INTRODUCTION TO COAGULATION

Blood is the liquid of life. Without it, we would die. Therefore, it is imperative that the body produces enough of it and, when injury occurs, that all efforts are made to prevent its loss. The term **hemostasis** refers to the process by which blood loss from an injured vessel is stopped. It originates from the Greek words *heme*, meaning blood, and *stasis*, meaning to halt. The end product of hemostasis is a blood clot that patches the wound and allows healing to occur without the threat of continued blood loss. Hemostasis occurs in two stages: primary hemostasis and secondary hemostasis.

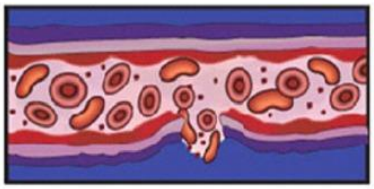
Primary hemostasis results in the formation of a platelet plug that temporarily stops blood loss from the injured vessel. It involves interactions of platelets with the injured blood vessel and with other platelets. The platelet plug does a great job of patching the injured vessel, but, unfortunately, it is fragile and can easily be dislodged from the blood vessel wall. Therefore, if it is to remain in place long enough to allow the injured vessel to heal, it must be reinforced. This is accomplished through secondary hemostasis.

Secondary hemostasis results in the formation of fibrin, an insoluble protein that serves as a mesh to hold the platelet plug together. The key players in secondary hemostasis are the coagulation factors. Once activated by various substances released from injured vessels and activated platelets, the coagulation factors interact with each other in a series of complex biochemical reactions to produce fibrin.

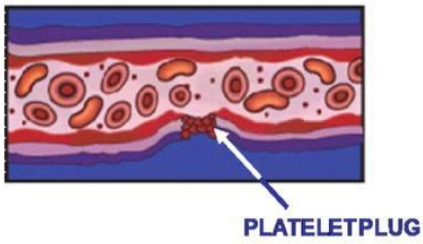
For many years, the **coagulation cascade** was used to explain fibrin formation. The coagulation cascade is a series of reactions that are connected in a cascading fashion, meaning that one reaction produces an end product that activates the next reaction, which produces another end product to activate another reaction, and so on until fibrin is formed. Recent clinical and experimental observations have demonstrated that, while the cascade hypothesis accurately reflects hemostatic mechanisms that occur in vitro (in the test tube), it does not fully and completely reflect the events of hemostasis in vivo. Today, the coagulation cascade has been replaced by the **cell-based theory** of coagulation. Although this new theory still incorporates many of the factors and reactions from the original coagulation cascade, it places greater emphasis on the role of cells, mainly tissue-factor bearing cells of the subendothelium and platelets, in fibrin formation.

Once a stable clot is formed, it will remain intact long enough to allow the wound to heal. Once the wound is healed and the clot is no longer needed, it must be removed. If it is not, there is a risk that it will break free and travel through the blood stream to become lodged in another area of the body, such as in a lung or the brain, where it will cause serious problems. The process by which the clot is removed is called **fibrinolysis**. It involves a series of reactions that break the clot into little pieces that pose no threat and are easily cleared from the blood by the liver.

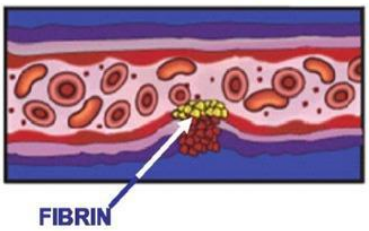
The entire process of clot formation and removal must be carefully controlled and precisely timed. If good, stable clots cannot be formed or if clots are removed too early, bleeding occurs. If clots are formed in excess or if they are allowed to hang around too long and ultimately break free and lodge elsewhere, thrombosis occurs. Interestingly, once clot formation begins, inhibitors to the clotting process spring into action and physiologic changes occur to slow down and stop the process so that it does get out of hand. Also, at the same time the hemostatic mechanisms are activated and clot formation begins, the fibrinolytic system is also activated and clot breakdown begins. Fortunately, the timing of the entire system is such that enough, but not too many, clots are formed, and that the clots are allowed to remain intact long enough for wound healing to occur.



Bleeding occurs after injury to a blood vessel. The hemostatic system is activated.



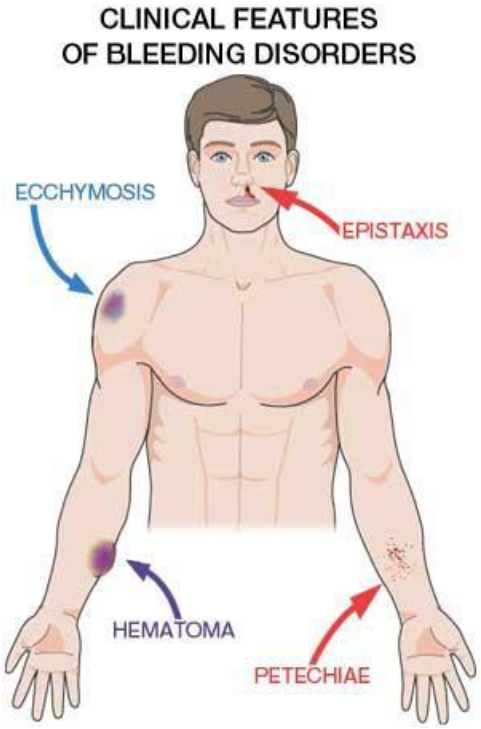
Primary hemostasis results in formation of a platelet plug.



Secondary hemostasis results in fibrin formation.

The entire coagulation system is very intricate and truly amazing. And, because of this, it can be difficult to understand. We will begin our study of coagulation by learning about the functions of the various components of the hemostatic mechanism, including the vascular system, platelets, coagulation factors, and fibrinolysis. Once we have a good understanding of the entire system, we will learn about all of the things that can go wrong with the system, causing either bleeding or thrombosis. Although we will classify disorders in terms of which component of the hemostatic mechanism is most affected, we will soon see that, due to the complex interactions that occur between these components and their dependency upon one another, this classification is somewhat arbitrary. We will identify disorders as being **hereditary** (caused by a defect in the genetic code that is passed from parent to child), **acquired** at some point after birth, or **idiopathic** (of unknown cause). Some disorders will be classified as **primary disorders** (not preceded by any other disorder) and others will be classified as **secondary disorders** (occur as a result of or in response to another disease or condition).

Coagulation defects can sometimes be **quantitative** in nature, meaning that there is too little or too much of a particular substance (protein or cell). At other times, they may be **qualitative**, meaning that the amount of a substance (protein or cell) is just right, but it is defective and doesn't function properly. Qualitative defects may also be referred to as **functional defects**.



Various terms will be used to describe the clinical features or symptoms that accompany these disorders.
They include:

Epistaxis: Nose bleed
Ecchymosis: Large bruise (>3mm) caused by bleeding into the subcutaneous tissue but without disruption of the skin
Petechiae: Small red dots within the skin caused by bleeding from capillaries
Purpura: Purple discoloration of the skin caused by petechiae and/or ecchymoses
Hematoma: A swelling or mass of blood confined to an organ, tissue, or space and caused by a break in a blood vessel

PRIMARY HEMOSTASIS

I. Vascular system
    A. Structure
    B. Role of the vascular system in hemostasis
        1. Endothelial cells
        2. Vessel wall response to injury
II. Platelets
    A. Production
    B. Structure
    C. Platelet participation in hemostasis
        1. Platelet participation in primary hemostasis
            a) Platelet adhesion
            b) Platelet activation
            c) Platelet aggregation
        2. Platelet participation in secondary hemostasis
        3. Platelet participation in blood vessel maintenance

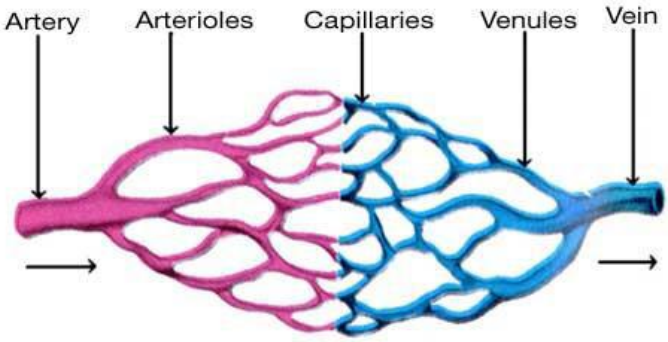
PRIMARY HEMOSTASIS

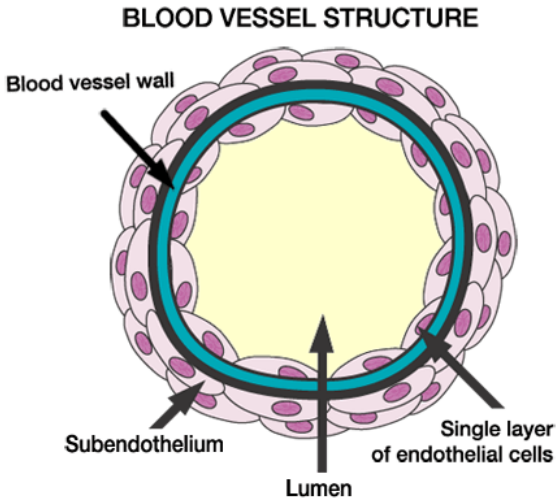
Primary hemostasis results in the formation of a platelet plug that temporarily stops blood loss from an injured vessel. It involves the vascular system and platelets.

VASCULAR SYSTEM

STRUCTURE

There are two types of blood vessels, arteries and veins. Arteries carry blood away from the heart, while veins carry blood back to the heart. Capillaries are tiny, thin walled vessels that serve as the bridges between the arteries and veins. Capillaries are formed when large arteries branch into smaller and smaller arteries called arterioles. Arterioles ultimately branch further to form the capillaries. Capillaries then connect with larger vessels called venules. Venules ultimately form progressively larger veins that will return blood to the heart. All blood vessels have a similar structure. The central area through which the blood flows is called the **lumen**. The lumen is lined with a single layer of endothelial cells. The tissues located below the endothelial cells are called the **subendothelium** or **subendothelial connective tissue**.





ROLE OF THE VASCULAR SYSTEM IN HEMOSTASIS

ENDOTHELIAL CELLS

The endothelial cells that line the blood vessels perform many functions; some that are related to hemostasis (**hemostatic functions**) and others that are not (**non-hemostatic functions**). Of the hemostatic functions, some are **non-thrombogenic**, meaning that they prevent blood coagulation, while others are **thrombogenic**, meaning that they induce or support blood coagulation. Usually, there is a good balance between the thrombogenic and non-thrombogenic functions, assuring that neither process overwhelms the other.

Endothelial cells also produce numerous substances that inhibit clot formation, including:
Heparin sulfate and thrombomodulin: which inhibit fibrin formation.

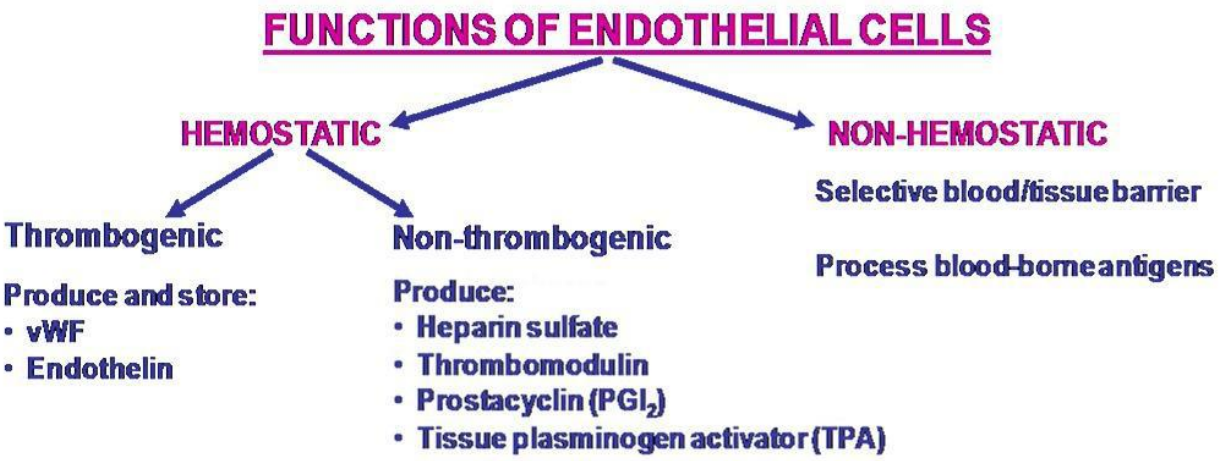
Prostacyclin (PGI_2): which inhibits platelet aggregation and also induces vasodilation to prevent vascular stasis that would enhance contact activation of both platelets and coagulation factors.

Tissue plasminogen activator (TPA): which activates the fibrinolytic system to dissolve clots that form.

Nitric oxide: which counteracts vasoconstriction.

The thrombogenic functions of the endothelial cells involve the production and storage of substances that each induce some aspect of blood coagulation.
von Willebrand factor (vWf), which supports the binding of platelets in the initial stage of clot formation, is produced by endothelial cells throughout the body. It is stored in the endothelial cells and in platelet alpha granules. It is also secreted into the subendothelial spaces and into the blood stream. In the blood, it circulates attached to factor VIII, a clotting factor that participates in the coagulation cascade to produce fibrin.

Endothelin, which causes blood vessel constriction (vasoconstriction), is released upon endothelial cell damage. The slowed blood flow resulting from vasoconstriction promotes contact activation of both platelets and coagulation factors.



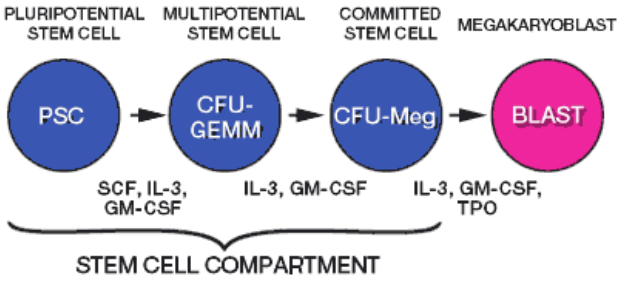
VESSEL WALL RESPONSE TO INJURY

The initial response of the blood vessel to injury is to constrict (vasoconstriction). This reduces the amount of blood escaping from the wounded vessel and also slows blood flow through the area, a condition that promotes contact activation of both platelets and coagulation factors. The exact mechanism by which vasoconstriction occurs is not fully understood, but it is known that endothelin, which is released from damaged endothelial cells, contributes to the process. Vasoconstriction will be enhanced upon activation of platelets and subsequent release of serotonin and thromboxane A_2 from the platelet granules.

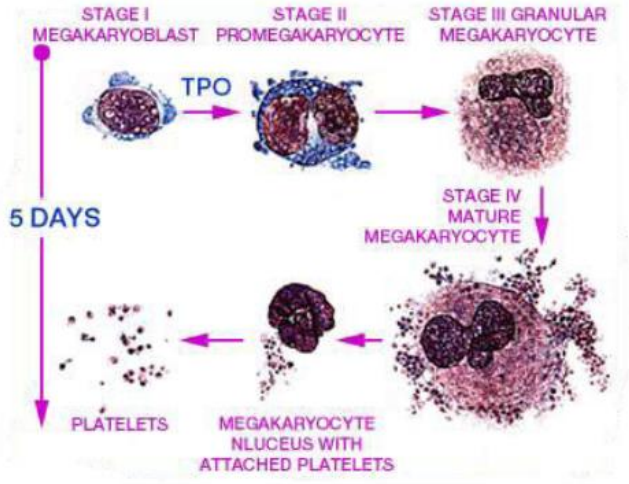
PLATELETS

PRODUCTION

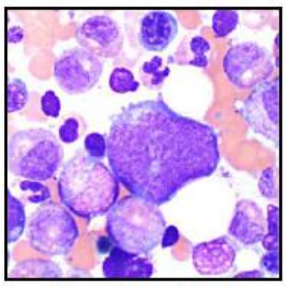
Platelets are produced in the bone marrow by a process called **thrombopoiesis** or **megakaryopoiesis**. It begins with primitive cells called stem cells that, under the influence of various cytokines, differentiate into the first recognizable cell of the megakaryocytic series, the **megakaryloblast**.

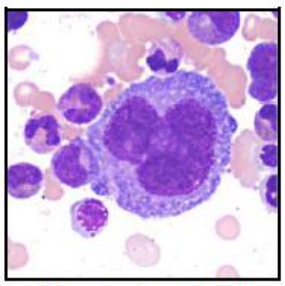


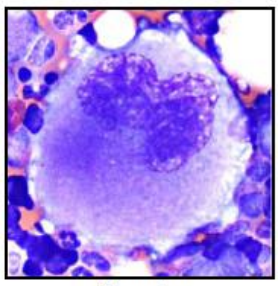
The megakaryoblast, under the influence of the cytokine **thrombopoietin**, matures into a **megakaryocyte** as shown below.

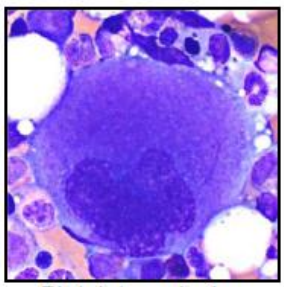


The mature megakaryocyte produces and releases from 2000 to 4000 platelets from its cytoplasm.


Megakaryoblast

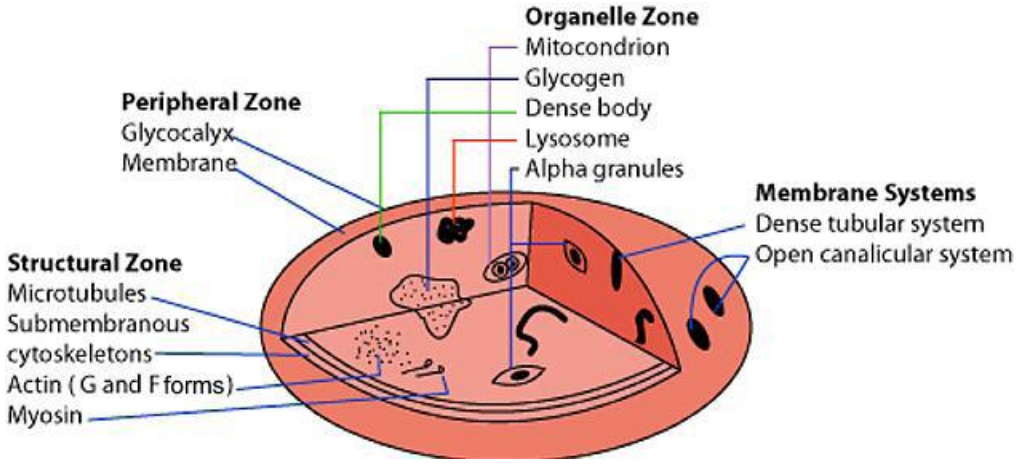

Promegakaryocyte


Granular megakaryocyte

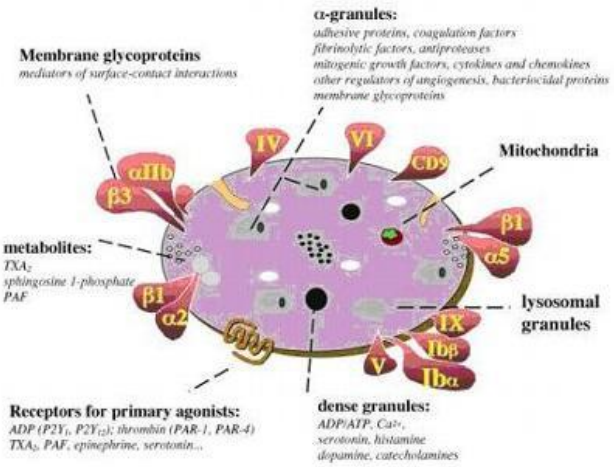

Platelet-producing megakaryocyte

STRUCTURE

It may be hard to believe just by looking at them, but platelets have quite complex structures. This isn't surprising considering all of the tasks that they perform to maintain vascular integrity and prevent blood loss. The platelet has four regions or zones: the peripheral zone, the structural zone, the organelle zone, and the membrane systems.



http://www.prolo.org/3.htmlThe functions of the **peripheral zone** include adhesion and aggregation. This zone consists of the fuzzy external surface, known as the glycocalyx, and the cytoplasmic membrane. The **structural zone** provides a cytoskeleton to maintain platelet shape and to control platelet contraction. It is composed of microtubules and a network of proteins. The **organelle zone** contains the structures that are needed for metabolism, in addition to the granules that contain the substances needed to perform its hemostatic and non-hemostatic functions. There are three types of granules in the organelle zone: **dense bodies**, **alpha granules**, and **lysosomal granules**. Two of these granules, dense bodies and alpha granules, contain substances that perform important hemostatic functions.



Hemostatic Functions of Platelet Granule Contents			
Dense Bodies		Alpha Granules	
Substance	Function	Substance	Function
ADP	Serves as an agonist for platelets. Also recruits and activates new platelets for platelet aggregation.	Fibrinogen	Platelet aggregation and conversion to fibrin
ATP	Function unknown	Factor V	Helps in fibrin formation
Calcium	Probable source of extracellular calcium needed for a variety of hemostatic reactions	Factor XI	Helps in fibrin formation
Serotonin	Vasoconstriction	vWF	Platelet adhesion
		PAI-1	Inhibits fibrinolysis
		Plasminogen	Converted to plasmin for fibrinolysis
		HMWK	Assists in conversion of prekallikrein to kallikrein
		Fibronectin	Adhesive protein for platelets
		Protein S	Cofactor for protein C, a coagulation factor inhibitor
		Thrombospondin	Interacts with many coagulation proteins and involved in platelet aggregation
		PF 4	Inhibits heparin
		Beta thromboglobulin	Neutralizes heparin
		Protein C inhibitor	Neutralizes protein C, a coagulation factor inhibitor

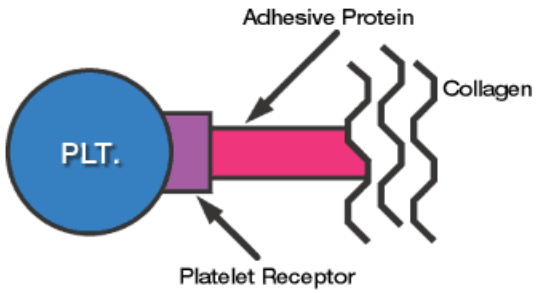
The fourth zone of the platelet, or **membrane system**, consists of an open canalicular system that serves as a route for release of the platelet's stored products, and the dense tubular system for storage of substances necessary for platelet function.

PLATELET PARTICIPATION IN HEMOSTASIS

PLATELET PARTICIPATION IN PRIMARY HEMOSTASIS

The most familiar contribution of platelets to the hemostatic mechanism is platelet plug formation. This is a two-step process by which activated platelets first adhere to the subendothelial tissue that is exposed following vessel injury (platelet adhesion) and then clump together with one another (platelet aggregation) to form a platelet mass that plugs the hole in the vessel.

PLATELET ADHESION



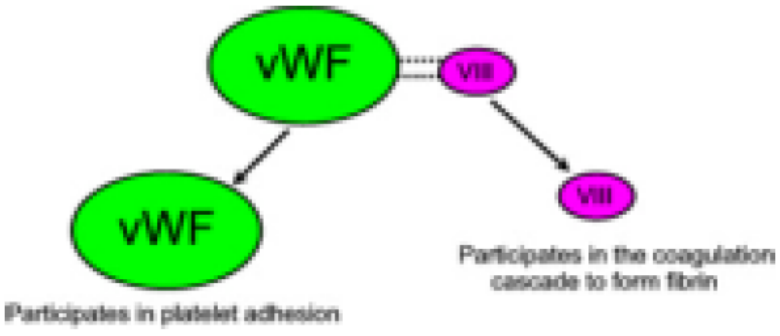
When endothelial cells are damaged, platelets escape from the blood and flow into the subendothelium where they stick (adhere) to exposed connective tissue, particularly collagen. Adhesion occurs when any one of a number of "adhesive" proteins present in the subendothelium, including fibronectin, laminin, vitronectin, thrombospondin, and von Willebrand factor, bind to the collagen fiber and also to a receptor on the platelet surface to form a "bridge" that attaches the platelet to the collagen.

There are many different receptors on the surface of the platelet, and each of the adhesive proteins binds with a particular type. As binding continues, the platelet attaches its receptors to various adhesive proteins of the connective tissue matrix and eventually spreads itself over the surface of the collagen.

One of the most important of the adhesive proteins is von Willebrand factor (vWf). vWf is produced by endothelial cells, and is stored in endothelial cell granules as well as being secreted into the subendothelial spaces and into the bloodstream. Some vWf is also stored in platelet alpha granules. vWf binds to both collagen and to a special receptor on the platelet surface known as glycoprotein Ib, causing the platelet to stick to the collagen.

Did You Know?

von Willebrand factor travels in the blood stream attached to the coagulation factor VIII, which is produced in the liver. Together, these two factors make a large macromolecular complex called the factor VIII complex. While vWf and factor VIII may travel together in the blood, once they arrive at a site of vessel injury they separate and perform their special tasks: vWf participates in platelet adhesion and factor VIII participates in the coagulation cascade. The relationship between the two factors is highly beneficial for factor VIII; if not attached to vWf, the larger component of the VIII complex, factor VIII will have a shortened survival rate. Imagine vWf as the big brother, carrying his little brother around and protecting him from harm.

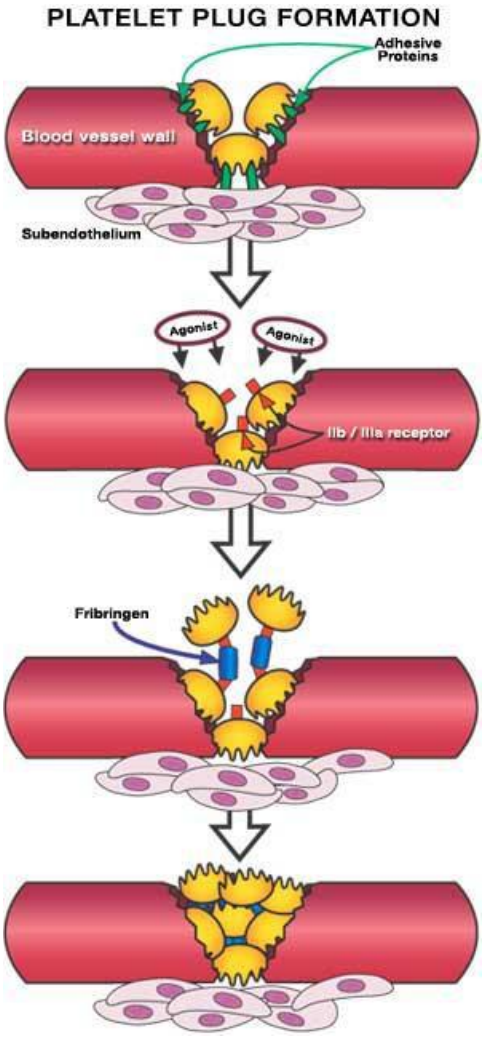


PLATELET ACTIVATION

Following adhesion, platelets must be activated before they can aggregate. Platelet activation will occur in response to a variety of substances, collectively called agonists. Some of these agonists are released from cells of the injured tissue while others are released from platelet granules as a result of the shape change that occurs during adhesion. Basically, all agonists cause the platelet to undergo a morphologic change, resulting in the appearance of the glycoprotein receptor IIb /IIIa on the platelet surface. With the appearance of this receptor, the platelets are now ready to stick to one another, or aggregate.

PLATELET AGGREGATION

Once the IIb /IIIa receptor appears on the surface of the activated platelet, fibrinogen that is present in the plasma and has also been released from platelet granules binds to receptors on adjacent platelets, forming a “bridge” that holds them together. This is platelet aggregation. Ionized calcium is also required to support fibrinogen binding to the IIb /IIIa receptors.



Platelets adhere to damaged endothelium and exposed subendothelium with the help of adhesive proteins.

Agonists released from injured tissue and platelet granules cause a morphologic change resulting in the appearance of IIb/ IIIa receptor on platelet surface.

Fibrinogen binds to IIb/IIIa receptors on adjacent platelets.

Platelet aggregation results in the formation of the platelet plug.

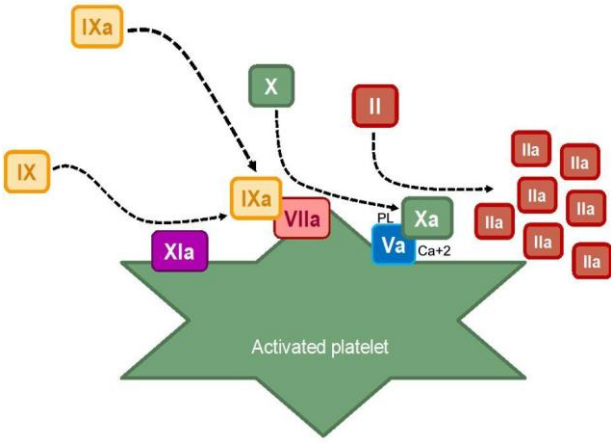
Platelet aggregation actually occurs in two phases: primary aggregation and secondary aggregation. Primary aggregation occurs in response to initial exposure to an agonist, mainly ADP that is released from the adherent platelets. During this phase of aggregation, fibrinogen binding is reversible and platelets are only loosely aggregated. Therefore, they may disaggregate, preventing secondary aggregation from occurring. Upon exposure to additional agonists, mainly ADP, serotonin, and thromboxane A2 released from the platelets involved in primary aggregation, the binding of fibrinogen becomes irreversible and the platelets become more tightly aggregated. This is secondary aggregation.

Did You Know?

You have probably heard that taking one aspirin per day reduces the risk of heart attack. And, indeed, this is the case. Aspirin prevents platelet synthesis of thromboxane A_2, one of the agonists that promotes secondary platelet aggregation. Exactly how does this prevent heart attacks? Most heart attacks are due to the inappropriate formation of platelet plugs within the arteries of the heart. By reducing the ability of platelets to aggregate, the risk of platelet plug formation in the heart is reduced. Once ingested, aspirin will affect thromboxane A_2 production for the entire lifespan of the platelet (9 to 12 days). It may also affect megakaryocytes in the bone marrow. Therefore, platelet aggregation and platelet plug formation will be impaired until new platelets from unaffected megakaryocytes can be produced.

PLATELET PARTICIPATION IN SECONDARY HEMOSTASIS

Platelet participation is hemostasis does not end with formation of the platelet plug. Platelets actively participate in secondary hemostasis, which involves the formation of the fibrin mesh that will stabilize and firmly anchor the platelet plug to the vessel wall. Activated platelets in the plug provide the phospholipid surface where the coagulation factors interact to produce thrombin (factor IIa), the proteolytic enzyme that is responsible for converting fibrinogen to fibrin. This capacity of the activated platelets to catalyze the coagulation process by providing phospholipid surfaces is known as **platelet factor 3 activity** or **platelet procoagulant activity**.



Platelets in the platelet plug provide the phospholipid surface where the coagulation factors interact to produce thrombin (factor IIa), the proteolytic enzyme that is responsible for converting fibrinogen to fibrin. This is referred to as platelet factor 3 activity.

Platelets release other substances that promote secondary hemostasis. They include:

- Fibrinogen, which is converted to fibrin.
- Factor V, which serves as a cofactor in fibrin clot formation.
- Factor XI, which interacts with other coagulation factors to produce fibrin.
- von Willebrand factor (vWf), which assists in platelet adhesion to provide a surface for the coagulation cascade and complexes with coagulation factor VIII to circulate in plasma.

Following fibrin formation, the platelets will contract the platelet plug-fibrin mass, making it a firmer, more cohesive clot that will adequately cover and protect the wounded vessel until repairs can be made. Clot retraction also serves to shrink the clot to allow for normal blood flow through the vessel. The clot retraction process requires calcium, thrombin (derived from the coagulation cascade), and energy in the form of ATP.

PLATELET PARTICIPATION IN BLOOD VESSEL MAINTENANCE

Even when not participating in clot formation, platelets remain busy. While they circulate in the blood, they constantly observe for the presence of small gaps in the vessels caused by separation of the endothelial cells. If necessary, they will fill the gaps to help prevent blood from escaping.

SECONDARY HEMOSTASIS

I. Coagulation cascade
    A. Historical perspective
    B. The coagulation cascade as we have known it for years
    C. Coagulation factors
        1. Description and classification
        2. Factor VIII-von Willebrand factor complex
    D. Formation and stabilization of fibrin
    E. Origin of thrombin
    F. Activation of factor X
        1. Extrinsic pathway
        2. Intrinsic pathway
    G. Problems with the coagulation cascade
II. Cell-based model of coagulation
    A. Overview of the cell-based model
    B. Initiation phase
    C. Amplification phase
    D. Propagation phase
III. Basal coagulation
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V. Complement system
VI. Physiologic control of hemostasis
    A. Blood flow
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    C. Feedback inhibition
    D. Biochemical inhibitors
        1. Tissue factor pathway inhibitor (TFPI)
        2. Protein C regulatory system
        3. Serine proteases
            a) Antithrombin
            b) Heparin cofactor II
            c) Alpha 1-antitrypsin, alpha 2-macroglobulin and protein Z-dependent protease inhibitor

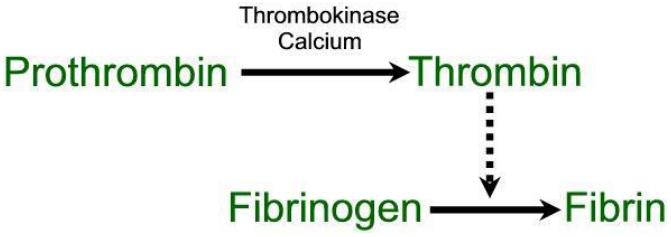
SECONDARY HEMOSTASIS

Secondary hemostasis results in the formation of fibrin, an insoluble protein that serves as a mesh to hold the platelet plug together. The key players in secondary hemostasis are the coagulation factors and the cells upon which the coagulation factors interact. Once activated by various substances released from injured vessels and activated platelets, the coagulation factors interact with each other on the surface of cells in a series of complex biochemical reactions to produce fibrin. For many years, the **coagulation cascade** was used to explain fibrin formation. The coagulation cascade is a series of reactions that are connected in a cascading fashion, meaning that one reaction produces an end product that activates the next reaction, which produces another end product to activate another reaction, and so on until fibrin is formed. Recent clinical and experimental observations have demonstrated that, while the cascade hypothesis accurately reflects hemostatic mechanisms that occur in vitro (in the test tube), it does not fully and completely reflect the events of hemostasis in vivo. Today, the coagulation cascade has been replaced by the **cell-based theory** of coagulation to explain hemostasis in vivo. Although this new theory still incorporates many of the factors and reactions from the original coagulation cascade, it places greater emphasis on the role of cells, mainly tissue-factor bearing cells of the subendothelium and activated platelets, in fibrin formation. Knowledge of the coagulation cascade still retains some value for explaining in vitro hemostasis and certain coagulation test results, and, as such, will be discussed.

COAGULATION CASCADE

HISTORICAL PERSPECTIVE

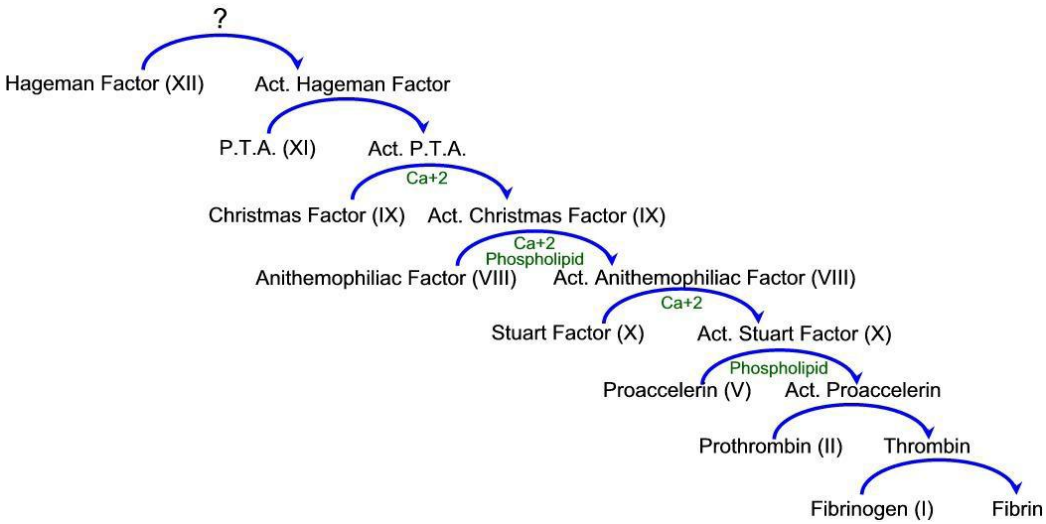
The mystery of blood clotting has fascinated man for hundreds of years. Both Aristotle and Hippocrates postulated that blood clotting occurred in response to cooling. Later, in the 1790s, a renowned Scottish physician by the name of John Hunter suggested that blood clotted in response to exposure to air. In 1832, German physiologist Johannes Muller identified the clotting protein fibrin while his contemporary Rudolf Virchow named fibrinogen as fibrin's hypothetical precursor. In 1856, fibrinogen was isolated by French physician Prosper Sylvain Denis and, a few years later, Estonian physiologist Alexander Schmidt demonstrated that the conversion of fibrinogen to fibrin was an enzymatic process. He named the enzyme thrombin and its precursor prothrombin. Based upon observations made earlier by other researchers, German physiologist and internist Paul Oskar Morawitz proposed the first coagulation model in 1904.



Early coagulation model proposed by Morawitz in 1904

As the groundwork for this first coagulation model was being laid, other researchers were focusing their attention on platelets and their role in blood clotting. In the 1880s, F. W. Zahn observed that an injured vessel was initially plugged by a “white thrombus”, not by fibrin, and other investigators observed that a “colorless blood corpuscle” that was smaller than red cells or white cells appeared to be involved in blood clotting. Bizzozero, an Italian pathologist, and Hayem, a French physician, both demonstrated that fibrin was associated with platelets, which led them to conclude that platelets supply a factor that is required for coagulation. Others later showed that the rate of prothrombin consumption and fibrin formation was low in platelet-poor plasma and increased as platelet numbers increased.

In the 1960s, two groups proposed a new, more complex model to explain blood clotting. This model, which was to become the first coagulation “cascade”, showed a sequential series of steps involving clotting factors in which activation of one clotting factor led to activation of another clotting factor and so on until it culminated in a burst of thrombin generation. At the time, it was believed that every clotting factor circulated in blood in an inactive form and was converted to an active enzyme. Platelet participation in the coagulation cascade was recognized by the inclusion of phospholipid (provided by the platelet membrane) at various steps.

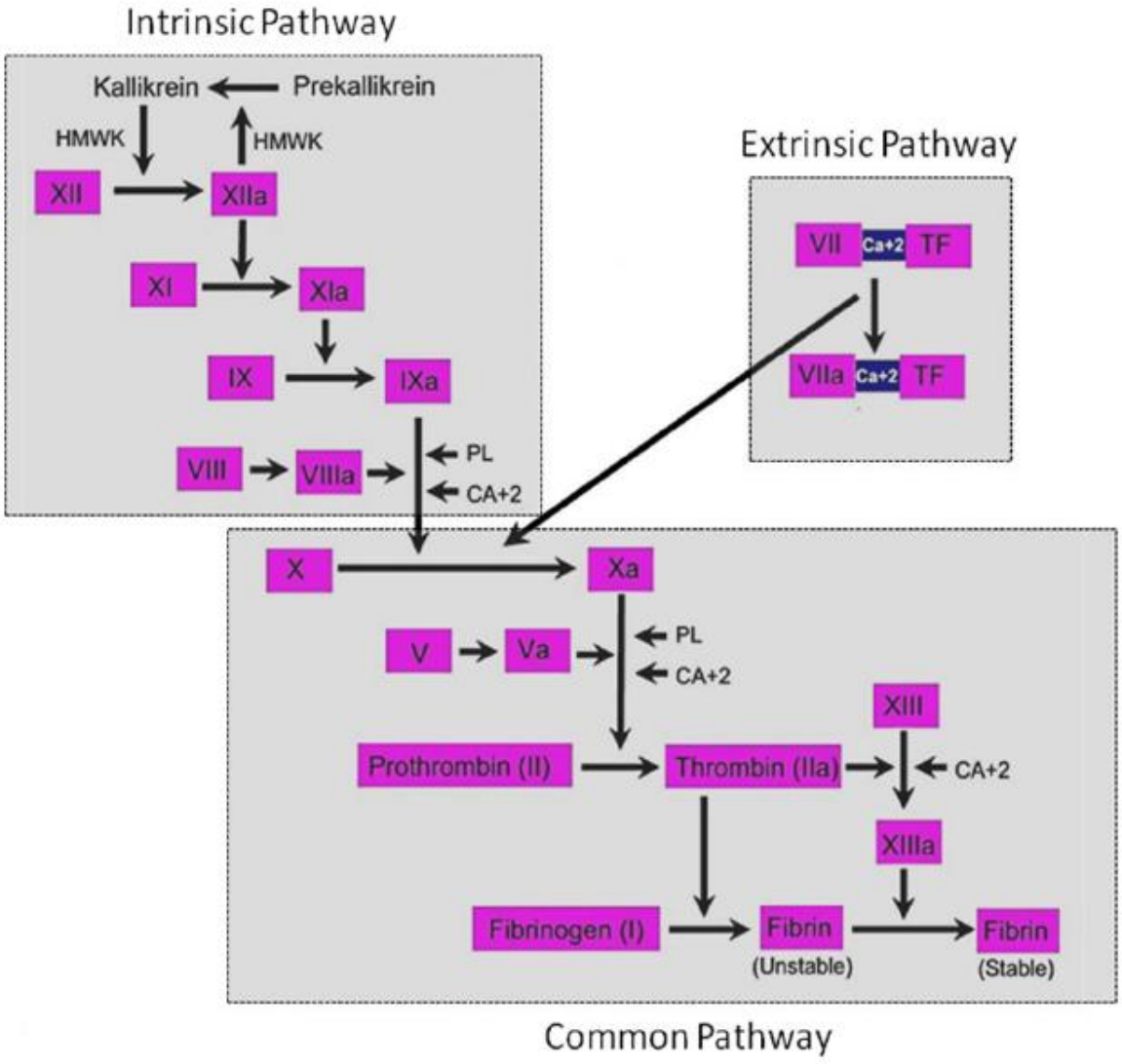


The first coagulation “cascade” proposed in the 1960’s

Over the years, the coagulation cascade underwent several modifications. First, it was recognized that some of the clotting factors were cofactors that did not possess enzymatic activity but supported the enzymatic activities of other factors. Second, the coagulation cascade was divided into intrinsic and extrinsic pathways that functioned independently of one another to activate factor X, which in a complex with its cofactor factor Va could convert prothrombin to thrombin. The intrinsic pathway was so named because all of the factors needed to form fibrin were within, or intrinsic to, the blood. The term extrinsic pathway acknowledged that a key component needed for fibrin formation, called tissue thromboplastin, came from tissue rather than blood. In other words, it originated from outside, or extrinsic to, the blood. With time, several researchers recognized that the two pathways could not operate independently of one another and that all coagulation factors and reactions were somehow interrelated.

THE COAGULATION CASCADE AS IT HAS BEEN KNOWN FOR MANY YEARS

After several modifications, the coagulation cascade evolved into the complex model shown below. The ultimate goal of the coagulation cascade is to form fibrin. Two different coagulation pathways, the **intrinsic pathway** and the **extrinsic pathway**, can accomplish this. The final steps of both the intrinsic and extrinsic pathways are the same. These common steps are referred to as the **common pathway**.



COAGULATION FACTORS

DESCRIPTION AND CLASSIFICATION

The coagulation factors, also called **procoagulants**, are the major players in the coagulation cascade. Most of the coagulation factors are glycoproteins produced by the liver; a few are produced by monocytes, endothelial cells, and megakaryocytes. Many of the coagulation factors are enzymes that circulate in an inactive form, called **zymogens**. When activated, these zymogens become **proteases**, or **proteolytic enzymes**, that are capable of splitting other proteins by hydrolysis of peptide bonds, resulting in the formation of smaller peptides and amino acid fragments. Some of the coagulation factors are **cofactors** that function to bind and stabilize their respective enzymes. Coagulation factors are identified by names and roman numerals. In the coagulation cascade, an activated factor is designated with its Roman numeral followed by an “a”.

THE COAGULATION FACTORS		
Factor	**Name**	**Function**
I**	Fibrinogen	Thrombin substrate; polymerizes to form fibrin
II**	Prothrombin	Serine protease
III**	Tissue factor	Cofactor for FVII
IV**	Ionized calcium (Ca+2)	Mineral
V	Labile factor	Cofactor
VII	Stable factor	Serine protease
VIII	Antihemophilic factor (AHF)	Cofactor
vWF	Von Willebrand factor (vWF)	Factor VIII carrier and platelet adhesion
IX	Christmas factor	Serine protease
X	Stuart-Prower factor	Serine protease
XI	Plasma thromboplastin antecedent (PTA)	Serine protease
XII	Hageman factor	Serine protease
XIII	Fibrin stabilization factor	Transglutaminase
Pre K	Prekallikrein Fletcher factor	Serine protease
HMWK	High molecular weight kininogen Fitzgerald factor	Cofactor
PF 3	Platelet factor 3 Phospholipid Phosphatidyl serine	Assembly molecule

** Indicates factors that are more commonly referred to by their name than their Roman numeral

Plasma Procoagulant SERINE PROTEASES			
Inactive Zymogen	**Active Protease**	**Cofactor**	**Substrate**
Prothrombin (II)	Thrombin (IIa)	---	Fibrinogen (I)
VII	VIIa	Tissue factor	IX, X
IX	IXa	VIIIa	X
X	Xa	Va	Prothrombin (II)
XI	XIa	---	IX
XII	XIIa	HMWK	XI
Prekallikreiin	Kallikrein	HMWK	XI

Plasma Procoagulant COFACTORS		
Inactive Form	**Active Form**	**Binds**
Tissue factor	Exposed tissue factor	VIIa
V	Va	Xa
VIII	VIIIa	IXa
HMWK	Kinin	XIIa, Pre-K

Each coagulation factor is assigned to a particular “pathway” in the coagulation cascade.

- **Extrinsic pathway** includes factors VII, I, II, V, and X (factors I, II, V, and X represent the common pathway)
- **Intrinsic pathway** includes factors VIII, IX, XI, XII, I, II, V, and X (factors I, II, V, and X represent the common pathway)

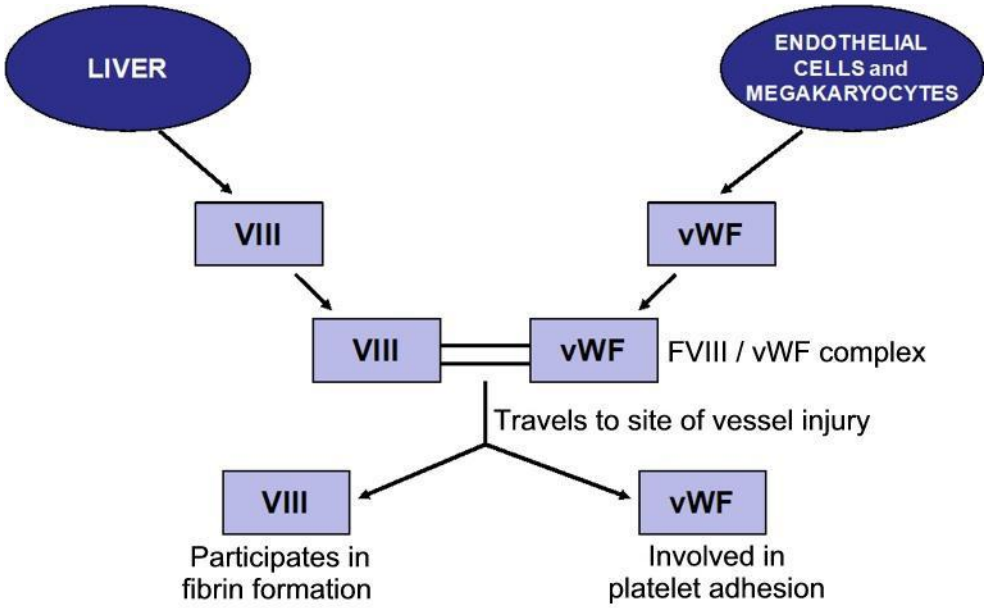
In addition to being assigned to a pathway, each factor can be placed into one of three groups based upon its physical properties.

- **Prothrombin group** includes factors II, VII, IX, and X: Liver synthesis of these factors requires vitamin K. Because of this, they are referred to as the **vitamin K dependent factors**.
- **Fibrinogen group** includes factors I, V, VIII, and XIII: Also referred to as the **consumable group** because they are consumed during formation of fibrin and, therefore, absent from serum.
- **Contact group** includes factors XI and XII as well as the plasma proteins prekallikrein and high molecular weight kininogen (HMWK): They are involved in the initial activation of the intrinsic coagulation pathway in vitro (in coagulation testing). They require contact with a negatively charged surface for their activity

FACTOR VIII – VON WILLEBRAND FACTOR COMPLEX

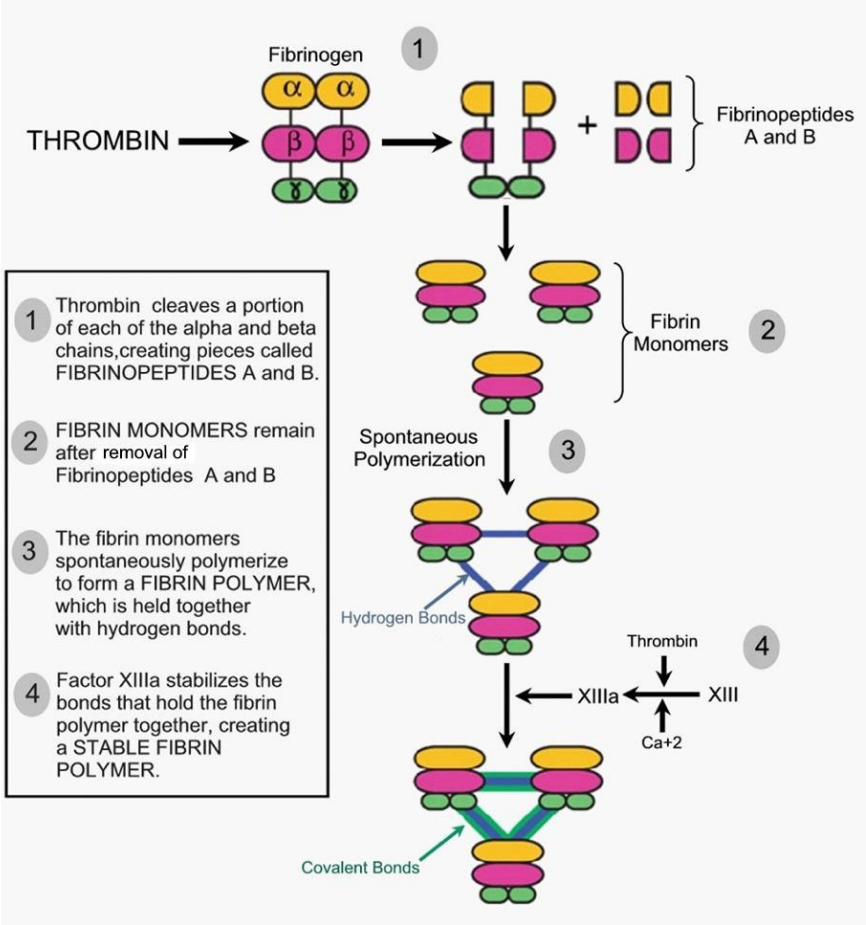
Factor VIII is somewhat unique in that it circulates in the blood as part of a large macromolecular complex composed of two distinct portions: factor VIII and von Willebrand factor (vWF). vWf, the larger portion, is produced by endothelial cells and megakaryocytes, and stored in the endothelial cells and in platelet alpha granules. It is also secreted into the

subendothelial spaces and into the blood stream. The Kupfer's cells of the liver appear to be the main site of synthesis of factor VIII, but small amounts may be produced by other tissues. It is the smaller portion of the large factor VIII - vWF complex. While vWf and factor VIII may travel together in the blood, once they arrive at a site of vessel injury they separate and perform their special tasks: vWf participates in platelet adhesion by providing receptor sites for platelets and collagen and factor VIII participates in the coagulation cascade, functioning as a cofactor for factor IX. The relationship between the two factors is highly beneficial for factor VIII. If not attached to vWf, factor VIII will have a shortened survival time. Imagine vWf as the big brother, carrying his little brother around and protecting him from harm.



FORMATION AND STABILIZATION OF FIBRIN

The culmination of the entire series of reactions in the coagulation cascade is the formation of fibrin. Fibrin is formed from fibrinogen through the action of the proteolytic enzyme thrombin. Fibrinogen is composed of three pairs of polypeptide chains (two alpha, two beta, and two gamma chains). The six chains are linked together by disulfide bonds. Thrombin cleaves a portion of each of the alpha and beta chains to create small pieces called fibrinopeptides A and B. The portion of the fibrinogen molecule that remains after removal of fibrinopeptides A and B is called a **fibrin monomer**. These fibrin monomers spontaneously aggregate, or polymerize, to form a **fibrin polymer**. At this point, the fibrin polymer is a rather unstable clot because it is held together with weak hydrogen bonds. In order to form a more stable fibrin polymer, factor XIIIa introduces covalent cross-links between the alpha and beta chains of the fibrin polymer to hold it firmly together. Factor XIII, also known as **fibrin-stabilizing factor**, is activated to factor XIIIa by thrombin and calcium (Ca^{+2}).

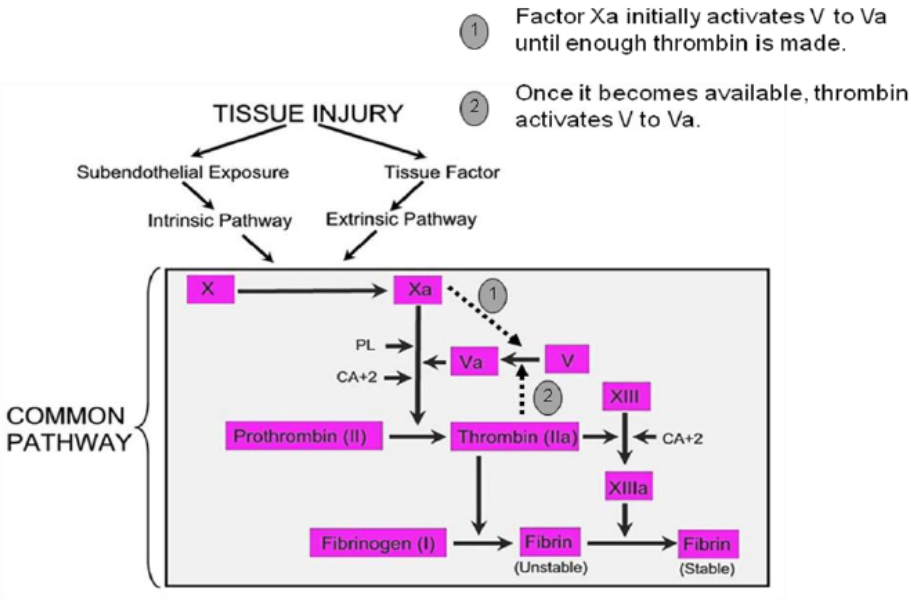


How does this compare to the cell-based model?

The method of fibrin formation remains the same for both the coagulation cascade and the cell-based model of coagulation, which will be discussed later. What differs between the two systems is the method by which thrombin (factor IIa), the proteolytic enzyme that converts fibrinogen to fibrin, is generated and where it is generated.

ORIGIN OF THROMBIN

The thrombin that is needed to convert fibrinogen to fibrin is formed from prothrombin (factor II). It is actually the activated form of prothrombin and, as such, is factor IIa. Prothrombin is converted to thrombin by the action of factor Xa, factor Va, Ca+2, and platelet phospholipid (platelet factor 3 activity) in the common pathway. As shown in the diagram below, factor X is activated to Xa via the intrinsic or extrinsic coagulation pathways. Factor V is initially activated to Va by factor Xa, but as the coagulation cascade progresses and enough thrombin is generated, continued activation of factor V becomes the responsibility of thrombin.



ACTIVATION OF FACTOR X

Factor Xa, which plays a crucial role in the conversion of prothrombin to thrombin, is derived from factor X as a result of either the intrinsic or extrinsic coagulation pathways. Activation of factor X via the extrinsic pathway occurs almost instantly, while it may take seconds or even minutes for the intrinsic pathway to accomplish the same goal. Although initial activation of factor X is accomplished through the extrinsic pathway, the principal method of continued, more efficient activation factor X occurs via the intrinsic pathway. Both pathways are activated in response to tissue injury. In the case of the intrinsic pathway, activation occurs when blood comes into contact with subendothelial connective tissue or other negatively charged surfaces that are exposed as a result of tissue damage. Activation of the extrinsic pathway occurs in response to the release of tissue thromboplastin or tissue factor from the injured tissues.

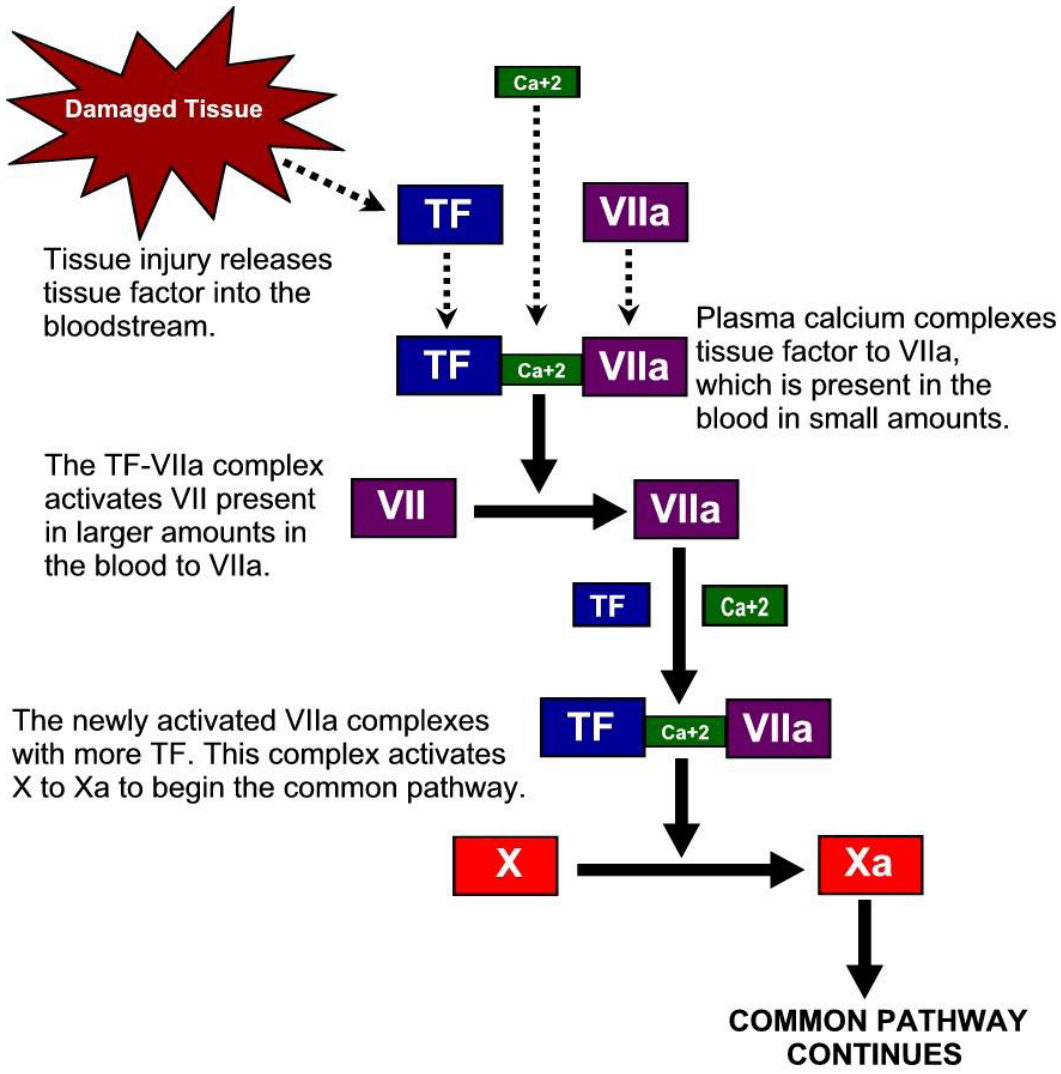
How does this compare to the cell-based model?

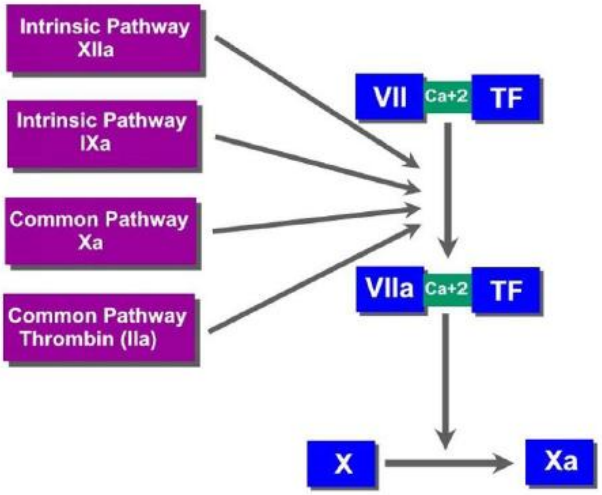
In the cell-based model of coagulation, activated platelets secrete some partially activated factor V from their alpha granules. Additional factor V is activated during the initiation phase by factor Xa and later, during the amplification phase, by thrombin. As for thrombin generation, both the coagulation cascade and the cell-based model recognize the **prothrombinase complex** (FXa-Va-PL-Ca+2) as the complex that converts prothrombin to thrombin. The difference once again is that the cell-based model places greater emphasis on the role of the tissue factor-bearing cell in this process.

EXTRINSIC PATHWAY

The extrinsic pathway consists of a series of reactions that lead to the activation of factor X to Xa and subsequent formation of fibrin by the common pathway. Remember that the extrinsic pathway is much faster than the intrinsic pathway. Therefore, fibrin formation occurs first as a result of the extrinsic pathway and is enhanced later by the intrinsic pathway.

Very small amounts of factor VIIa, the activated form of VII, are present in normal plasma. This information becomes critical when attempting to understand how the extrinsic coagulation pathway is activated. The main method of activation of the extrinsic pathway occurs when tissue injury releases tissue factor (tissue thromboplastin). Calcium (Ca+2), which is present in the plasma, acts as a bridge to complex factor VIIa, present in very small amounts in plasma, to tissue factor (TF) to form the VIIa-TF complex. This complex then activates more factor VII to VIIa. The newly activated VIIa complexes with TF to activate X to Xa.





Following initial activation, a variety of positive feedback mechanisms will sustain and enhance the extrinsic pathway in order to produce sufficient fibrin. Some of these mechanisms occur in response to activated factors produced via the intrinsic pathway.

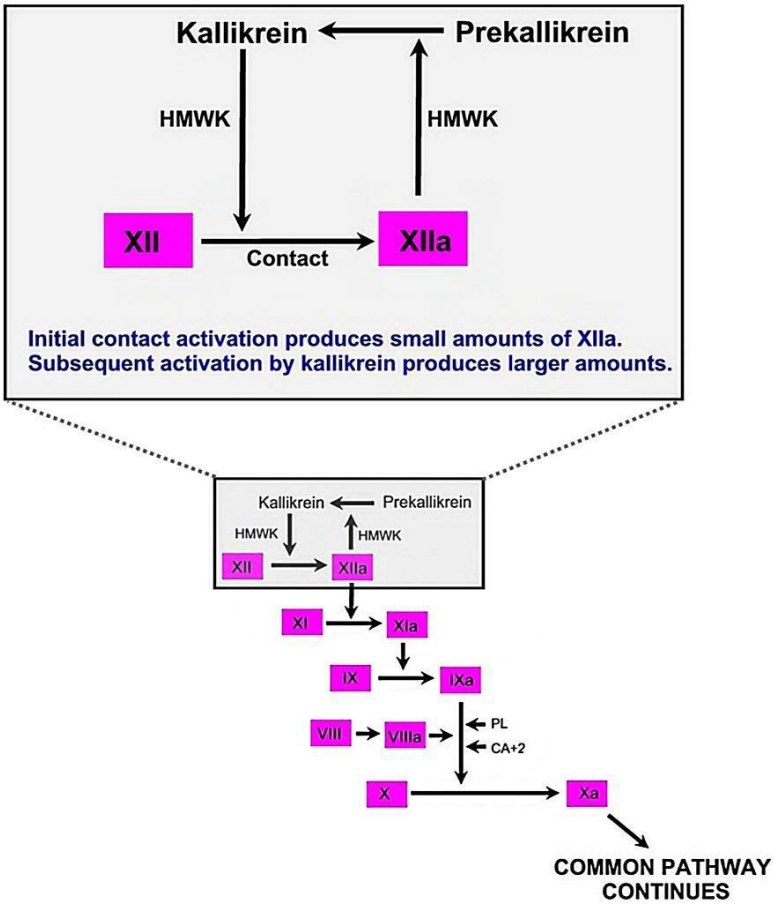
- XIIa formed via the intrinsic pathway will activate VII to VIIa.
- Factor Xa, formed by either the intrinsic or extrinsic pathways, activates more VII to VIIa.
- Factor IXa, formed by the intrinsic pathway, activates more VII to VIIa.
- Thrombin, formed by either the intrinsic or extrinsic pathways, activates more VII to VIIa.

How does this compare to the cell-based model?

It is now known that factor XII plays no role in *in vivo* blood clotting but is involved in *in vitro* (in the test tube) coagulation reactions. Therefore, activation of factor VII by factor XIIa occurs only in coagulation testing and not within the body. The cell-based model supports the theory that factor VII is largely activated by the FVIIa-TF complex as indicated above. It also supports the theory that factor VII is activated by proteases that are converted from their zymogen forms by the initial FVIIa-TF complex, mainly factor Xa and to a lesser extent thrombin (IIa) and possibly factor IXa.

INTRINSIC PATHWAY

As with the extrinsic pathway, the intrinsic pathway consists of a series of reactions that lead to the activation of factor X to factor Xa and subsequent formation of fibrin by the common pathway. The factors involved in these reactions are VIII, IX, XI, and XII. The initial method of activation of the intrinsic pathway involves activation of factor XII upon its exposure to negatively charged subendothelium or collagen. This is called **contact activation**. Factor XII is only partially activated by contact with the subendothelium. To enhance activation of the intrinsic pathway, production of greater quantities of XIIa occurs when the original XIIa works with high molecular weight kininogen (HMWK) to convert prekallikrein to kallikrein. Kallikrein, in combination with HMWK, activates more factor XII.

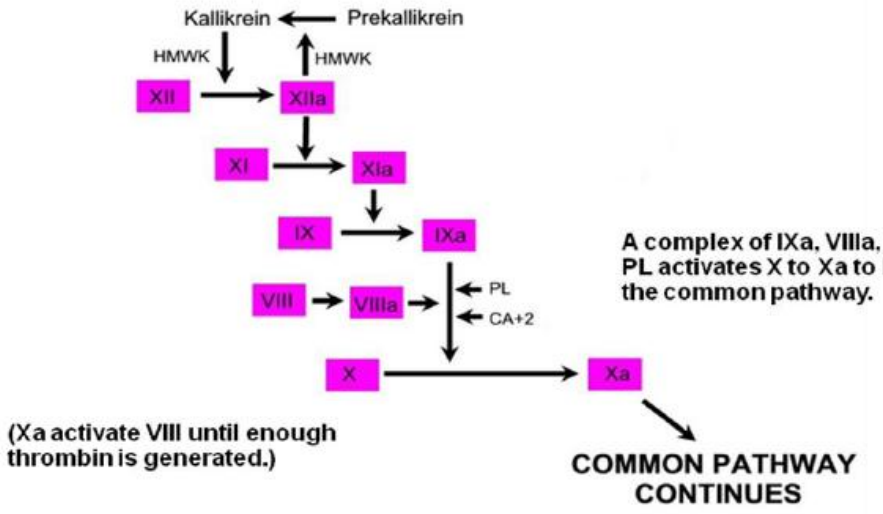


Once activated, XIIa activates XI to IXa. As shown in the diagram below, this step requires platelet phospholipid (PL). Activated platelets in the plug provide the phospholipid surface where the coagulation factors interact to produce thrombin (factor IIa), the proteolytic enzyme that is responsible for converting fibrinogen to fibrin. This capacity of the activated platelets to catalyze the coagulation process by providing phospholipid surfaces is known as **platelet factor 3** or **platelet procoagulant activity**.

Once factor XIa is formed, it in turn activates IX to IXa. IXa in combination with VIIIa, platelet phospholipid, and calcium (Ca^{+2}) activates X to Xa to signal the start of the common pathway. Note that factor VIII is activated to VIIIa by thrombin and to a lesser extent by factor Xa.

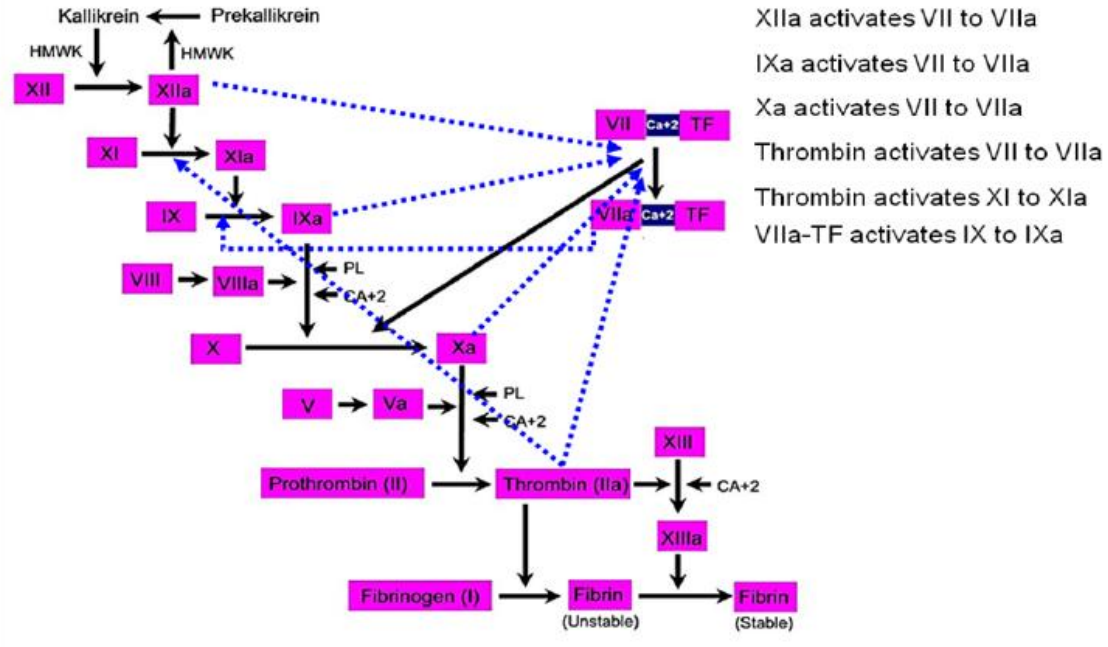
Just as the intrinsic pathway produces factors that enhance the extrinsic pathway, the extrinsic pathway boosts intrinsic pathway activity.

- VIIa-TF formed in the extrinsic serves as a positive feedback mechanism to the intrinsic pathway by activating factor IX to IXa.
- Thrombin formed via the extrinsic pathway activates factor XI to XIa.



How does this compare to the cell-based model?

It is now known that factor XII plays no role in *in vivo* blood clotting but is involved in *in vitro* (in the test tube) coagulation reactions. This explains why an individual with a factor XII deficiency does not have a bleeding problem but will have a prolonged result for the activated partial thromboplastin time (APTT) test, a laboratory test that monitors factors in the intrinsic pathway. Intrinsic pathway factors other than factor XII, including factors I, II, V, VIII, IX, X and XI, are involved in the propagation phase of the cell-based coagulation model. Factors I, II, V, IX, and X are also involved in the initiation phase of the cell-based model.



Summary of alternate methods of coagulation pathway activation

PROBLEMS WITH THE COAGULATION CASCADE

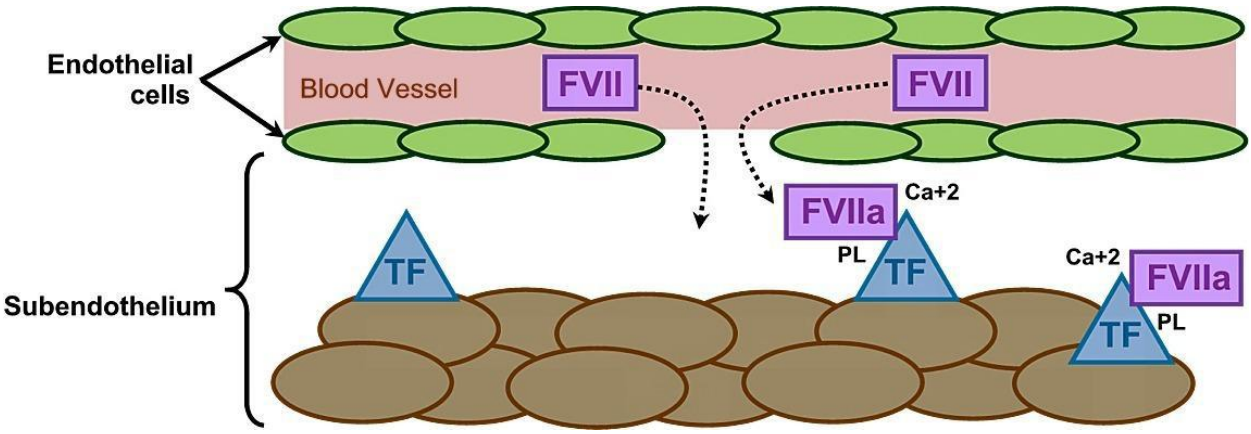
Recent clinical and experimental observations have demonstrated that, while the coagulation cascade accurately reflects hemostatic mechanisms that occur *in vitro* (in the test tube), it does not fully and completely reflect the events of hemostasis *in vivo*. This explains why patients with a deficiency of factor XII or its cofactors prekallikrein and high-molecular weight kinnogen (HMWK) do not have bleeding problems but do have prolonged activated partial thromboplastin time (APTT) results. This implies that while factor XII and its cofactors are not necessary for in vivo hemostasis, they are necessary for the contact activation phase of the intrinsic pathway in laboratory testing. Also, the fact that patients deficient in factors VIII or IX (both involved in the intrinsic pathway) have serious bleeding tendencies despite intact extrinsic pathways and patients deficient in factor VII have serious bleeding tendencies despite intact intrinsic pathways suggests that the intrinsic and extrinsic pathways do not operate as independent, redundant pathways as the coagulation cascade suggests.

CELL-BASED THEORY OF COAGULATION

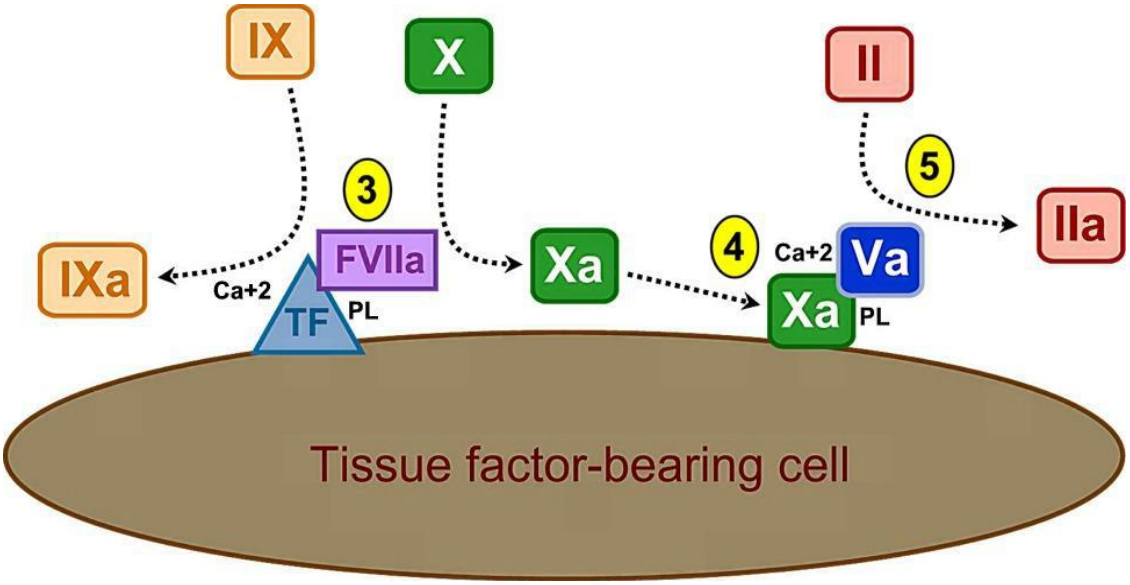
The new cell-based theory of coagulation more accurately reflects how coagulation occurs within the body than does the coagulation cascade. Although this new theory still incorporates many of the factors and reactions from the original coagulation cascade, it places greater emphasis on the role of cells, mainly tissue-factor bearing cells of the subendothelium and activated platelets, in fibrin formation. In the cell-based model, the intrinsic and extrinsic pathways really do exist but function differently than we originally thought. Cells, rather than coagulation proteins, direct and control the coagulation process. The “extrinsic” or **tissue factor pathway** occurs on the surface of the initiating cells (tissue factor bearing cells) while the “intrinsic pathway” occurs on the surface of activated platelets. Having reactions occur on the surface of cells ensures that the coagulation process remains localized to the site of injury. The cell-based theory describes hemostasis as occurring in three distinct yet overlapping phases: initiation, amplification, and propagation.

INITIATION PHASE

Blood coagulation is initiated by blood vessel injury and subsequent exposure of blood to cells expressing tissue factor (TF). TF is expressed on cells that exist in the subendothelium and are not normally in contact with blood, such as fibroblasts and smooth muscle cells. It was previously called tissue thromboplastin and factor III. Upon blood vessel injury, factor VII leaves the bloodstream and enters the subendothelial space where it binds with TF in the presence of calcium and phospholipid on the surface of the cells and becomes activated. The methods by which factor VII is activated are not completely understood but are most likely dependent on the small amount of factor VII that circulates in plasma in the active form (FVIIa). This small amount of circulating FVIIa, which represents approximately 1% of all circulating FVII, has very poor proteolytic efficiency in the absence of tissue factor, its cofactor. As such, it cannot initiate widespread hemostasis within the intact blood vessel. Upon vessel injury, this preexisting FVIIa binds with TF on cells in the subendothelial space, forming a complex that activates more VII that is bound to TF, a phenomenon known as **autoactivation**. Additional activation of FVII can occur by other proteases that are converted from their zymogen forms by the initial FVIIa-TF complex, mainly factor Xa and to a lesser extent thrombin (IIa). (See diagram below)



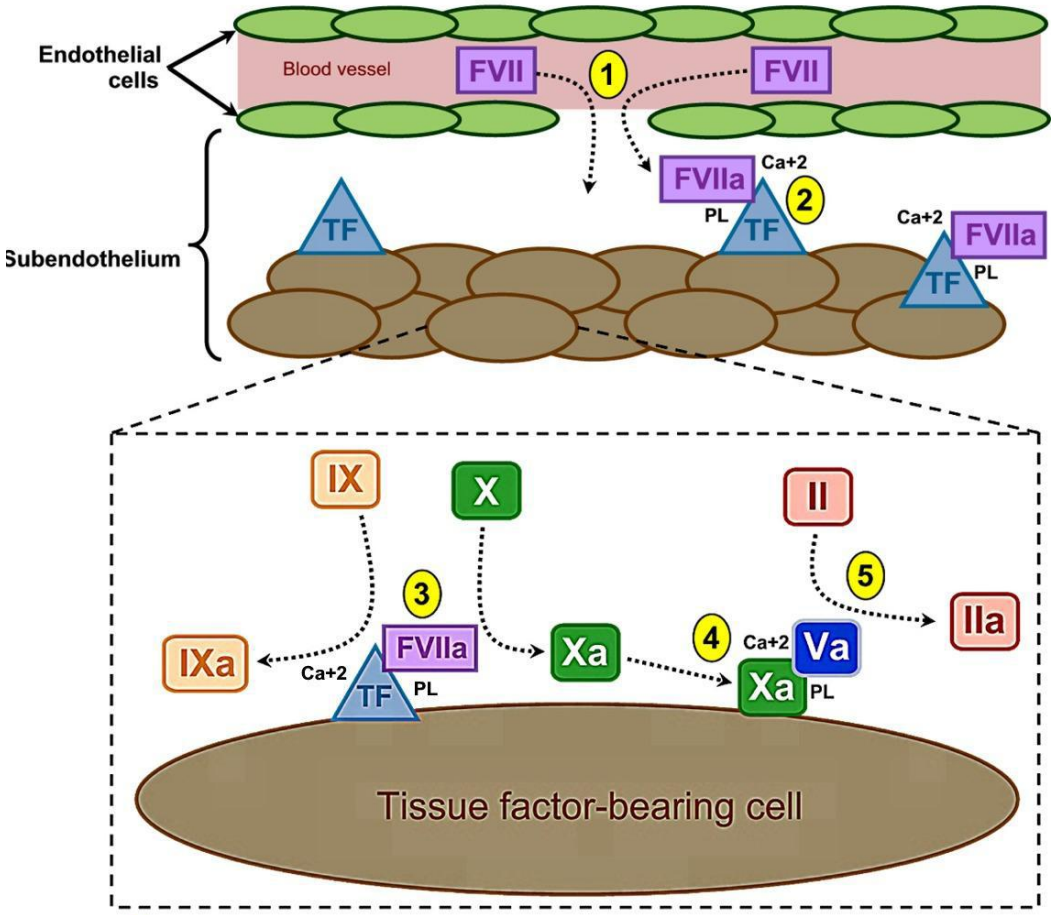
The FVIIa-TF complex activates small amounts of factors IX and X. FXa associates with FVa in the presence of calcium and phosphospholid to form the **prothrombinase complex** that activates small amounts of prothrombin (II) to thrombin (IIa). (See diagram below)



Factor Va, the activated form of factor V, is a critical component of the prothrombinase complex. The factor Va that is used during the initiation phase comes from one of following sources:

- Platelets that are partially activated by adhesion to collagen and other extracellular matrix components secrete partially activated FV from their alpha granules.
- Factor V that enters the subendothelial space from the blood stream can be activated by factor Xa.
- Non-coagulation proteases can activate factor V.

Overview of the INITIATION PHASE



1. Blood vessel injury allows FVII to enter the subendothelial space.
2. FVII binds with TF on the surface of the cell and is activated.
3. The FVIIa-TF complex activates factors IX and X.
4. FXa binds with FVa to form the prothrombinase complex.
5. The prothrombinase complex converts a small amount of prothrombin to thrombin.

AMPLIFICATION PHASE

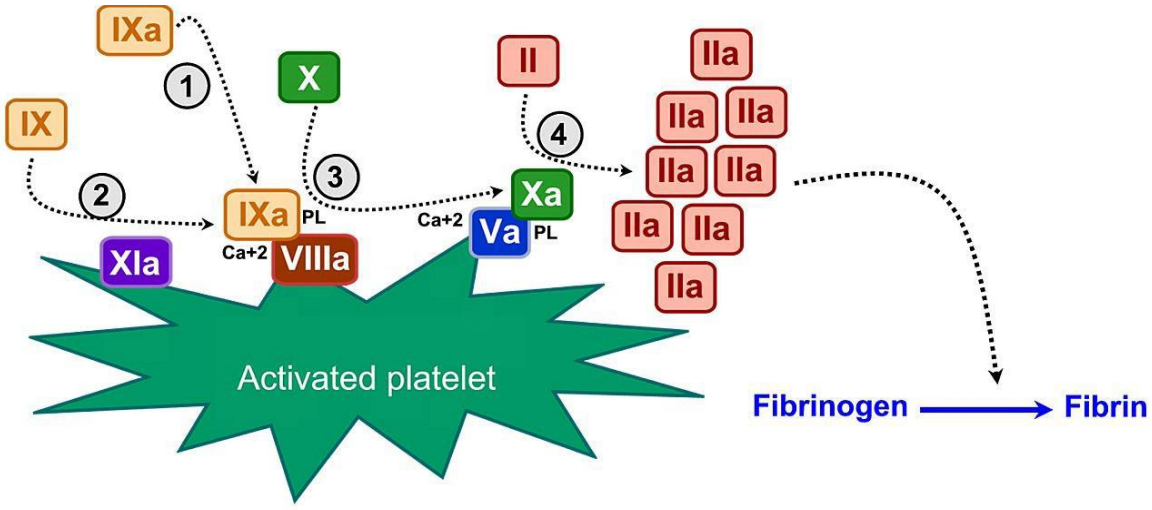
The small amount of thrombin produced on the surface of the TF-bearing cell during the initiation phase is not enough to form fibrin. The goal of the amplification phase is to utilize the small amount of thrombin that is produced during the initiation phase to set the stage for full-scale thrombin generation during the propagation stage. The thrombin generated during the initiation phase is used to:

- Fully activate platelets that were partially activated upon adhesion to the site of injury. Activated platelets release factor Va from their alpha granules.
- Activate cofactor V (that originated from the blood) on the platelet surface.
- Activate cofactor VIII.
- Release von Willebrand factor (vWF) from cofactor VIII, allowing vWF to mediate additional platelet adhesion and aggregation at the site of injury.
- Activate factor XI on the platelet surface.

PROPAGATION PHASE

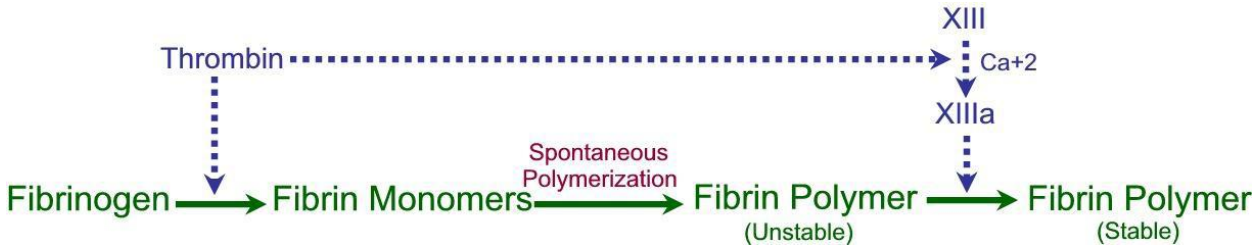
The propagation phase occurs on the surfaces of activated platelets. There are four key events that occur during the propagation phase.

1. FIXa generated during the initiation and amplification phases binds to FVIIIa on the platelet surface.
2. FXIa bound to the platelet surface activates more FIX.
3. The FIXa-FVIIIa-Ca+2-PL complex (called the **tenase complex**) on the platelet surface activates FX.
4. FXa rapidly associates with FVa that bound to the platelet surface during the amplification phase to form the FXa-FVa-Ca+2-PL complex (called the **prothrombinase complex**). This complex converts enough prothrombin (FII) to thrombin (FIIa) for fibrin formation to occur.



1. FIXa generated duing the initiation phase binds to the FVIIIa on the platelet surface.
2. FXIa bound to the platelet surface activates more FIX.
3. The FIXa / FVIIIa complex on the platelet surface activates FX.
4. FXa rapidly associates with FVa that bound to the platelet surface during the amplificaiton phase and the FXa /FVa complex (called the prothrombinase complex) converts enough prothrombin (FII) to thrombin (FIIa) for fibrin formation to occur.

The method by which fibrinogen is converted to fibrin by the proteolytic enzyme thrombin during the propagation phase is the same as was described in the coagulation cascade. Fibrinogen is composed of three pairs of polypeptide chains (two alpha, two beta, and two gamma chains) that are linked together by disulfide bonds. Thrombin cleaves a portion of each of the alpha and beta chains to create small pieces called fibrinopeptides A and B. The portion of the fibrinogen molecule that remains after removal of fibrinopeptides A and B is called a **fibrin monomer**. These fibrin monomers spontaneously aggregate, or polymerize, to form a **fibrin polymer**. The unstable fibrin polymer is stabilized by the action of factor XIIIa. Factor XIIIa introduces covalent cross-links between the alpha and beta chains of the fibrin polymer to hold it firmly together. Factor XIII, also known as **fibrin-stabilizing factor**, is activated to factor XIIIa by thrombin and calcium (Ca^{+2}).



BASAL COAGULATION

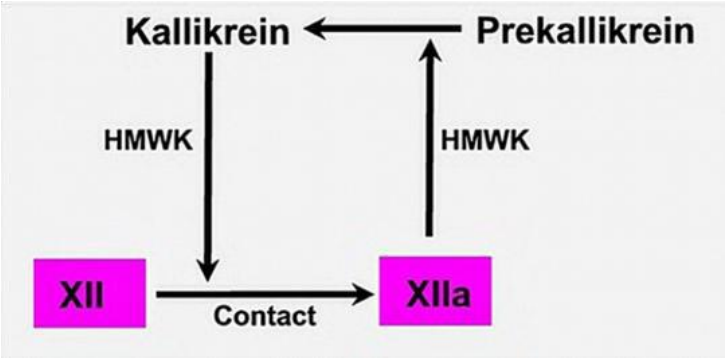
The presence of low levels of activated coagulation factors in the blood at all times, called **basal coagulation**, allows the blood to remain fluid while at the same time permitting small, exquisitely controlled and confined fibrin clots to plug small leaks in the vasculature. The following scenario explains how these activated factors come to be in the bloodstream.

1. A low level of activity of the TF pathway probably occurs at all times in the extravascular space. Some factor VII is probably bound to extravascular TF to form a few FVIIa-TF complexes.
2. Factors X and IX leave the blood vessels and travel through the extravascular space, where they become activated by their contact with the TF-FVIIa complex. This does not lead to clot formation in the extravascular space because platelets and factor VIII complexed with vWF are unable to traverse the intact blood vessels.
3. Low levels of activated factors X and IX reenter the blood vessels where they interact with other coagulation factors to form small, confined fibrin clots that repair small tears in the blood vessels.

KININ SYSTEM

The kinin system, also called the kinin-kallikrein system, is important in inflammation, vascular permeability, and chemotaxis. It is activated by both the coagulation and fibrinolytic systems. Members of the kinin system include kininogens, kinins, and enzymes. The kininogens, which are the precursors of kinins, include high molecular weight kininogen (HMWK) and low molecular weight kininogen (LMWK). Kallikrein, a proteolytic enzyme of the kinin system, acts upon HMWK to release the kinin called bradykinin and upon LMWK to release the kinin called kallidin. Both bradykinin and kallidin function as potent vasodialators in addition to increasing vascular permeability, stimulating pain receptors and causing contraction of smooth muscle. In addition to liberating kinins from kininogens,

kallikrein minimally activates plasminogen to plasmin in the fibrinolytic system. Kallikrein is derived from prekallikrein through the action of factor XIIa. Other enzymes of the kinin system include carboxypeptidase, angiotensin converting enzyme, and neutral endopeptidase.



COMPLEMENT SYSTEM

The complement system is a group of approximately 22 proteins in the blood that play a vital role in the body's immune defenses through a cascade of interactions. The complement system also participates in or is influenced by the coagulation and fibrinolytic systems. Examples include:

- Plasmin, the proteolytic enzyme that degrades fibrin, cleaves the complement protein C3 into C3a and C3b. C3a causes increased vascular permeability while C3b functions as an opsonin by causing immune adherence.
- The complement protein C5a is an anaphylatoxin and chemotactic agent for WBCs as well as a potent platelet aggregator.
- C1 esterase inhibitor is an inhibitor of the complement sequence as well as an inhibitor of thrombin and plasmin.

PHYSIOLOGIC CONTROL OF HEMOSTASIS

Once blood coagulation has begun, it is vitally important to restrict its activity to the site of vascular injury and to shut it down after the task of producing a fibrin clot has been completed. If allowed to expand beyond the site of injury and to proceed unchecked, life-threatening thrombosis may result. There are a variety of physiologic controls that prevent excessive clot formation.

BLOOD FLOW

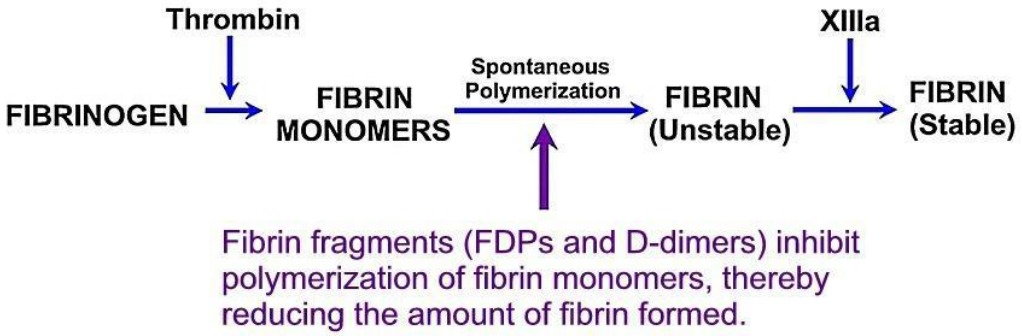
Immediately following blood vessel injury, the injured vessel constricts (vasoconstriction). This reduces the amount of blood escaping from the wound and also slows blood flow through the area, a condition that promotes activation of both platelets and coagulation factors. Once fibrin production begins to occur, blood flow in the wounded vessel returns to normal. This serves to limit coagulation by washing activated factors away and limiting activation of additional factors.

LIVER CLEARANCE

Activated coagulation factors are cleared from the blood by hepatocytes of the liver, thereby limiting additional fibrin formation.

FEEDBACK INHIBITION

Some of the activated coagulation factors have the ability to destroy other factors. This usually occurs only after blood coagulation is in full swing and the concentration of activated factors is high. For example, as the concentration of thrombin increases, it will destroy factors V and VIII. This is very interesting since, during the early stages of the fibrin formation, thrombin helped to activate these same factors. Perhaps one of the best examples of feedback inhibition is the ability of fibrin fragments to inhibit further conversion of fibrinogen to fibrin. Fibrin fragments, including fibrin degradation products (FDPs) and D-dimers, are formed when the fibrinolytic system breaks down the fibrin clot. It is important to remember that the fibrinolytic system is activated simultaneously with the coagulation cascade. Therefore, as clots are forming, preparations are being made to break them down. As this occurs, fibrin fragments accumulate in the blood and interfere with fibrin formation as well as platelet aggregation.



BIOCHEMICAL INHIBITORS

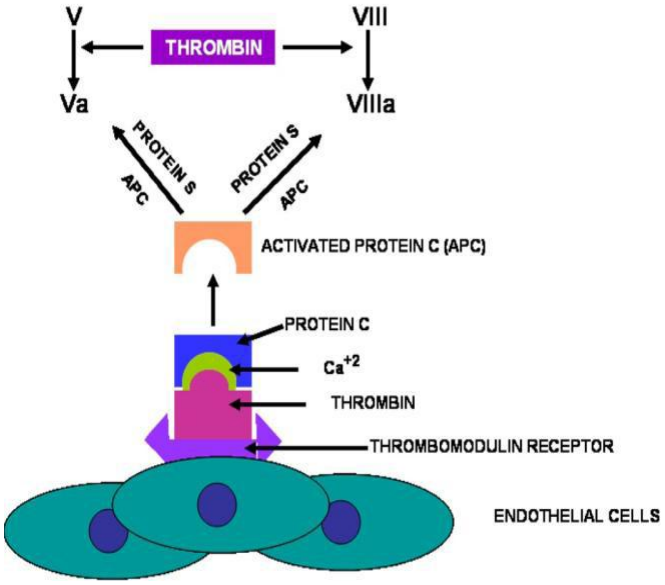
The biochemical inhibitors are proteins that have the ability to regulate the enzymatic activities of the activated coagulation factors, thereby slowing and stopping the coagulation cascade. The biochemical inhibitors include tissue factor pathway inhibitor, the protein C regulatory system (involves proteins C and S), and the serine protease inhibitors, including antithrombin, heparin cofactor II, alpha 1-antitrypsin, alpha 2-macroglobulin, and Z-dependent protease inhibitor. Deficiencies or defects in any of these inhibitors are associated with thrombotic disorders.

TISSUE FACTOR PATHWAY INHIBITOR (TFPI)

TFPI is produced by several cell lines, including lung, liver, bladder, and endothelial tissue cells. Platelets also produce and secrete TFPI after stimulation with calcium or thrombin. In the initiation phase of coagulation, factor VIIa and tissue factor (TF) form a complex that activates factors IX and X. Factor Xa reacts with the FVIIa/TF complex to bind TFPI. TFPI inactivates factor VIIa, making the FVIIa/TF reaction short liver. Heparin enhances the inhibitory abilities of TFPI by 40-fold.

PROTEIN C REGULATORY SYSTEM

Protein C is activated by thrombin. Once activated, it inhibits the activity of factors Va and VIIIa. In order to be optimally activated, protein C must bind to thrombin (IIa) on the thrombomodulin receptor on the surface of the endothelial cell. Calcium serves as a bridge to bind thrombin and protein C together. Protein S facilitates the activation of protein C by promoting its binding to the thrombomodulin receptor. It also works with activated protein C to accelerate the inactivation of factors Va and VIIIa.



SERINE PROTEASES

ANTITHROMBIN

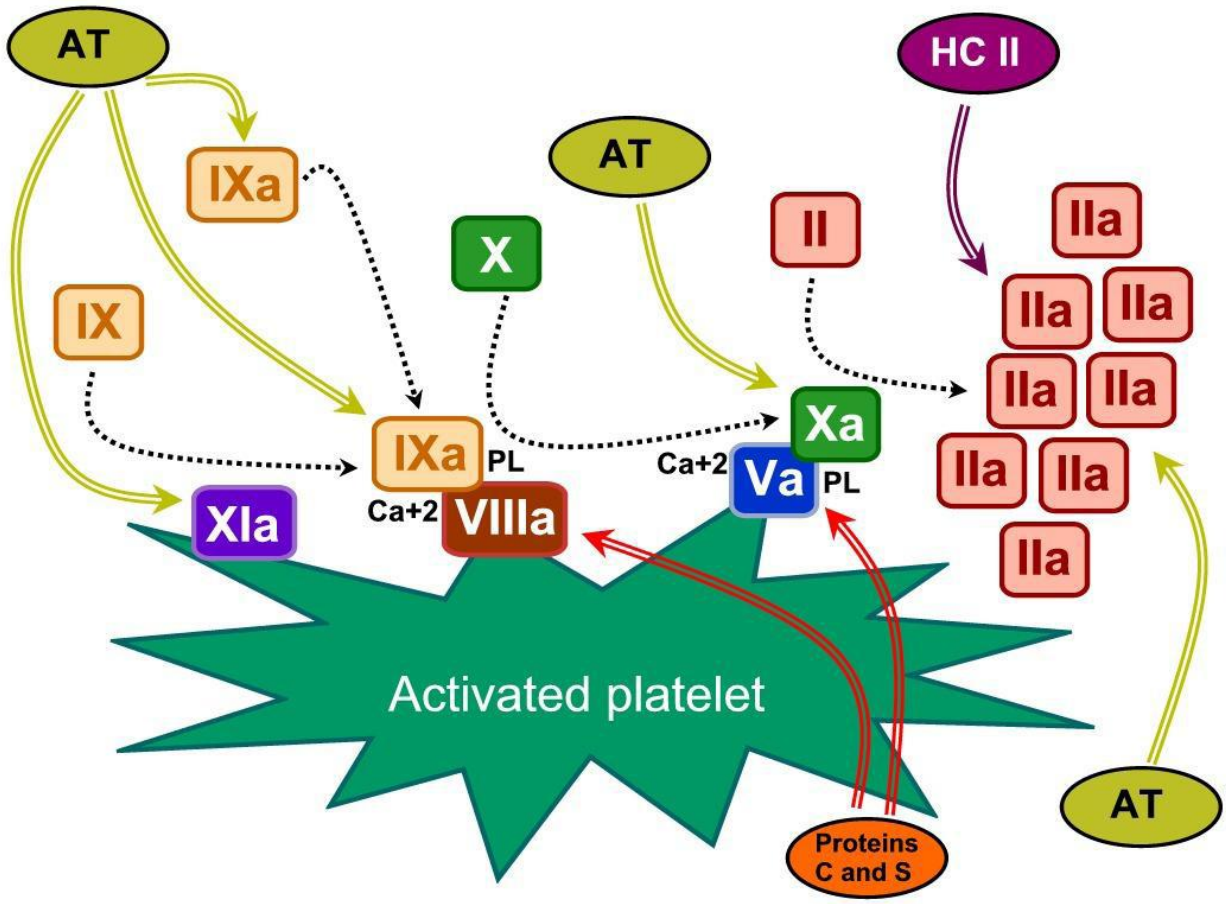
Antithrombin (AT) is produced in the liver, by endothelial cells, and possibly by megakaryocytes. It is the most important of the protease inhibitors because of its ability to neutralize numerous activated factors, including IIa (thrombin), IXa, Xa, XIa, XIIa, and kallikrein, by forming 1:1 complexes with each of these activated factors. It also neutralizes plasmin, the proteolytic enzyme of the fibrinolytic system that degrades the fibrin clot. AT plays an important role in heparin anticoagulant therapy. Heparin binds with AT, causing a conformational change that greatly increases AT activity. In fact, the change enhances the rate with which AT binds with and deactivates thrombin by a factor of 1000 times. It is important to remember that heparin in and of itself does not anticoagulate the blood. Rather, it is heparin's effect of AT that produces the anticoagulant effect. Therefore, individuals who are AT deficient will not respond to heparin therapy.

HEPARIN COFACTOR II

Heparin cofactor II inhibits the activity of thrombin only. Heparin enhances the activity of heparin cofactor II but not to the same extent that it enhances AT-III activity.

ALPHA 1-ANTITRYPSIN, ALPHA 2-MACROGLOBULIN AND PROTEIN Z-DEPENDENT PROTEASE INHIBITOR

These serine proteases play a less significant role in inhibiting coagulation than do antithrombin and heparin cofactor II. Alpha 1-antitrypsin inhibits factor Xa. Alpha 2-macroglobulin is a non-specific serine protease that targets a broad spectrum of protease substrates. Protein Z dependent protease inhibitor binds protein Z and factor Xa in a complex with calcium and phospholipid to inhibit factor Xa-induced thrombin formation.



Summary of biochemical inhibitors

FIBRINOLYSIS

I. Overview of the fibrinolytic system
    A. Plasminogen and plasmin
    B. Plasminogen activators
        1. Tissue plasminogen activator (TPA)
        2. Urokinase (UPA)
        3. Kallikrein, factor XIa, and factor XIIa
II. Mechanism of fibrinolysis
III. Physiologic control of fibrinolysis
    A. Plasmin inhibitors
    B. Plasminogen activator inhibitors

FIBRINOLYSIS

Fibrinolysis is the process by which the fibrin clot is enzymatically dissolved. Defects in the fibrinolytic system can result in bleeding, if the system is too active and there is uncontrolled fibrin degradation, or thrombosis, if there is ineffective fibrin breakdown.

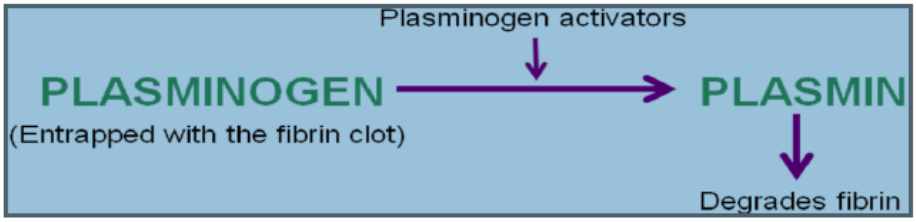
OVERVIEW OF THE FIBRINOLYTIC SYSTEM

PLASMINOGEN AND PLASMIN

The proteolytic enzyme that actually degrades the fibrin clot is plasmin. The inactive precursor of plasmin is plasminogen. Plasminogen, a liver-synthesized protein, circulates in the blood and is entrapped within the fibrin clot as it forms. Upon tissue injury, substances that will activate plasminogen and cause its conversion to plasmin are released simultaneously with substances that initiate coagulation. This means that as soon as the body begins to make a clot, it is initiating systems that will dissolve the clot. Fibrinolysis does not normally begin until a few hours after fibrin polymerization and cross-linking. Fortunately, it is a slow acting process that gradually breaks down the clot, allowing sufficient time for complete tissue repair to occur before the clot is totally removed.

PLASMINOGEN ACTIVATORS

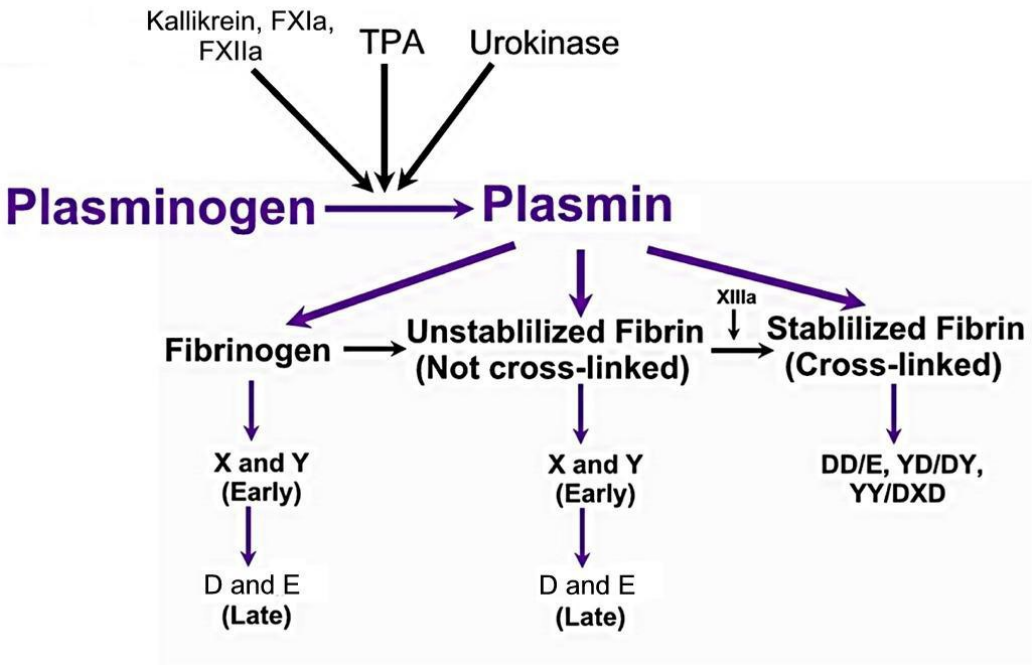
Plasminogen in converted to plasmin by various plasminogen activators. The major plasminogen activators are tissue plasminogen activator (t-PA) and urokinase (u-PA). Under certain circumstances, proteases traditionally classified within the intrinsic coagulation pathway, including kallikrein, factor XIa, and factor XIIa, are capable of activating plasminogen. However, these proteases normally account for less than 15 percent of plasmin-generating activity.



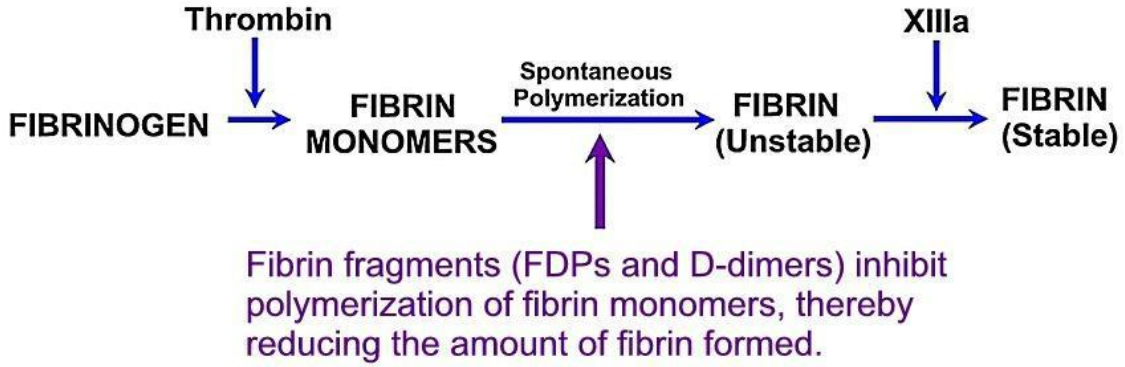
Tissue plasminogen activator (t-PA) appears to be the major activator of plasminogen. It is synthesized primarily by endothelial cells and released upon cell damage to be incorporated within the fibrin clot along with plasminogen. t-PA is a relatively poor plasminogen activator if it is not associated with fibrin. However, its strong affinity for plasminogen in the presence of fibrin greatly enhances its ability to generate plasmin. Urokinase (u-PA), which is synthesized by epithelial cells and kidney cells, is present in small amounts in plasma. As with t-PA, u-PA is incorporated along with plasminogen into the fibrin clot as it forms. It is an effective plasminogen activator both in the presence and in the absence of fibrin.

MECHANISM OF FIBRINOLYSIS

As the fibrin clot forms, plasminogen, which is circulating in the plasma, adheres to the surface of the fibrin. Plasminogen is then activated to plasmin by a plasminogen activator. Plasmin degrades both the unstabilized and stabilized forms of fibrin, resulting in the formation of fragments of low molecular weight that are rapidly clear from the plasma by the mononuclear phagocytic cells of the liver. It also degrades fluid-phase fibrinogen (fibrinogen that is circulating in plasma).



Degradation of fluid-phase fibrinogen by plasmin serves to inhibit further fibrin formation and, as such, serves as one of the many feedback mechanisms that prevent uncontrolled clot formation. Another feedback mechanism occurs as the number of fibrin fragments, known as fibrin degradation products (FDPs) and D-dimers, increases. These fibrin fragments inhibit further clot formation by interfering with fibrin monomer polymerization, a crucial step in the conversion of fibrinogen to fibrin. They also inhibit platelet aggregation.



Plasmin has other functions in addition to degrading fibrin and fibrinogen that it performs. It destroys fluid-phase factors V and VIII, thereby inhibiting further fibrin formation at the site of injury. It also indirectly enhances or amplifies the conversion of factor XII to factor XIIa, which in turn results in more conversion of prekallikrein to kallikrein. Kallikrein, in turn, converts more plasminogen to plasmin, enhancing fibrinolysis. Finally, plasmin can liberate kinins from kininogens, and activate the complement system.

FUNCTIONS OF PLASMIN
• Degrades stabilized fibrin and unstabilized fibrin • Destroys fluid-phase fibrinogen • Destroys fluid-phase factors V, VIII, IX, and XI • Indirectly enhances or amplifies the conversion of factor XII to factor XIIa • Enhances or amplifies conversion of prekallikrein to kallikrein (occurs in response to factor XIIa generation) • Liberates kinins from kininogen (occurs in response to kallikrein generation) • Activates the complement system by cleaving C3 into fragments

PHYSIOLOGIC CONTROL OF FIBRINOLYSIS

Various inhibitors function to control the fibrinolytic system so that it does not get out of hand.

ALPHA 2 - ANTIPLASMIN

Plasmin that is bound to fibrin digests the clot and restores blood flow through the vessel. Plasmin that is circulating freely, however, digests fibrinogen, factor V, factor VIII, and fibronectin, causing a potentially dangerous situation of uncontrolled fibrinolysis and bleeding. Plasmin inhibitors function to eliminate free plasmin, thereby preventing bleeding. Alpha 2-antiplasmin is the major inhibitor of plasmin. It is synthesized by the liver and kidneys and circulates in plasma at relatively high concentrations. It rapidly and irreversibly binds with free (not bound to fibrin) plasmin and deactivates it. It also links to fibrin during polymerization to render it resistant of digestion by plasmin.

Alpha 2-macroglobulin is another plasmin inhibitor that functions in a manner similar to alpha 2-antiplasmin. It is synthesized by endothelial cells and macrophages and stored in platelet alpha granules. It inactivates plasmin with approximately 10% of the efficiency of alpha 2-antiplasmin.

THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR

Thrombin-activatable fibrinolysis inhibitor (TAFI) becomes activated by the thrombin-thromoblomudulin complex that also activates the protein C regulatory pathway. Activated TAFI inhibits fibrinolysis by altering the structure of partially degraded fibrin, thereby preventing the binding of tissue plasminogen activator (TPA) and plasminogen to fibrin and blocking the formation of plasmin.

PLASMINOGEN ACTIVATOR INHIBITOR-1

Plasminogen activator inhibitor – 1 (PAI -1) is found in endothelial cells, monocytes, macrophages, hepatocytes, adipocytes, and platelets. Its release from these cells is stimulated by many cytokines, growth factors, and lipoproteins associated with the inflammatory response. PAI-1 is present in plasma in amounts that exceed normal plasma tissue plasminogen activator (TPA) levels. Therefore, all circulating TPA is normally bound to PAI-1 to prevent inappropriate plasmin generation. Following trauma and endothelial cell damage, however, the level of TPA exceeds that of PAI-1, resulting in sufficient free (not bound to PAI-1) TPA to initiate fibrinolysis. Other plasminogen activator inhibitors exist but play a far less significant role in preventing plasminogen activation than does PAI-1.

BLEEDING DISORDERS DUE TO DEFECTS IN PRIMARY HEMOSTASIS

I. Disorders of the vascular system
    A. Disorders characterized by inadequate support of the blood vessels
    B. Disorders characterized by poorly constructed blood vessels
    C. Disorders in which blood vessel walls are damaged
        1. Damage by antibodies
            a) Allergic purpura
            b) Deposition of immune complexes
        2. Damage by non-antibody mechanisms

II. Platelet disorders
    A. Quantitative platelet disorders
        1. Decreased platelet production
        2. Increased platelet destruction
            a) Platelet destruction by immune mechanisms (immune thrombocytopenia)
                (1) Immune thrombocytopenic purpura (ITP)
                    (a) Acute ITP
                    (b) Chronic ITP
                (2) Immunologic drug-induced thrombocytopenia
                (3) Neonatal alloimmune thrombocytopenia
                (4) Neonatal autoimmune thrombocytopenia
            b) Platelet destruction by non-immune mechanisms (non-immune thrombocytopenia)
                (1) Hemolytic uremic syndrome (HUS)
                (2) Thrombotic thrombocytopenic purpura (TTP)
                (3) Disseminated intravascular coagulation (DIC)
    B. Qualitative platelet disorders
        1. Hereditary disorders of platelet adhesion
            a) von Willebrand disease
                (1) Description
                (2) Classification
                (3) Diagnostic criteria
                (4) Treatment
            b) Bernard-Soulier syndrome
                (1) Description
                (2) Clinical picture
                (3) Diagnostic criteria
                (4) Treatment
        2. Hereditary disorders of platelet aggregation
        3. Hereditary disorders of platelet secretion
        4. Hereditary disorders of platelet membrane receptors and procoagulant activity
        5. Acquired disorders of platelet function

BLEEDING DISORDERS DUE TO DEFECTS IN PRIMARY HEMOSTASIS

Primary hemostasis involves reactions between the vascular system and platelets and results in the formation of a platelet plug that temporarily halts blood flow from an injured vessel. Defects of the blood vessels and defects or deficiencies of platelets will result in bleeding disorders. Clinically, these disorders are characterized by less severe forms of bleeding, such as bleeding into the mucous membranes or skin. Petechiae and ecchymoses are common presenting symptoms.

DISORDERS OF THE VASCULAR SYSTEM

A variety of disorders, both inherited and acquired, can adversely affect the integrity of the blood vessels, resulting in bruising and bleeding. There are various mechanisms by which these disorders cause vessel damage or weakness. In some of these disorders, inadequate support of the blood vessels renders the vessels more susceptible to damage. In other disorders, the blood vessels are poorly constructed. And, in still others, the blood vessels are directly or indirectly damaged as a result of a disease process.

The diagnosis of blood vessel disorders is most often made by exclusion. Routine laboratory tests, including platelet count, prothrombin time, and activated partial thromboplastin time, will be normal. Two additional tests, the bleeding time and the capillary fragility test, can be performed and may provide helpful diagnostic information. Consult the *Laboratory Evaluation of Coagulation Disorders* section of this manual to review the principles of these tests.

DISORDERS CHARACTERIZED BY INADEQUATE SUPPORT OF BLOOD VESSELS

Normally, blood vessels are supported and protected by strong subendothelial connective tissue structures. When there is insufficient production of connective tissue or when the connective tissue is defective, the blood vessels are more susceptible to damage. Disorders in which this occurs are summarized in the table below.

DISORDERS CHARACTERIZED BY INADEQUATE SUPPORT OF BLOOD VESSELS			
Disorder	**Inherited or Acquired**	**Probable Cause**	**Significant Laboratory Findings**
Ehlers-Danlos syndrome	Inherited	Decreased synthesis of subendothelial connective tissue, mainly collagen and possibly elastin	Bleeding Time: Prolonged Capillary Fragility Test: Positive
Marfan syndrome	Inherited	Defective collagen formation	Bleeding Time: Prolonged Capillary Fragility Test: Variable
Osteogenesis imperfecta	Inherited	Defective bone matrix and abnormal procollagen	Bleeding Time: Prolonged Capillary Fragility Test: Variable
Senile purpura	Acquired	Decreased elasticity of supporting connective tissue; occurs with aging	Bleeding Time: Normal Capillary Fragility Test: Normal
Cushing's syndrome	Acquired	Oversecretion of corticosteroids results in loss of subcutaneous connective tissue	Bleeding Time: Normal Capillary Fragility Test: Positive
Scurvy (Vitamin C deficiency)	Acquired	Decreased synthesis of collagen	Bleeding Time: Normal Capillary Fragility Test: Positive

DISORDERS CHARACTERIZED BY POORLY CONSTRUCTED BLOOD VESSELS

Poorly constructed blood vessels are more easily injured, resulting in bleeding into the surrounding tissues. Disorders in which vessel construction is affected are summarized below.

DISORDERS CHARACTERIZED BY POORLY CONSTRUCTED BLOOD VESSELS			
Disorder	**Inherited or Acquired**	**Probable Cause**	**Significant Laboratory Findings**
Hereditary hemorrhagic helangiectasia (Osler-Weber-Rendu disease)	Inherited	Lack of elastic fibers in walls of capillaries and small venules results in abnormally dilated vessels and mucous membrane lesions that bleed easily	Bleeding Time: Normal Capillary Fragility Test: Positive
Pseudoxanthoma elasticum	Inherited	Abnormal elastic fibers in all arterial vessels	Bleeding Time: Prolonged

DISORDERS IN WHICH BLOOD VESSEL WALLS ARE DAMAGED

A variety of disorders can cause damage to the endothelial cell layer that lines the blood vessel walls. Some of these conditions are characterized by the presence of antibodies that damage the vascular endothelium while others produce damage by some non-antibody mechanism.

DAMAGE BY ANTIBODIES

ALLERGIC PURPURA This condition, also known as Henoch-Schonlein purpura or anaphylactoid purpura, is characterized by the production of an autoantibody that is directed against the vascular endothelium. It usually occurs in children older than two years of age with a recent history of infection. It is speculated that the infectious agent, or perhaps a drug that is administered to treat the infection, alters or damages the vascular endothelium. The immune system perceives the damaged endothelium as foreign and produces an antibody against it. Bleeding from the damaged vessels produces characteristic lesions in the skin. Laboratory tests are usually negative, with the exception of an occasional positive capillary fragility test.

DEPOSITION OF IMMUNE COMPLEXES

Infectious agents, including bacteria and viruses, and various drugs act as antigens in that they induce antibody formation. The antibodies produced will attach to the antigen, forming antigen-antibody complexes, or immune complexes. If these immune complexes attach to the vascular endothelium, they will initiate an inflammatory response that will damage the blood vessel wall. Some immune complexes will even activate the complement system, resulting in more serious damage to the vascular endothelium. In these conditions, the bleeding time is frequently normal and the capillary fragility test is variable depending upon the degree of vessel damage.

Paraprotein disorders also cause blood vessel damage through immune complex formation. **Paraproteins** are abnormal immunoglobulins (antibodies) produced in excess in certain malignant disorders such as multiple myeloma and various lymphoproliferative diseases. Excess antibody results in excess immune complex formation, thereby increasing the likelihood of vessel damage. Although the bleeding encountered in these paraprotein disorders is in part due to the effect of immune complexes on the vascular endothelium, numerous other factors, such as qualitative platelet defects and acquired inhibitors to the coagulation factors, contribute to the hemostasis problems. Coagulation test results are variable, depending upon the number and severity of mechanisms involved.

DAMAGE BY NON-ANTIBODY MECHANISMS

A variety of conditions cause vessel damage through mechanisms not related to antibody formation.

- Several drugs, including aspirin, quinine, and warfarins, have been known to cause **vasculitis** (inflammation of the blood vessels) through an allergic mechanism rather than an immune reaction.
- Certain bacterial toxins can directly damage the vascular endothelium.
- Some conditions are characterized by the formation of amyloid, an abnormal starch-like compound. Deposition of amyloid on the vessel walls results in vascular fragility and bleeding.

PLATELET DISORDERS

Platelets serve many important functions in blood coagulation, from platelet plug formation to participation in the fibrin formation. Therefore, it is not surprising that bleeding will occur if there are insufficient numbers of platelets available to perform these functions (quantitative defect) or if any aspect of platelet function is impaired (qualitative defect).

QUANTITATIVE PLATELET DISORDERS

The term **thrombocytopenia** is used to describe a platelet count that is below normal. General causes for thrombocytopenia include decreased production of platelets by the bone marrow or increased destruction of platelets by any one of several mechanisms.

Platelets are produced by megakaryocytes in the bone marrow. Megakaryocytes originate from primitive cells called stem cells. The earliest stem cell, the pluripotential stem cell, matures into a multipotential stem cell (CFU-GEMM) and finally into a stem cell committed to the platelet cell line (CFU-Meg). The first recognizable cell of the megakaryocytic cell line, the megakaryoblast, immediately follows the committed stem cell stage. Through a process called **endomitosis**, the megakaryoblast matures into a megakaryocyte. The mature megakaryocyte produces and releases platelets from its cytoplasm.

Whenever thrombocytopenia exists, it is important to determine if it is due to decreased platelet production or increased platelet destruction. This is most commonly accomplished by performing a bone marrow evaluation. If there are inadequate numbers of megakaryocytes in the bone marrow, the thrombocytopenia is due to decreased production. If, on the other hand, there are sufficient or increased numbers of megakaryocytes in the bone marrow, platelets are being produced in adequate numbers and the cause of the thrombocytopenia in the peripheral blood is increased platelet destruction. Once the general cause of the thrombocytopenia is determined, the specific cause will be determined by additional testing.

DECREASED PLATELET PRODUCTION

There are a variety of conditions that impair platelet production. They are categorized according to general cause as follows:

- Damage to bone marrow stem cells by agents such as drugs, radiation, chemicals and viruses. Other cell lines in addition to platelets will be affected depending upon which type of stem cell is affected. A bone marrow evaluation will reveal a decrease in one or more cell lines.
- Bone marrow replacement by abnormal cells as seen in leukemia, lymphoma, and metastatic carcinoma. Other cell lines in addition to platelets will be affected and, as a result, **pancytopenia** (a decrease in all cell lines) will frequently develop. A bone marrow evaluation will reveal evidence of an abnormal cell line and further testing will be performed to obtain a definitive diagnosis.
- Ineffective thrombopoiesis in conditions such as megaloblastic anemia. Other cells lines in addition to platelets will be affected; pancytopenia will frequently develop. A bone marrow evaluation will reveal abnormalities consistent with the disease that is affecting hematopoiesis.
- Hereditary disorders that affect platelet production, such as May-Hegglin anomaly, Bernard-Soulier syndrome, and Wiscott-Aldrich syndrome. Platelet function may also be impaired in some of these disorders.

INCREASED PLATELET DESTRUCTION

Once released from the bone marrow, platelets survive in the peripheral circulation for approximately 9 to 12 days. There are a variety of conditions that can result in platelets being destroyed prematurely. Some of these conditions involve the production of antibodies that cause platelet destruction. These conditions are collectively referred to as immune thrombocytopenia. Other conditions are characterized by increased platelet destruction or utilization by non-immune mechanisms.

PLATELET DESTRUCTION BY IMMUNE MECHANISMS (IMMUNE THROMBOCYTOPENIA)

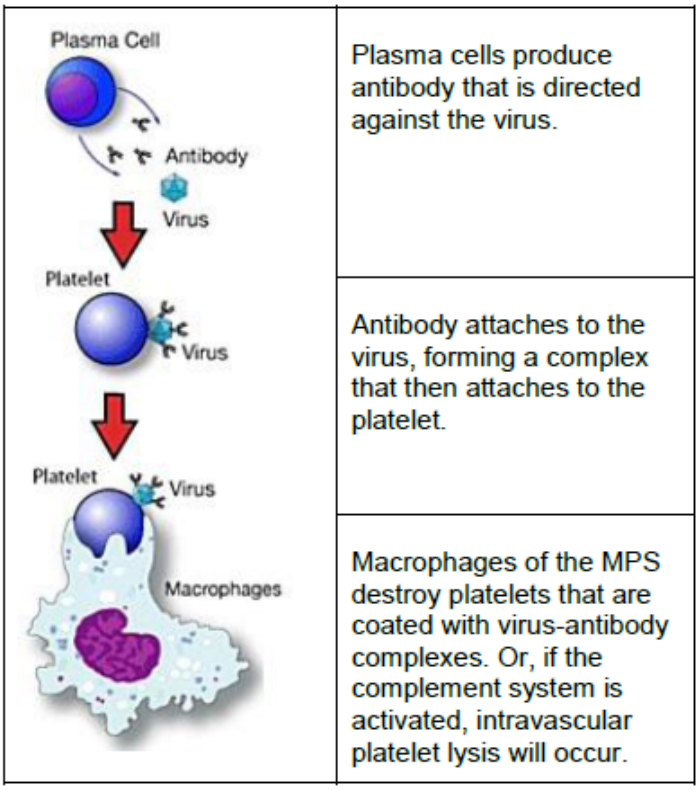
Immune (Idiopathic)Thrombocytopenic Purpura (ITP)

Immune thrombocytopenic purpura was previously referred to as *idiopathic* thrombocytopenic purpura because, at one time, the majority of cases appeared to arise without apparent etiology or underlying disease state. It is not known that all forms of the disease are immunologically mediated in some way. There are three forms of ITP: acute ITP, chronic ITP, and intermittent (recurrent) ITP.

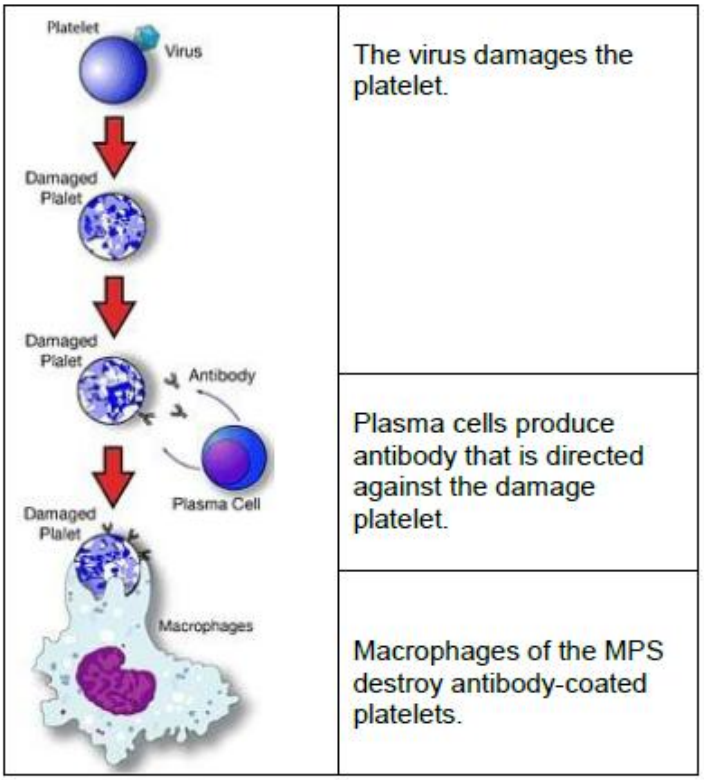
Acute ITP occurs most frequently in children between the ages of 2 years and 6 years of age. It is commonly preceded by a viral infection 1 to 3 weeks prior to the development of thrombocytopenia. Symptoms include an abrupt onset of bleeding from mucous membranes, easy bruising, and petechiae on the extremities. A peripheral blood count often reveals a platelet count of less than 20 X 10^3/uL. Platelet survival time in acute ITP is only 1 to 3 hours as opposed to the normal lifespan of 9 to 12 days.

There are two possible mechanisms by which platelets are destroyed in acute ITP.

- The virus induces antibody formation by the immune system. The anti-virus antibody attaches to the virus forming a complex that then attaches to the surface of the platelet. The spleen and other organs of the mononuclear phagocyte system remove the coated platelets, or, if the antibody activates the complement system, intravascular platelet lysis will occur.



- The virus damages the platelets and an immune response is directed against the altered platelets. Antibody coated platelets are removed by the mononuclear phagocyte system, predominantly the spleen.



Following discovery of a low platelet count, a bone marrow examination may be performed to determine the cause of the thrombocytopenia. In the

case of acute ITP, the number of bone marrow megakaryocytes will be increased. The megakayocytes will also be larger and will have a nucleus with more lobes than normal. Both of these changes occur in response to stimulation by thombopoietin, the cytokine (growth factor) that regulates maturation of megakaryocytes and production of platelets. The levels of thrombopoietin will rise in response to the decreasing platelet count and the body's increasing need for platelets.

Most patients with acute ITP experience a spontaneous remission within 2 to 6 weeks. Treatment is usually limited to precautionary measures aimed at preventing trauma and reducing the risk of bleeding. In some cases, corticosteroids, usually prednisone, are administered to suppress the immune system and slow antibody production.

Chronic ITP occurs predominantly in young to middle aged adults, with a female to male ratio of 3 to 1. It is characterized by a gradual onset of mild bleeding in the form of recurring epistaxis, menorrhagia, and easy bruisability. Spontaneous remission is uncommon. Platelet destruction in chronic ITP occurs as the spleen and other organs of the mononuclear phagocyte system remove and destroy antibody-coated platelets. The antibody involved is believed to be autoimmune in nature, meaning that the body has produced an antibody against its own platelets.

The autoantibody production can occur as a primary disorder, or can occur secondary to other diseases such as chronic lymphocytic leukemia, Hodgkin lymphoma, systemic lupus erythematosus, and HIV infection. Platelet survival times in chronic ITP are 1 to 3 days as opposed to the normal 9 to 12 days. Platelet counts are usually in the range of 30 to 80 x 10^3/uL. As with chronic ITP, a bone marrow examination will reveal an increase in the number and size of megakaryocytes. These megakaryocyte changes result in greater platelet production and occur in response to increased production of thrombopoietin.

The first line of therapy for patients with chronic ITP is the use of corticosteroids, usually prednisone. These drugs act in a variety of ways on the immune system to suppress splenic destruction of platelets. If rapid elevation in platelet count is needed, such as in the case of critical bleeding or in preparation for surgery, intravenous immunoglobulin (IVIG), pooled IgG immunoglobulins, may be administered. The precise mechanism by which IVIG suppresses autoimmune disorders such as ITP is not clear. While IVIG is quick and effective at alleviating autoimmune platelet destruction, its effect is usually short lived, usually wearing off within a few weeks. Splenectomy will also reduce platelet destruction since the spleen is a major site of antibody production as well as being the MPS system organ that is most active in platelet destruction. For those patients whose platelet counts do not increase following drug therapy and splenectomy, high doses of immunosuppressive drugs can be administered but tend to have limited success.

In some patients with chronic ITP, there are alternating intervals of thrombocytopenia and periods during which the platelet count is normal. This is referred to as **intermittent (recurrent) ITP**.

Comparison of Acute and Chronic ITP		
Characteristics	**Acute ITP**	**Chronic ITP**
Age of onset	2 – 6 years	20 – 50 years
Sex predominance	None	Female to male ratio: 3 to 1
Prior infection	Common	Unusual
Onset of bleeding	Sudden	Gradual
Platelet count	< 20 x 10^3/uL	30 to 80 x 10^3/uL
Duration	2 – 6 weeks	Months to years
Spontaneous remission	90% of patients	Uncommon
Seasonal pattern	Higher incidence in winter and spring	None
Therapy • Steroids • Splenectomy	 70% response rate Rare	 30% response rate < 45 years of age: 90% response > 45 years of age: 40% response

Immunologic Drug-Induced Thrombocytopenia

Another form of immune thrombocytopenia is **drug-induced thrombocytopenia**. The list of drugs that are capable of causing thrombocytopenia through immune mechanisms is quite extensive. Some of the more commonly implicated drugs include quinine, quinidine, digitoxin, gold, thiazides, salicylates, and various sulfa drugs.

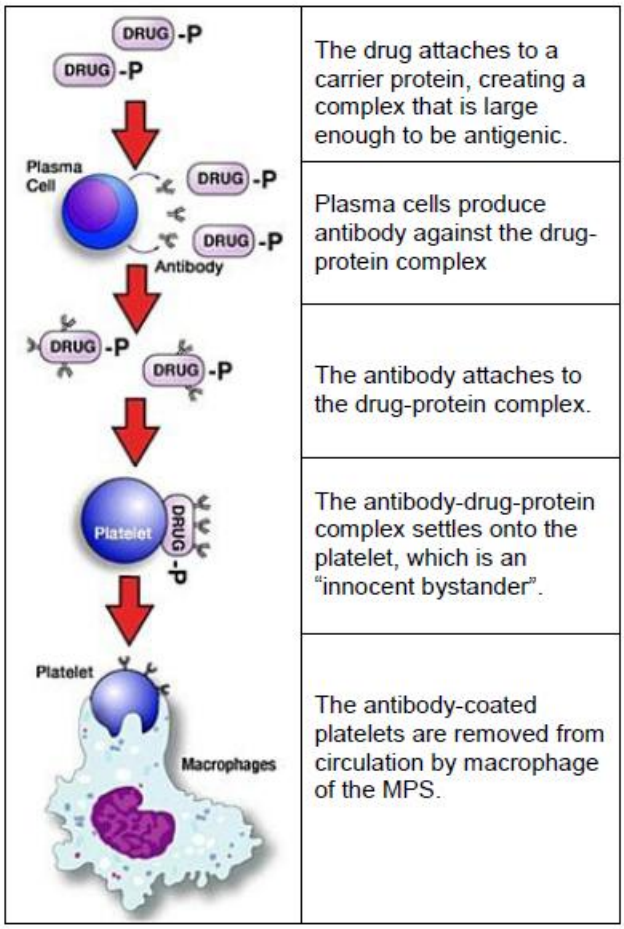
There are a variety of mechanisms by which antibody formation and subsequent immune destruction of platelets occurs. Because most drugs are too small to be antigenic, the first step in any of the mechanisms is for the drug to bind with a larger structure to produce a complex that is big enough to elicit antibody production. The structure may be a carrier protein present in the blood or may be the platelets themselves.

By one mechanism, the drug first attaches to a carrier protein in the blood.

The drug-protein complex stimulates the production of an antibody which then attaches to the complex.

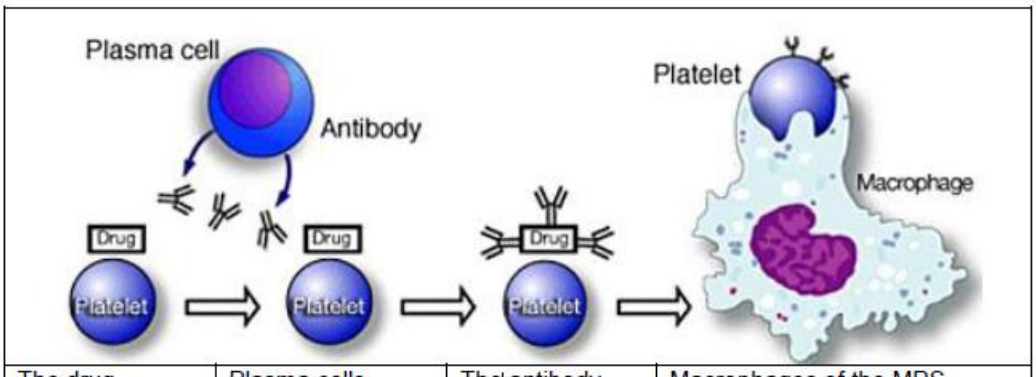
The circulating drug-protein-antibody complex attaches to the surface of the platelet, which is the "innocent bystander".

The antibody-coated platelet will be removed and destroyed by the spleen, or, if the antibody activates complement, intravascular lysis of the platelet occurs.



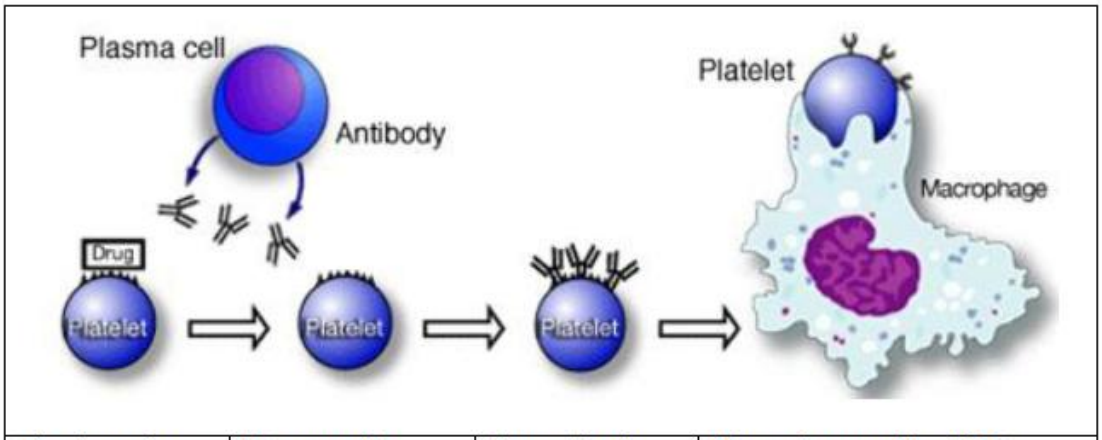
	The drug attaches to a carrier protein, creating a complex that is large enough to be antigenic.
	Plasma cells produce antibody against the drug-protein complex
	The antibody attaches to the drug-protein complex.
	The antibody-drug-protein complex settles onto the platelet, which is an "innocent bystander".
	The antibody-coated platelets are removed from circulation by macrophage of the MPS.

Another mechanism involves the binding of the drug to the platelet. The drug-platelet complex is antigenic and stimulates antibody production. The antibody attaches to the antigen on the platelet surface. The antibody-coated platelet is removed and destroyed by the spleen.



The drug attaches to the platelet.	Plasma cells produce antibody against the drug-coated platelet.	The antibody attaches to the drug-coated platelet.	Macrophages of the MPS remove coated platelets from circulation.

A third mechanism involves the platelet surface being altered by the drug. The altered platelet stimulates the production of antibody. The antibody attaches to the surface of the platelet, and the antibody-coated platelet is removed and destroyed by the spleen.



The drug alters the platelet surface.	Plasma cells produce antibody against the altered platelet.	The antibody attaches to the altered platelet.	Macrophages of the MPS remove antibody-coated platelets from circulation.

Did You Know?

Heparin, a drug used for the prevention and treatment of thrombosis, can cause thrombocytopenia in some patients. This condition, called heparin-induced thrombocytopenia or HIT, occurs in up to 5% of patients receiving unfractionated heparin and in up to 1% of patients receiving low molecular weight heparin. The mechanism by which thrombocytopenia develops is immunologic in nature. First, the heparin induces antibody formation. One end of the anti-heparin antibody attaches to the heparin molecule, while the other end of the antibody attaches to the platelet. The spleen destroys the antibody-coated platelets. The platelet counts in affected individuals may drop as low at 50 x 10^3/uL. The antibody also causes thrombosis in some patients by causing platelet activation and the formation of microvascular thrombi, which can be life-threatening.

PLATELET DESTRUCTION BY NON-IMMUNE MECHANISMS (NON-IMMUNE THROMBOCYTOPENIA)

There are three disorders in which thrombocytopenia develops because platelets are being destroyed or consumed in clots. Collectively, these three disorders are referred to as the **consumption coagulopathies**. In two of these disorders, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), clot formation is platelet mediated. This means that activation of platelets results in clots that are composed largely of platelets. In the third disorder, disseminated intravascular coagulation (DIC), clot formation is coagulation factor mediated. This means that something activates the coagulation factors, resulting in clots composed largely of fibrin. In DIC, platelets are consumed secondarily as they get caught up in the fibrin strands.

Hemolytic Uremic Syndrome (HUS)
Hemolytic uremic syndrome (HUS) occurs predominantly in children following an episode of gastroenteritis (especially E. coli or Shigella), upper respiratory infection, urinary tract infection, or viral disease (e.g., varicella or measles). Toxins produced by the microorganisms cause damage to the vascular endothelium, particularly of the kidney and, to a lesser extent, the central nervous system, heart, and liver. The damaged endothelium releases substances that activate and aggregate platelets, resulting in the formation of platelet thrombi in the microvasculature of the affected organs. The platelet-mediated clots are responsible for the major clinical findings associated with HUS, including acute renal failure, variable central nervous system symptoms, thrombocytopenia (due to platelet consumption), and microangiopathic hemolytic anemia (due to fragmentation of red blood cells as they encounter intravascular thrombi).

Thrombotic Thrombocytopenic Purpura (TTP)
Thrombotic thrombocytopenic purpura (TTP) is an acute disease that affects young adults. The clinical findings are similar to those found in HUS, including thrombocytopenia, renal dysfunction, neurologic abnormalities (more common than in HUS), and microangiopathic hemolytic anemia. As with HUS, these clinical findings are due to the presence of small intravascular clots composed predominantly of platelets. The primary stimulus that causes platelet activation and aggregation is unknown.

Disseminated Intravascular Coagulation (DIC)
Disseminated intravascular coagulation (DIC) differs from TTP and HUS in that clot formation is coagulation factor mediated as opposed to being platelet mediated. In this disorder, something activates the coagulation cascade, resulting in fibrin formation. While DIC is considered a thrombotic disorder, it ultimately transitions to a bleeding disorder due to the following events.

- Thrombocytopenia develops as platelets are consumed as they get caught in the fibrin strands.

- Coagulation factors are consumed as clot formation progresses, ultimately resulting in prolonged prothrombin time, prolonged activated partial thromboplastin time, and decreased fibrinogen.

- In response to clot formation, the fibrinolytic system is activated. Increased clot breakdown will result in increased levels of fibrin breakdown products (FDPs and D-dimers) in the peripheral blood. Fibrin breakdown products impede blood clotting by interfering with fibrin monomer polymerization and platelet aggregation.

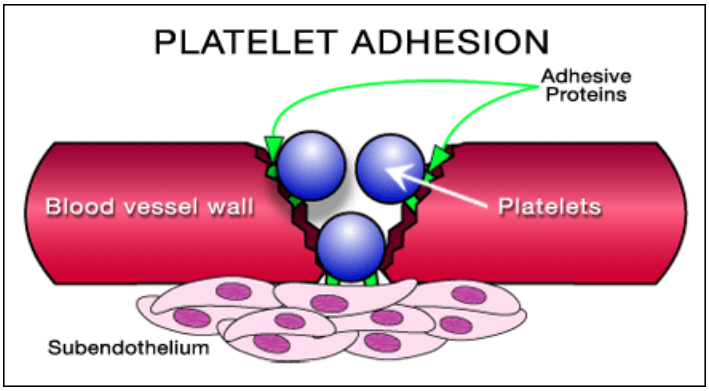
QUALITATIVE PLATELET DISORDERS

Qualitative platelet disorders are those in which there are problems with platelet function. Platelets perform a variety of hemostatic functions, including adhesion and aggregation to form a platelet plug, participation in the fibrin formation, and blood vessel maintenance. A platelet defect that affects any one of these functions will result in a bleeding disorder. As with vascular defects, the bleeding encountered in qualitative platelet disorders tends to be less severe in nature and occurs predominantly in the form of bleeding into the mucous

membranes or skin. Qualitative platelet disorders can be either hereditary or acquired. The hereditary disorders are characterized by defects in platelet adhesion, platelet aggregation, or secretion of granule contents.

HEREDITARY DISORDERS OF PLATELET ADHESION

When endothelial cells are damaged, platelets escape from the blood and flow into the subendothelium where they stick (adhere) to exposed connective tissue, particularly collagen. Adhesion occurs when any one of a number of “adhesive” proteins present in the subendothelium (including fibronectin, laminin, vitronectin, thrombospondin, and von Willebrand factor) bind to the collagen fiber and also to a receptor on the platelet surface to form a “bridge” that attaches the platelet to the collagen. There are many different receptors on the surface of the platelet, each of which binds to a particular type of adhesive protein.

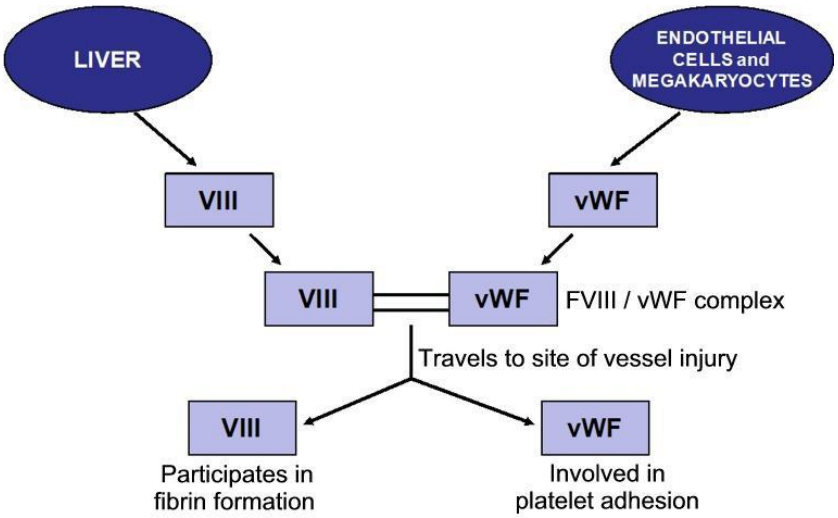


One of the most important of the adhesive proteins is von Willebrand factor (vWf). vWf binds to both collagen and to a special receptor on the platelet surface known as glycoprotein Ib/IX/V, causing the platelet to stick to the collagen. A deficiency or defect in either von Willbrand factor or the glycoprotein Ib/IX/V receptor will result in defective platelet adhesion. There are two hereditary disorders in which platelets fail to adhere to collagen by this mechanism. They are von Willebrand disease and Bernard-Soulier disease.

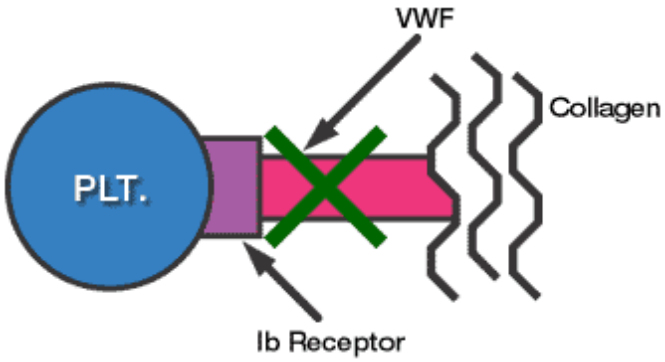
VON WILLEBRAND DISEASE

Description

von Willebrand disease is the most common inherited bleeding disorder in humans. It is caused by a deficiency of von Willebrand factor (vWF). vWF is produced by endothelial cells and is stored in the granules of endothelial cells and platelets as well as being secreted into the subendothelial spaces and into the bloodstream. In the bloodstream, it travels attached to factor VIII, a coagulation factor that participates in fibrin formation. When the large factor VIII complex arrives at the site of vessel injury, the two factors separate to perform their specialized hemostatic functions; factor VIII interacts with other coagulation factors to produce fibrin while vWF functions as an adhesive protein to facilitate platelet adhesion.



vWF actually serves as a bridge between the subendothelial collagen and the platelet by simultaneously binding to the collagen and to the glycoprotein Ib/IX/V receptor on the platelet surface. Platelet adhesion initiates a series of reactions that ultimately result in formation of the platelet plug.



In von Willebrand disease, a deficiency or defect of the von Willebrand factor (an adhesive protein) affects the ability of the platelets to adhere to the subendothelial surface.

In light of the role of vWF in platelet adhesion, it is apparent that a deficiency of vWF will result in problems with platelet adhesion. However, patients with von Willebrand disease also experience problems with fibrin formation. This is due to the fact factor VIII is also decreased. The decrease in VIII is not related to a decreased ability to produce this factor, but rather, to decreased survival time. Remember that VIII and vWf are produced separately but travel together in the bloodstream as a large factor VIII complex. The vWF portion of the complex is much larger than the C portion, and serves to protect the C portion from proteolysis. In von Willebrand disease, decreased production of vWF results in some of the factor VIII traveling alone in the blood stream. Factor VIII that is not attached to vWF has a decreased survival time.

Classification

von Willebrand disease is generally classified into 3 types (types I, II and III) based upon whether the defect is quantitative or qualitative in nature and, if qualitative, the exact nature of the structural defect. Types I and III are quantitative defects, with patients having decreased levels of normal vWF. Type I is characterized by moderate decreases in vWF while type III is characterized by marked decreases. Type II von Willebrand disease is qualitative in nature. Both types I and II have several different subtypes. In all, approximately 20 clinical variants of von Willebrand disease have been described. Most types are inherited as autosomal dominant traits while a few are autosomal recessive traits. The most severe bleeding occurs in type III and subtype 2B vonWillebrand disease.

Clinical Picture

A hallmark of von Willebrand disease is its clinical variability. Most cases are heterozygous and, as such, tend to be mild in nature with symptoms usually not appearing until the second decade of life. The occasional homozygous patient experiences more severe bleeding that begins earlier in life but may decrease with age.

Symptoms normally include mild bleeding into the skin and mucous membranes (e. g., nose bleeds, mouth and gum bleeding, and easy bruising), heavy menstrual bleeding in adolescent females, and occasional severe bleeding following surgery. Patients with more severe forms of the disease may experience bleeding, including hemarthroses and spontaneous deep tissue bleeding, that is generally more consistent with other inherited factor deficiencies.

Diagnostic Criteria

The laboratory findings in von Willebrand disease include:

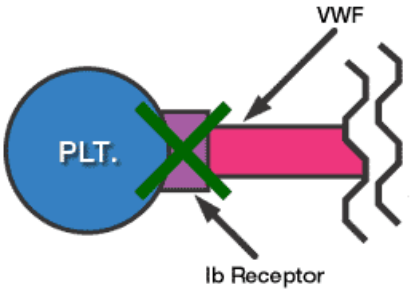
- Platelet aggregation is abnormal with ristocetin, but normal with ADP, collagen, and epinephrine. This aggregation pattern is consistent with platelet adhesion problems.
- Bleeding time is prolonged due to decreased platelet adhesion.
- The APTT is prolonged but the PT is normal. This is due to decreased levels of factor VIII which is involved in the intrinsic coagulation pathway (measured by the APTT) but not the extrinsic pathway (measured by the PT).
- An assay for factor VIII reveals moderately decreased levels. This is due to decreased survival of factor VIII rather than decreased production.
- Assays for factor vWF reveal either decreased levels of normal vWF or the presence of structurally abnormal vWF depending upon the classification of the disease. An enzyme immunoassay is performed to quantify vWF to identify quantitative defects (types I and III vWF disease). A ristocetin cofactor activity assay is performed to determine the factor's ability to bind to platelets and, as such, identifies qualitative defects (type II vWF disease).

Treatment

Patients with von Willebrand disease that experience mild bleeding usually require only local measures, such as limb elevation, pressure, and ice packs. Cases with moderate bleeding are commonly treated with drugs such as estrogen and DDAVP that trigger release of vWF from storage organelles. Commercial preparations containing vWF and factor VIII from human plasma fractionation are used to treat patients who experience severe bleeding.

BERNARD-SOULIER SYNDROME (GIANT PLATELET SYNDROME)

Description
Bernard-Soulier syndrome is inherited as an autosomal-recessive trait. In this disorder, platelets have an insufficient number of glycoprotein Ib/IX/V receptors or possess abnormal glycoprotein Ib receptors. Lack of functional glycoprotein Ib/IX/V receptors prevents interaction with von Willebrand factor and subsequent adhesion to collagen.



In Bernard-Soulier syndrome, a deficiency or defect of the glycoprotein Ib/IX/V receptors of the platelet affects the ability of vWf to bind to the platelet. This, in turn, affects the ability of platelets to adhere to the **subendothelial surface.**

Clinical Picture
Symptoms normally include mild bleeding into the skin and mucous membranes (e. g., nose bleeds, mouth and gum bleeding, and easy bruising), heavy menstrual bleeding, and excessive bleeding following surgery. Symptoms appear early in life and tend to decrease with age.

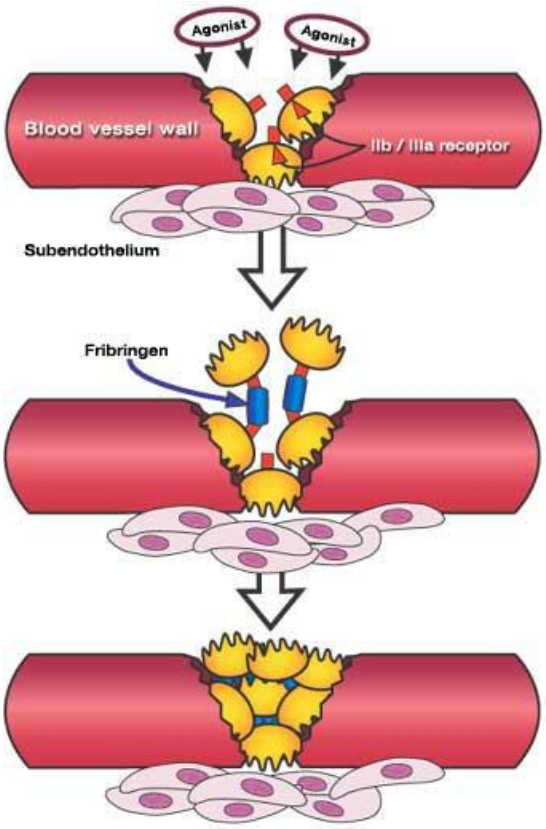
Diagnostic Criteria
The laboratory findings in Bernard-Soulier syndrome are as follows:

- The platelet count is normal or slightly decreased and is variable from time to time in the same patient.
- Platelet morphology is abnormal with more than 60% of platelets being increased in size (between 2.5 and 8 microns in diameter) and having increased numbers of dense granules.
- Platelet aggregation is abnormal with ristocetin and botrocetin, but normal with ADP, collagen, arachadonic acid, and epinephrine. This aggregation pattern is consistent with platelet adhesion problems.
- Bleeding time is prolonged due to decreased platelet adhesion.
- Clot retraction is normal.

Treatment
Treatment for the patient with Bernard-Soulier syndrome is supportive in nature. Blood transfusions are administered as needed following uncontrolled hemorrhage. Iron therapy will be given to the patient who develops iron deficiency anemia due to chronic blood loss. Platelet transfusions can be given if the platelet count drops dramatically, but should be used judiciously to avoid antibody production against the glycoprotein Ib/IX/V receptor (alloimmunization).

HEREDITARY DISORDERS OF PLATELET AGGREGATION

Following adhesion, platelets must be activated before they can aggregate. Platelet activation occurs in response to substances called agonists that are released from cells of the injured tissue and from the granules of adherent platelets. The agonists cause the platelet to undergo a morphologic change, resulting in the appearance of the glycoprotein receptor IIb /IIIa on the platelet surface. Once the IIb /IIIa receptor appears on the surface of the activated platelet, fibrinogen that is present in the plasma and has also been released from the granules of adherent platelets binds to receptors on adjacent platelets, forming a "bridge" that holds them together. This is platelet aggregation.



Following platelet adhesion, agonists released from injured tissue and platelet granules cause a morphologic change resulting in the appearance of IIb/IIIa receptor on platelet surface.

Fibrinogen binds to IIb/IIIa receptors on adjacent platelets.

Platelet aggregation results in the formation of the platelet plug.

Platelet aggregation actually occurs in two phases: primary aggregation and secondary aggregation.

Primary aggregation occurs in response to initial exposure to an agonist, mainly ADP that is released from the adherent platelets. During this phase of aggregation, fibrinogen binding is reversible and platelets are only loosely aggregated. Therefore, they may disaggregate, preventing secondary aggregation from occurring. Upon exposure to additional agonists, mainly ADP, serotonin, and thromboxane A2 released from the platelets involved in primary aggregation, the binding of fibrinogen becomes irreversible and the platelets become more tightly aggregated. This is **secondary aggregation**.

From this description, it is clear to see that both fibrinogen and the platelet IIb/IIIa receptor play important roles in platelet aggregation. In the absence of one or more of these components, platelet aggregation will be abnormal. Two hereditary disorders fall into this category: Glanzmann's thrombasthenia and afibrinogenemia.

GLANZMANN'S THROMBASTHENIA

Glanzmann's thrombasthenia is a rare autosomal recessive disorder of platelet function associated with an absence or deficiency of the platelet IIb/IIIa receptor. As a result, platelets cannot bind fibrinogen and will not aggregate. The clinical picture of this disorder is quite variable, with some patients experiencing only minor bleeding and bruising and other patients experiencing life-threatening hemorrhage. Laboratory findings are as follows:

- Platelet count and morphology are normal.
- The bleeding time is markedly prolonged, indicating platelet dysfunction.
- Clot retraction is abnormal.
- Platelet aggregation is abnormal with ADP, collagen, and epinephrine, but normal with ristocetin. This aggregation pattern is consistent with platelets that are able to adhere normally (normal platelet adhesion) but have an impaired ability to aggregate.

AFIBRINOGENEMIA

Fibrinogen serves as the "bridge" that attaches to the IIb/IIIa receptors on adjacent platelets, resulting in platelet aggregation. Therefore, it is logical that lack of fibrinogen, or afibrinogenemia, is characterized by platelet aggregation problems. The bleeding time in patients with afibrinogenemia will be prolonged, and platelet aggregation studies will be abnormal with ADP, epinephrine, and collagen. In addition, tests that monitor the coagulation cascade, including the PT and APTT, will be prolonged.

HEREDITARY DISORDERS OF PLATELET SECRETION

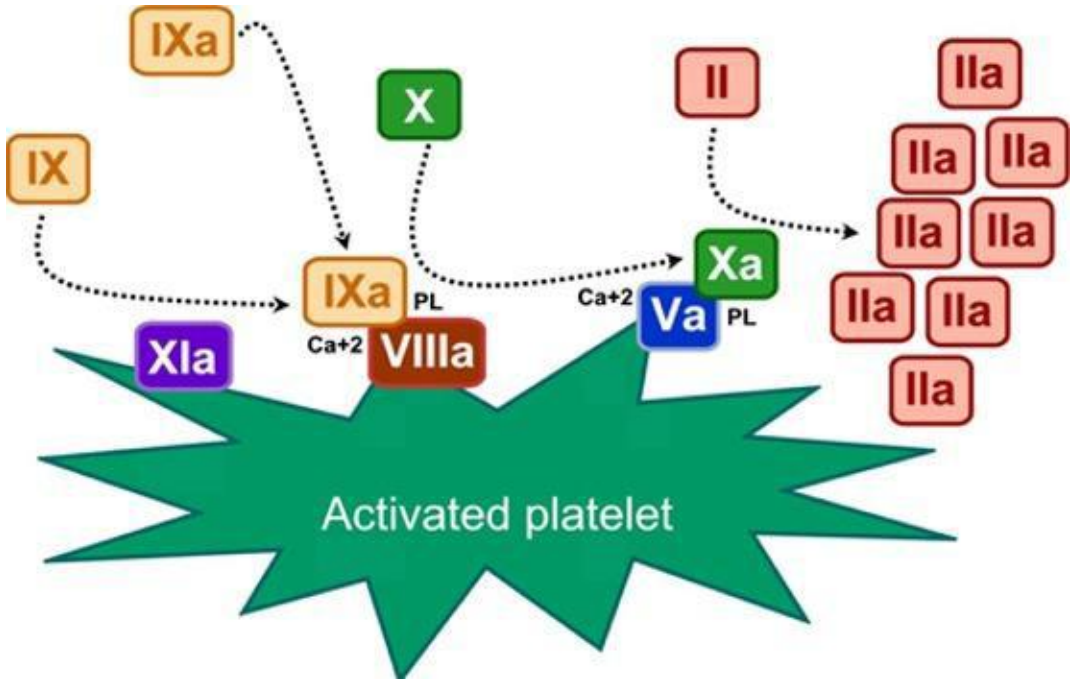
Platelet granules contain many substances that are necessary for proper platelet aggregation. These substances, called agonists, are released from platelet granules as a result of the morphologic changes that occur during adhesion. ADP, serotonin, and thromboxane A_2 are types of agonists. Conditions characterized by a deficiency of any of these substances within the platelet or an inability of the platelet to release these substances will result in abnormal platelet aggregation.

Conditions known as **storage pool diseases** fall in this category. Two types of disorders are classified as storage pool diseases: **dense granule deficiencies** and **gray platelet syndrome**. In dense granule deficiencies, platelets show a decrease or absence of dense bodies on electron morphology. Platelet morphology on the peripheral blood smear, however, appears normal. The bleeding time is prolonged and platelet aggregation studies are abnormal with 19
ADP, epinephrine, and collagen. Dense granule deficiencies can occur as an isolated problem and can also seen in association with several disorders, such as Chediak-Higashi syndrome, Hermansky-Pudlak syndrome, and Wiskott-Aldrich syndrome. In **gray platelet syndrome**, platelets lack alpha granules. Because alpha granules are normally numerous, their absence causes platelets to appear agranular and gray in color. Patients generally present with mild bleeding symptoms. Typical laboratory findings include thrombocytopenia, prolonged bleeding time, and nonspecific abnormal platelet aggregation studies.

An **enzymatic defect in the thromboxane A_2 synthesis pathway** is another disorder of platelet secretion. Thromboxane A_2 is an agonist that is produced and released from platelet granules following primary aggregation. Therefore, an inability to synthesize thromboxane A_2 results in abnormal secondary platelet aggregation.

HEREDITARY DISORDERS OF PLATELET MEMBRANE RECEPTORS AND PROCOAGULANT ACTIVITY

In addition to their roles in primary hemostasis and formation of the platelet plug, platelets also participate in secondary hemostasis, which involves the formation of the fibrin mesh that will stabilize and firmly anchor the platelet plug to the vessel wall. Activated platelets in the plug provide the phospholipid surface where the coagulation factors interact to produce thrombin (factor IIa), the proteolytic enzyme that is responsible for converting fibrinogen to fibrin. This capacity of the activated platelets to catalyze the coagulation process by providing phospholipid surfaces is known as **platelet factor 3** or **platelet procoagulant activity**.



Platelets in the platelet plug provide the phospholipid surface where the coagulation factors interact to produce thrombin (factor IIa), the proteolytic enzyme that is responsible for converting fibrinogen to fibrin.

ACQUIRED DISORDERS OF PLATELET FUNCTION

Abnormal platelet function is induced in a variety of conditions and in response to certain drugs.

UREMIA

Renal insufficiency results in accumulation of waste products that adversely affect platelet function.

HEMATOLOGIC DISORDERS

A variety of hematologic disorders, including myeloproliferative disorders and myelodysplastic syndromes, are characterized by the production of abnormal blood cells. If the disorder affects the stem cells responsible for platelet production, platelets will be affected. Disorders characterized by excessive production of abnormal proteins, including multiple myeloma and Waldenstrom's macroglobulinemia, are characterized by abnormal platelet function because the abnormal proteins coat the platelet surface and interfere with membrane reactions of platelet stimulation.

DRUGS

Many drugs have been shown to contribute to platelet dysfunction. Some antibiotics alter platelet function. It is believed that the antibiotic coats the platelet membrane and blocks ADP and epinephrine receptors, thereby interfering with the platelets ability to respond to these agonists. Aspirin interferes with the thromboxane A_2 synthesis pathway by irreversibly altering a pathway enzyme. Although aspirin is cleared from the blood rapidly (within 30 minutes), platelet function is defective for the life of the platelet. Alcohol ingestion over a long period of time may contribute to platelet dysfunction. Several mechanisms have been proposed including inhibition of prostaglandin synthesis and alteration of granule contents.

BLEEDING DISORDERS DUE TO DEFECTS IN SECONDARY HEMOSTASIS

I. Inherited factor deficiencies
    A. Von Willebrand Disease
        1. Description
        2. Classification
        3. Clinical picture
            a) Type 1
            b) Type 2
            c) Type 3
        4. Diagnostic criteria
        5. Treatment
            a) Type 1 vWD
            b) Type 2 and 3 vWD
    B. Hemophilia A
        1. Description
        2. Clinical picture
        3. Diagnostic criteria
        4. Treatment
    C. Hemophilia B
        1. Description
        2. Clinical picture and diagnostic criteria
        3. Treatment
    D. Other inherited factor deficiencies
        1. Fibrinogen (factor I)
        2. Prothrombin (factor II)
        3. Factor V
        4. Factor X
        5. Factor XI
        6. Factor XII
        7. Factor XIII
II. Acquired factor deficiencies
    A. Vitamin K deficiency
    B. Liver disease
    C. Disseminated intravascular coagulation (DIC)
        1. Definition
        2. Etiology
        3. Clinical manifestations
        4. Laboratory findings
III. Acquired pathological coagulation inhibitors
    A. Specific inhibitors
    B. Non-specific inhibitors

BLEEDING DISORDERS DUE TO DEFECTS IN SECONDARY HEMOSTASIS

Secondary hemostasis involves the interactions of coagulation factors to form fibrin. Deficiencies or defects of the coagulation factors result in insufficient fibrin formation and ultimately bleeding. Factor deficiencies or defects can be either inherited or acquired. In inherited conditions, a defect in a gene that regulates the synthesis of a particular factor results in either insufficient production of the factor (quantitative defect) or production of a nonfunctional molecule (qualitative defect). Inherited conditions usually involve only one coagulation factor. Acquired factor deficiencies, on the other hand, occur in response to another condition that occurs during the lifetime of the individual. They are more common than the inherited conditions and frequently result in multiple factor deficiencies. The bleeding associated with factor deficiencies tends to be more severe than the bleeding associated with disorders of primary hemostasis (vascular and platelet defects), and is usually related to the level of factor. Symptoms usually include:

- Deep tissue bleeding, including bleeding into the joints (hemarthrosis).
- Deep intramuscular bleeding.
- Intracranial bleeding.
- Moderate mucosal bleeding (e.g., gastrointestinal tract, genitourinary tract, intrapulmonary, etc.)

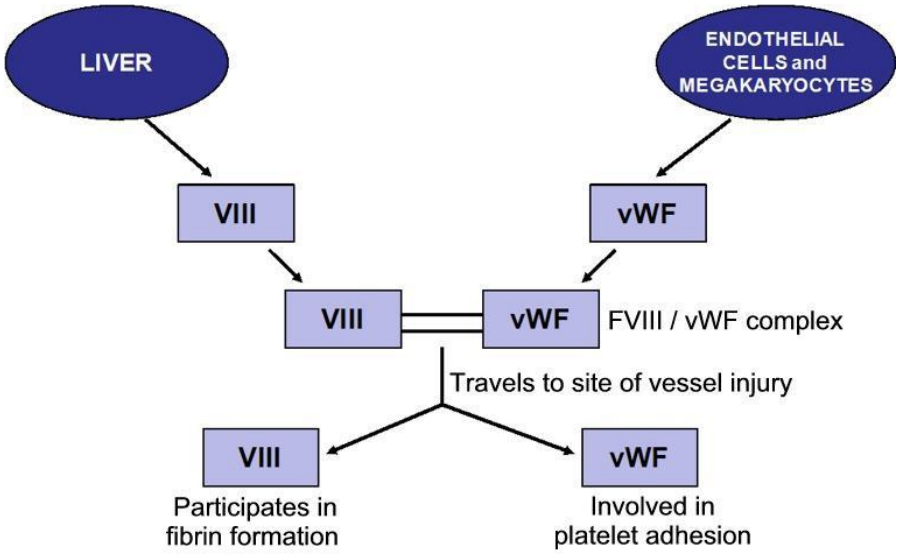
INHERITED FACTOR DEFICIENCIES

Inherited factor deficiencies can be due to decreased or absent production of functionally normal factor (quantitative defect) or production of abnormally functioning factor (qualitative defect). Deficiencies have been described for each of the factors of the coagulation cascade. However, the most common hereditary disorders are hemophilia A (defect or deficiency of factor VIII:C), von Willebrand disease (defect or deficiency of factor VIII:vWf), and Christmas disease (defect or deficiency of factor IX).

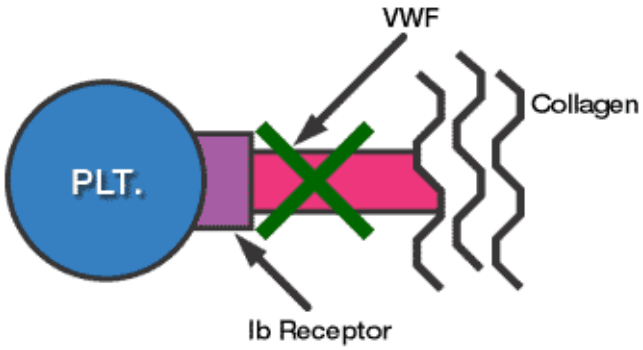
VON WILLEBRAND DISEASE

DESCRIPTION

von Willebrand disease is the most common inherited bleeding disorder in humans. It is caused by a deficiency of von Willebrand factor (vWF). vWF is produced by endothelial cells and is stored in the granules of endothelial cells and platelets as well as being secreted into the subendothelial spaces and into the bloodstream. In the bloodstream, it travels attached to factor VIII, the coagulation factor that interacts with other coagulation factors to form fibrin. When the large factor VIII complex arrives at the site of vessel injury, the two factors separate to perform their specialized hemostatic functions; factor VIII participates in the fibrin formation while vWF functions as an adhesive protein to facilitate platelet adhesion.



vWF actually serves as a bridge between the subendothelial collagen and the platelet by simultaneously binding to the collagen and to the glycoprotein Ib/IX/V receptor on the platelet surface. Platelet adhesion initiates a series of reactions that ultimately result in formation of the platelet plug.



In von Willebrand disease, a deficiency or defect of the von Willebrand factor (an adhesive protein) affects the ability of the platelets to adhere to the subendothelial surface.

In light of vWF's role in platelet adhesion, it is apparent that a deficiency of vWF will result in problems with platelet adhesion. However, patients with von Willebrand disease also experience problems with the coagulation cascade and fibrin formation. This is due to the fact that levels of factor VIII are also decreased. The decrease in VIII is not related to a decreased ability to produce this factor, but rather, to decreased survival time. Remember that factor VIII and vWf are produced separately but travel together in the bloodstream as a large factor VIII complex. The vWF portion of the complex is much larger than the factor VIII portion, and serves to protect the factor VIII portion from harm. In von Willebrand disease, decreased production of vWF results in some of the factor VIII traveling alone in the blood stream. Factor VIII that is not attached to vWF has a decreased survival time.

CLASSIFICATION

von Willebrand disease is generally classified into 3 types (types 1, 2, and 3) based upon whether the defect is quantitative or qualitative in nature, and, if qualitative, the exact nature of the structural defect. Types 1 and 3 are quantitative defects, with patients having decreased levels of normal vWF. Type 1is characterized by moderate decreases in vWF and is seen in more than 70% of patients with von Willebrand disease. Type 3 is characterized by marked decreases of vWF. Type 2 von Willebrand disease is qualitative in nature. There are several subtypes within the type 2 classification. The most significant of these subtypes are subtypes 2A and 2B. In subtype 2A, the abnormality renders the vWF more susceptible to proteolysis, resulting in smaller molecules which have lower activity than larger forms. 10% to 20% of patients with von Willebrand disease have subtype 2A. In subtype 2B, the abnormality increases the affinity of vWF to platelet glycoprotein receptor Ib/IX/V. vWF binds resting platelets and is unavailable for normal platelet adhesion.

Most types of von Willebrand disease are inherited as autosomal dominant traits while a few are autosomal recessive traits.

CLINICAL PICTURE

TYPE 1

Most people with type 1 von Willebrand disease display very mild symptoms. In fact, many type 1 individuals do not realize that they have the disease until another family member is diagnosed. Symptoms are variable from patient to patient and include easy bruising, recurrent nosebleeds, mucocutaneous hemorrhage, and prolonged posttraumatic bleeding. In women suffering from types 1 von Willebrand disease, the most frequent symptom is menorrhagia. Pregnancy within women suffering from type 1 is well tolerated since plasma levels of von Willebrand factor and factor VIII increase during pregnancy.

TYPE 2

Individuals with type 2 von Willebrand disease usually have symptoms from early childhood; symptoms may even be present at birth. They usually experience prolonged bleeding from cuts, easy bruising, nose bleeds, skin hematomas, and prolonged bleeding from the gums following teeth extraction and minor trauma. More than 50% of women with type 2 von Willebrand experience heavy periods that may require blood transfusion. Gastrointestinal bleeding is rare but can be life-threatening. Some women with type 2 von Willebrand disease exhibit prolonged bleeding during delivery.

TYPE 3

Individuals with type 3 von Willebrand disease have more serious symptoms, including spontaneous mucous membrane and gastrointestinal bleeding, bleeding into the joints, and deep tissue bleeding. These bleeding episodes can be life threatening. For women suffering from this type and for those people whom have just undergone surgery, bleeding is controlled by hormone replacement therapy.

DIAGNOSTIC CRITERIA

- Platelet aggregation is abnormal with ristocetin, but normal with ADP, collagen, and epinephrine. This aggregation pattern in consistent with problems with platelet adhesion.
- Bleeding time is prolonged due to decreased platelet adhesion.
- The APTT is prolonged but the PT is normal. This is due to decreased levels of factor VIII, which is involved in the intrinsic coagulation pathway (measured by the APTT) but not the extrinsic pathway (measured by the PT).
- An assay for factor VIII reveals moderately decreased levels. This is due to decreased survival of factor VIII rather than decreased production.
- Assays for factor vWF reveal either decreased levels of normal vWF or the presence of structurally abnormal vWF depending upon the classification of the disease.
- Additional testing, including DNA sequencing and polymerase chain reaction (PCR), can be performed to identify the genetic mutation that is causing the disease. Information obtained by this type of testing is valuable in determining disease classification which, in turn, dictates the type of treatment that will be administered.

TREATMENT

Treatment varies with disease classification.

FOR TYPE 1 VON WILLEBRAND DISEASE

The most common treatment for type 1 von Willebrand disease is desmopressin (DDAVP), a synthetic copy of antidiuretic hormone that raises the level of von Willebrand factor in the blood by releasing it from endothelial storage sites. This drug has no value in the treatment of type 3 vonWillebrand disease (due to the fact that there is little or no stored vWf to be released) and limited value in the treatment of patients with type 2B (due to the fact that the vWf is this form of the disease is structurally defective).

Cyklokapron (tranexamic acid) and Amicar (aminocaproic acid) can be used as supplements to desmopressin. These drugs suppress the activity of plasmin, the enzyme that dissolves blood clots, and thus allow a formed clot to remain in place longer. Since Cyklokapron and Amicar do not help to actually form a clot, they cannot be used in place of other drugs, such as desmopressin or vWF concentrate, that induce clot formation.

Hormonal treatments, particularly estrogen therapy, have been used to treat some patients, particularly females who experience heavy menstrual bleeding. The mechanism by which hormones increase levels increase levels of von Willebrand factor and factor C is not known.

FOR TYPES 2 AND 3 VON WILLEBRAND DISEASE

Factor VIII concentrate with Von Willebrand factor in high molecular weight form is the treatment of choice for type 3, some forms of type 2, and for all types during episodes of serious bleeding or following major surgery. This concentrate, which replaces the missing vWF in the blood long enough to allow clotting to take place, is made from pooled human plasma that has been screened for bloodborne pathogens, such as HIV, hepatitis B, and hepatitis C, and then pasteurized to destroy any remaining viruses. It is administered intravenously. It is imperative that the product used contain vWF of the high molecular weight form in order to be effective. Unfortunately, most available factor VIII concentrates do not contain sufficient high molecular weight vWF to be used to treat von Willebrand disease. In the past, cryoprecipitate, another blood component made from plasma that contains both factor VIII and vWF, was commonly used to treat von Willebrand disease.

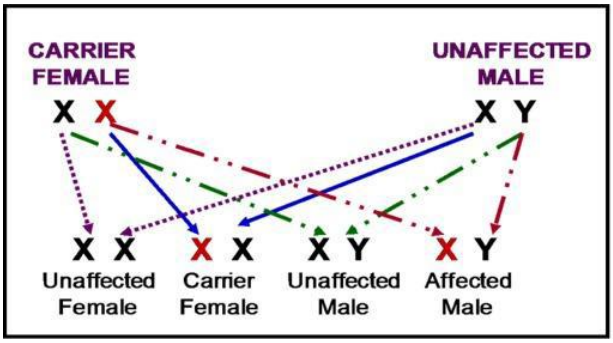
However, because there is no effective method to kill viruses in cryoprecipitate, it is no longer recommended.

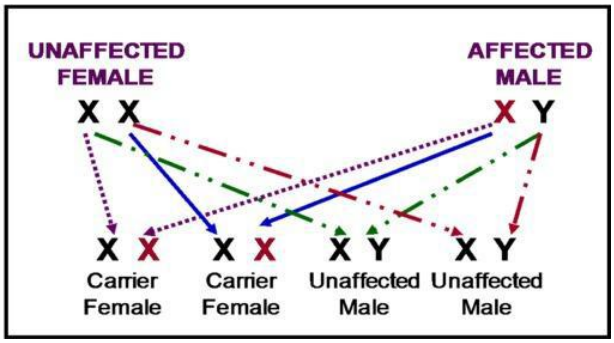
In all forms of the disease, bleeding in the mouth or nose can be stopped by applying powder-form thrombin directly to the bleeding site.

HEMOPHILIA A

DESCRIPTION

Hemophilia A results from a deficiency of factor VIII. It is inherited as a sex-linked recessive trait. This means that the gene controlling production of factor VIII is located at the terminal end of the long arm of the X chromosome. As such, females are carriers of the disease. Female carriers usually have approximately 50% of factor VIII activity and, therefore, do not usually exhibit clinical bleeding. Carrier women produce sons with the disease and daughters who are obligatory carriers of the disease. Sons of affected men are not affected (because they pass along the unaffected Y chromosome to their sons), but their daughters are obligatory carriers (because they pass along the affected X chromosome to their daughters).





Hemophilia A can be caused by any one of a variety of defects in the gene controlling factor VIII production. The severity of the disease depends upon the level of factor VIII activity that remains. Factor VIII activity varies with the type of genetic mutation and the molecular function that is disrupted by the mutation.

CLINICAL PICTURE

Symptoms of hemophilia A include:

- Deep tissue bleeding, including bleeding into the joints (hemarthrosis).
- Deep intramuscular bleeding.
- Intracranial bleeding.
- Hematuria.
- Moderate mucosal bleeding (e.g., gastrointestinal tract, genitourinary tract, intrapulmonary, etc.)

Severity of symptoms varies with the level of factor VIII activity.

- Less than 1% factor activity results in severe hemophilia with spontaneous bleeding.
- Between 1% and 5% factor activity results in moderate hemophilia characterized by excessive bleeding following trauma.
- Between 5% and 20% factor activity results in mild hemophilia with moderate bleeding during surgery or following trauma.

DIAGNOSTIC CRITERIA

Hemophilia A should be suspected if a male child with a family history of bleeding presents with a prolonged APTT in conjunction with a normal PT. A factor VIII assay should be performed to confirm a decreased level of factor activity. Since the bleeding associated with hemophilia A is due solely to decreased factor VIII activity, coagulation tests that monitor other aspects of hemostasis, such as platelet number and function, vascular integrity, and other coagulation factors, will be normal. One such test is the bleeding time. It is important to remember that the bleeding time, when properly performed, is dependent upon platelet function (adhesion and aggregation) and, to a lesser degree, vascular integrity. It is not dependent upon the coagulation factors and fibrin formation. For this reason, the bleeding time, when properly performed, will be normal for the patient with hemophilia A.

TREATMENT

Mild hemophilia A may be treated with desmopressin (DDAVP), which helps the body release factor VIII that is stored in endothelial cells that line the blood vessels. More severe cases of hemophilia A require treatment with concentrates of human plasma such as antihemophilic factor (AHF) or purified, heat-treated lyophilized preparations of factor VIII concentrate. Under normal circumstances when the hemophilia patient is not experiencing or suspecting a bleeding episode, prophylactic doses of factor VIII concentrate are administered to maintain the level of factor activity above 1% of normal. Due to the relatively short half-life of factor VIII (approximately 8 to 12 hours), infusions are required at least twice per day to maintain this level of factor activity. When a hemostatic challenge such as a surgical procedure is anticipated, additional factor VIII concentrate is administered. The factor VIII activity level achieved depends upon the nature of the bleeding, but is seldom greater than 70%. The level should remain high until the threat of bleeding is resolved.

As a result of repeated treatment with factor VIII concentrate, 10% to 20% of patients develop antibodies or inhibitors that are capable of neutralizing human factor VIII. If the inhibitor level is low, administering larger doses of factor VIII will control bleeding. Patients with high levels of inhibitor are treated with plasma-derived concentrates of activated vitamin-K dependent factors, called **activated prothrombin complex concentrates**. These complexes generate thrombin in the presence of factor VIII inhibitors. Patients with inhibitors may also be treated with recombinant activated factor VII (FVIIa), which bypasses factor VIII and promotes thrombin formation through the tissue factor pathway.

HEMOPHILIA B

DESCRIPTION
Hemophilia B, also known as Christmas disease, results from a deficiency of factor IX. Like hemophilia A, it is a sex-linked recessive trait. Therefore, females are rarely affected by the disease, but serve as carriers who pass the disease to their sons.

CLINICAL PICTURE AND DIAGNOSTIC CRITERIA
Clinically, hemophilia B is similar to hemophilia A, with the severity of symptoms being related to the level of factor IX activity. Laboratory results are also similar to those seen in hemophilia A.

TREATMENT
Hemophilia B is treated with purified plasma-derived factor IX concentrates. Since factor IX has a longer half-life than factor VIII, treatment for hemophilia B is administered less frequently than treatment for hemophilia A; repeat doses of factor IX concentrate are given every 24 hours. Therefore, the incidence of developing an antibody or inhibitor to factor IX is only 2% to 3%.

LABORATORY EVALUATION OF VON WILLEBRAND DISEASE AND X-LINKED RECESSIVE DISORDERS

Laboratory Test	Von Willebrand Disease	X-Linked Recessive Disorders	
		Hemophilia A (Factor VIII Deficiency)	Hemophilia B (Factor IX Deficiency)
Platelet Tests:			
Platelet Count	Normal	Normal	Normal
Bleeding Time	Prolonged	Normal	Normal
Platelet Aggregation			
• ADP	Normal	Normal	Normal
• Collagen	Normal	Normal	Normal
• Epinephrine	Normal	Normal	Normal
• Ristocetin	Absent	Normal	Normal
Coagulation Factor Tests:			
PT	Normal	Normal	Normal
APTT	Increased	Increased	Increased
Factor VIII assay	Decreased	Decreased	Normal
Factor IX assay	Normal	Normal	Decreased

OTHER INHERITED FACTOR DEFICIENCIES
FIBRINOGEN (FACTOR I)
There are three forms of inherited fibrinogen deficiency: afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia. Both afibrinogenemia and hypofibrinogenemia are quantitative defects, meaning that insufficient amounts of structurally normal fibrinogen are produced. Dysfibrinogenemia, on the other hand, is a qualitative defect, meaning that structurally abnormal fibrinogen is produced.

The characteristics of each of these disorders are summarized in the table below. As you review the laboratory results for these disorders, remember that many of the more commonly performed coagulation tests, including the PT, APTT, and thrombin time, are clot-based assays. This means that the endpoint of the test is dependent upon the formation of a fibrin clot. Therefore, these tests will be abnormal (prolonged) when there is insufficient fibrinogen to produce a fibrin clot.

You should also remember that fibrinogen is required for platelet aggregation. Therefore, the bleeding time, which is most dependent upon proper platelet function (adhesion and aggregation) will be prolonged in disorders with very low fibrinogen levels, as will platelet aggregation studies.

DISORDER	MODE OF INHERITANCE	NATURE OF DEFECT	CLINICAL PICTURE	LABORATORY RESULTS
Afibrinogenemia	Autosomal recessive – Homozygous	Quantitative: Fibrinogen <5 mg/dL	At birth, bleeding from umbilical cord. Joint bleeds are rare. Moderate to severe bleeding following trauma.	PT, APTT and thrombin clotting time are abnormal. Bleeding time is prolonged. Platelet aggregation abnormal with epinephrine and ADP.
Hypofibrinogenemia	Autosomal recessive – Heterozygous	Quantitative: Fibrinogen between 20 and 100 mg/dL	Few, if any, bleeding symptoms.	PT, APTT and thrombin clotting time are abnormal if fibrinogen is <100 mg/dL, but normal if fibrinogen is >100 mg/dL.
Dysfibrinogenemia	Autosomal dominant	Qualitative: Over 300 abnormal fibrinogens have been identified.	Most patients (>50%) are asymptomatic. If present, bleeding occurs following trauma.	Only thrombin clotting time and reptilase time are abnormal (prolonged).

PROTHROMBIN (FACTOR II)

Inherited factor II deficiency is an extremely rare condition. Both quantitative and qualitative disorders have been identified. The severity of clinical symptoms is related to the amount of functional prothrombin. Homozygous patients experience severe bleeding following trauma or surgery in addition to menorrhagia, hematuria, bruising, and epistaxis. Heterozygous patients experience only epistaxis and bleeding following tooth extraction. Because factor II is involved in the common coagulation pathway, both the PT and APTT values are prolonged.

FACTOR V

The incidence of factor V deficiency, also called parahemophilia, is extremely rare; it affects only 1 in one million individuals. It is a relatively mild hemorrhagic disorder that is characterized by post-traumatic bruising, menorrhagia, epistaxis, and bleeding from mucous membranes. Because factor V is involved in the common coagulation pathway, both the PT and APTT values are prolonged.

FACTOR X

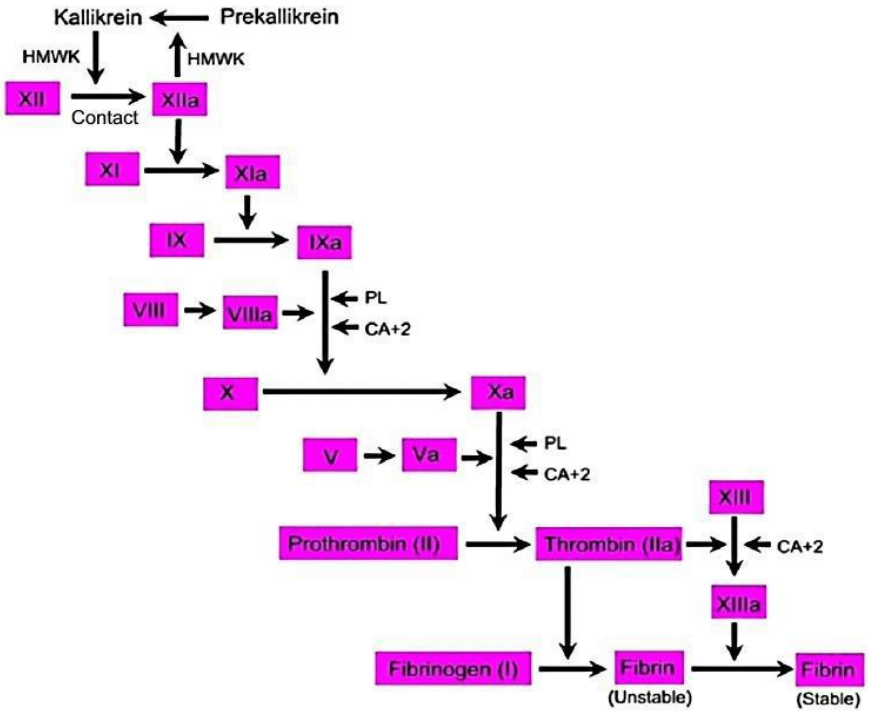
Factor X deficiency has been reported in approximately 50 families. Severity of bleeding depends upon the degree of depression of factor X. Because factor X is involved in the common coagulation pathway, both the PT and APTT values will be prolonged.

FACTOR XI

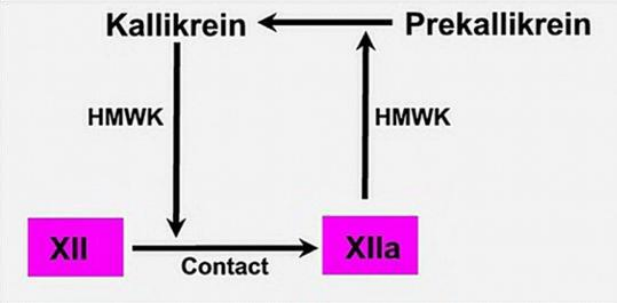
This disorder affects approximately 1 in 100,000 persons. Symptoms tend to be mild, with bleeding usually occurring only following trauma, surgery, or childbirth. Spontaneous bleeding is uncommon.

FACTOR XII

Factor XII deficiency, also called Hageman trait, is inherited as an autosomal recessive trait. Factor XII plays no role in *in vivo* blood clotting but is involved in *in vitro* (in the test tube) coagulation reactions. This explains why an individual with a factor XII deficiency does not have a bleeding problem but will have a prolonged result for the activated partial thromboplastin time (APTT) test, a laboratory test that monitors factors in the intrinsic pathway. The intrinsic coagulation pathway begins with factor XII being activated by contact with a negatively-charged particulate activator in the APTT reagent.



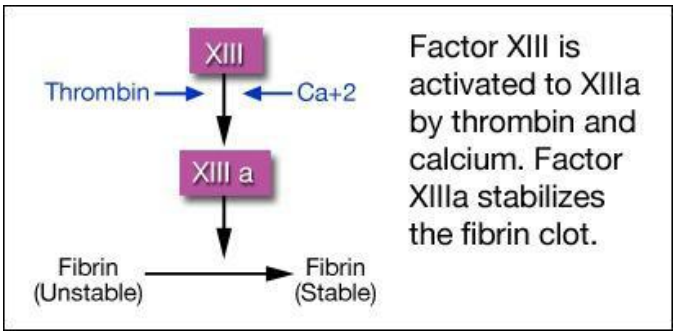
Factor XII also plays a role in the kinin system, which is important in inflammation, vascular permeability, and chemotaxis. Factor XII, which is initially activated by contact with a negatively charged surface, converts prekallikrein to kallikrein, its active enzyme form. Kallikrein in turn activates more factor XII.



Kallikrein also acts upon high molecular weight kininogen (HMWK) to release the kinin called bradykinin and upon LMWK to release the kinin called kallidin. Both bradykinin and kallidin function as potent vasodialators in addition to increasing vascular permeability, stimulating pain receptors and causing contraction of smooth muscle. In addition to liberating kinins from kininogens, kallikrein minimally activates plasminogen to plasmin in the fibrinolytic system.

FACTOR XIII

Factor XIII is not involved in the initial formation of fibrin (unstable fibrin). Its function is to stabilize the fibrin clot. Therefore, factor XIII deficiency is characterized by initial stoppage of bleeding with recurrence of bleeding when the unstable clot dissolves, usually 36 hours or more after the traumatic event. Only patients who are homozygous for factor XIII deficiency experience bleeding problems, including umbilical cord bleeding at birth, severe bleeding following surgery or trauma, and intracranial hemorrhage. For reasons not fully understood, some patients also have abnormal scar formation.



ACQUIRED FACTOR DEFICIENCIES

Acquired factor deficiencies, which occur secondary to or in response to another disease state or condition, are more common than inherited factor deficiencies. These induced deficiencies usually involve more than one coagulation factor and are produced by a variety of mechanisms, including decreased production of factor, production of nonfunctional factor, and increased consumption or utilization of factor.

VITAMIN K DEFICIENCY

Vitamin K is required for liver synthesis of factors II, VII, IX and X. Without sufficient vitamin K, the liver produces biologically inactive forms of these factors. There are two major sources of vitamin K: dietary intake of green, leafy vegetables, and synthesis by bacteria in the gastrointestinal tract. Vitamin K is a fat soluble vitamin and, therefore, is dependent upon pancreatic lipases and bile for absorption into the plasma from the gastrointestinal tract.

The most common causes of vitamin K deficiency in the adult are:

- Fat malabsorptive syndromes (e.g., sprue, biliary obstruction, pancreatic disease) that impair the absorption of fat which in turn affects the absorption of vitamin K.
- Prolonged broad-spectrum antibiotic therapy that abolishes normal flora of the intestinal tract, thereby reducing bacterial synthesis of vitamin K.

Vitamin K deficiency in the newborn causes a condition known as **hemorrhagic disease of the newborn**. This disorder, which occurs in the first few days of life, is characterized by bleeding from the umbilicus or circumcision, generalized bruising, intramuscular hemorrhage, and intracranial bleeding. It results from a combination of reduced stores of vitamin K and the inability of the immature liver to produce sufficient factors. In the United States, hemorrhagic disease of the newborn is prevented by administration of vitamin K to all newborns.

LIVER DISEASE

A number of mechanisms may contribute to the hemostatic defect that is commonly encountered in liver disease. They include:

- Defective liver synthesis of coagulation factors: This is the most important factor contributing to the bleeding tendencies encountered in liver disease. The factors most affected are the vitamins D dependent factors II, VII, IX, and X. Because of this, vitamin K therapy is used to treat some cases of bleeding associated with liver disease.
- Thrombocytopenia: Damage to the liver causes hepatic portal hypertension, which, in turn, leads to congestive splenomegaly and splenic pooling of platelets. In alcohol related liver disease, alcohol toxicity also suppresses platelet production.
- Increased fibrinolytic activity: Fibrinolytic activity is enhanced in liver disease for two reasons:
    - Plasmin inhibitors, including alpha-2-antiplasmin and alpha-2-macroglobulin, are liver synthesized. Impaired synthesis of these inhibitors results in plasmin activity proceeding unchecked. Increased destruction of fibrin clots, fibrinogen, factor V, and factor VIII by plasmin ultimately causes bleeding.

- The bleeding problem caused by increased fibrinolytic activity is further exacerbated by the accumulation of fibrin degradation products (FDPs). FDPs, which arise from the breakdown of both fibrin and fibrinogen by plasmin, interfere with further fibrin formation and also inhibit platelet function.

- Disseminated intravascular coagulation (DIC): DIC can occur in liver disease for two reasons.
    - Activated procoagulants that are released from degenerating liver cells begin fibrin formation.
    - Impaired synthesis by the liver of coagulation inhibitors, including antithrombin, proteins C and S, and heparin cofactor II allows the coagulation cascade proceeds unchecked.

As a result of the overwhelming clot formation that occurs in DIC, the coagulation factors are depleted and bleeding ultimately occurs.

DISSEMINATED INTRAVASCULAR COAGULATION

DEFINITION

Disseminated intravascular coagulation (DIC) is not a disease in itself, but rather, a syndrome that occurs in response to other conditions or diseases. Each of the conditions that are associated with the development of DIC is able to activate the coagulation system by some mechanism, thereby causing massive clot formation. Clot formation occurs primarily in the microvasculature, including the capillaries, venules and arterioles, and is systemic in nature. As clotting occurs, the coagulation factors are consumed in the clots. When the coagulation factors are depleted, bleeding occurs. The bleeding in DIC is exacerbated by other events that occur simultaneously with clot formation and consumption of the coagulation factors. When the coagulation system is activated, the fibrinolytic system is also activated. Activation of the fibrinolytic system involves converting plasminogen to plasmin, the proteolytic enzyme that degrades the fibrin clot. Upon tissue injury, substances that activate plasminogen and cause its conversion to plasmin are released simultaneously with substances that activate the coagulation pathways. This means that as soon as the body begins to make a clot, it is initiating systems that will dissolve the clot. In DIC, excessive, systemic clotting results in increased clot breakdown, which in turn results in more bleeding.

Another factor that contributes to bleeding in DIC is thrombocytopenia. While the primary problem in DIC is consumption of coagulation factors through extensive clot formation, bleeding will worsen as platelets get caught in the fibrin clots and thrombocytopenia develops.

ETIOLOGY

DIC affects approximately 1 in 1000 hospitalized patients and occurs most commonly in the very young and in the elderly. There are various mechanisms by which the coagulation can be activated and cause DIC.

- Release of tissue factor activates the tissue factor pathway of coagulation. Tissue factor, also referred to as tissue thromboplastin, is present in most tissues, but certain tissues, including the placenta and brain, are particularly rich sources. Therefore, conditions that injure these tissues, including complications of pregnancy (e.g., abruptio placenta, intrauterine fetal death, toxemia, retained placenta, etc.) and massive tissue trauma (e.g., extensive surgery, traumatic injury, etc.) can cause DIC by this mechanism. Also, certain cells, such as red blood cells and some tumor cells, contain tissue factor-like procoagulants. This explains why DIC occurs in some cases of hemolytic anemia and in certain malignancies (e.g., mucinous adenocarcinomas).
- Endothelial cell damage and subsequent exposure of tissue factor activates the tissue factor pathway. A variety of conditions can injure the vascular endothelium, including immune complex disease, liver disease, burns, vasculitis, and sepsis.
- Direct activation of the factor II or X by proteolytic enzymes results in fibrin formation via the common pathway. The venoms of certain snakes act as proteolytic enzymes and cause DIC by this mechanism. For example, Russell's viper venom activates factor X whereas the venom of the sand rattlesnake causes direct conversion of prothrombin (factor II) to thrombin.
- Certain malignancies are also capable of causing DIC by releasing substances that directly activate factors II or X.

CLINICAL MANIFESTATIONS

The clinical picture of DIC can vary substantially from case to case. Approximately 20% of the cases are asymptomatic and are suspected only on the basis of laboratory data. Other cases involve life-threatening hemorrhage.

The clinical course of DIC can be either acute or chronic. **Acute DIC**, which represents 80% to 90% of the cases, is characterized by the sudden onset of severe bleeding from at least 3 sites simultaneously. The bleeding may be profuse and may result in death if immediate action is not taken. Symptoms related to tissue anoxia and microinfarcts stemming from obstruction of the microvasculature of the heart, kidney, brain, liver, and pancreas by thrombi are also present.

Chronic DIC, on the other hand, is a low-grade disorder in which the triggering mechanism is not as powerful as that seen in acute DIC. As a result, clot formation, while still systemic, occurs more slowly and on a smaller scale. Due to the slow nature of clot formation, production of coagulation factors and platelets keeps pace with (or compensates for) those that are lost. As a result, bleeding due to factor depletion and thrombocytopenia does not occur. For this reason, chronic DIC is sometimes referred to as "compensated" DIC. Symptoms related to thrombosis are more likely to occur than is bleeding.

LABORATORY FINDINGS

Laboratory results vary depending upon whether the DIC is acute or chronic in nature.

LAB TEST	ACUTE DIC	CHRONIC DIC	EXPLANATION
PT	Increased	Normal	In acute DIC, coagulation factors are consumed quickly, resulting in insufficient factors and prolonged values for PT and APTT. In chronic DIC, production of factors keeps pace with loss and the PT and APTT are unaffected.
APTT	Increased	Normal	
Fibrinogen	Decreased	Normal	In acute DIC, fibrinogen is consumed during rapid clot formation. In chronic DIC, fibrinogen production keeps pace with loss.
FDP or D-Dimer	Increased	Increased	In both acute and chronic DIC, fibrinolytic activity is increased in response to increased clot formation. Fibrin breakdown products increase as fibrin is degraded.
AT	Decreased	Normal or Decreased	In acute DIC, AT levels decline as it combines with and deactivates thrombin and other activated proteins that are being formed rapidly. In chronic DIC, production of AT may or may not keep pace with consumption.
Plasminogen	Decreased	Normal or Decreased	In both acute and chronic DIC, fibrinolytic activity is increased and plasminogen is converted to plasmin at an increased rate. In acute DIC, the process is rapid and plasminogen is depleted. In chronic DIC, the process is slower; production of plasminogen may or may not keep pace with loss.
Platelet Count	Decreased	Normal	In both acute and chronic DIC, platelets will be caught up in the fibrin clots. In acute DIC, the process is rapid and thrombocytopenia develops. In chronic DIC, the process is slower; platelet production usually keeps pace with loss
Blood Smear	RBC fragments (schistocytes) and occasional microspherocytes occurs in half of patients	RBC fragments (schistocytes) and occasional microspherocytes occurs in 90% of patients	RBCs are ripped apart as they are forced through the fibrin webs that clog the blood vessels. Microspherocytes form as some of the larger fragments reseal.

Did You Know?

Disseminated intravascular coagulation (DIC) is one of three disorders that are collectively referred to as consumption coagulopathies. The two other disorders included in this classification are hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). These disorders are so named because increased clot formation results in the consumption of coagulation factors and platelets. In DIC, clot formation is coagulation cascade mediated, resulting in clots that are composed largely of fibrin. In HUS and TTP, clot formation is platelet mediated, resulting in clots that are composed largely of platelets.

ACQUIRED PATHOLOGIC COAGULATION INHIBITORS

Acquired inhibitors are also called circulating anticoagulants because they inhibit or impair blood coagulation. They may develop in response to various disease states, drugs, or clinical situations. Some inhibitors are directed against a single coagulation factor (specific inhibitors) while others are not (non-specific inhibitors).

SPECIFIC INHIBITORS

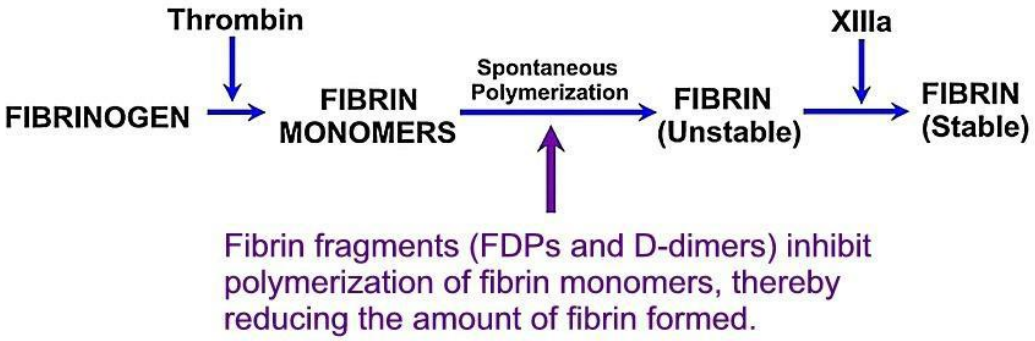
Specific inhibitors are immunoglobulins (antibodies) that are directed against a single coagulation factor. While inhibitors have been described for many factors, those directed against factors VIII and IX are by far the most common. Approximately 10% to 20% of patients with hemophilia A develop antibodies to factor VIII while 2% to 3% of patients with hemophilia B (Christmas disease) develop antibodies to factor IX. In both of these conditions, production of the inhibitor, an IgG class antibody, occurs as a result of treatment with factor concentrates.

Factor VIII inhibitors have been described in patients who do not have hemophilia A. Usually, an underlying disease, such as a lymphoproliferative disorder, multiple myeloma, or autoimmune disorder, is responsible for inhibitor development. Occasionally, an otherwise healthy female will develop factor VIII inhibitor during or after pregnancy, or an elderly patient will develop inhibitor for no apparent reason.

The severity of bleeding varies with the strength of the inhibitor and the amount of factor destruction. Treatment for patients with low levels of inhibitor consists of administering higher doses of factor concentrate in hopes of overcoming the inhibitor. Patients with high levels of inhibitor are treated with plasma-derived concentrates of activated vitamin-K dependent factors, called **activated prothrombin complex concentrates**. These complexes generate thrombin in the presence of factor inhibitors. Patients with inhibitors may also be treated with recombinant activated factor VII (FVIIa), which promotes thrombin formation through the tissue factor pathway.

NON-SPECIFIC INHIBITORS

Non-specific inhibitors are those that are not directed against a single coagulation factor. The most common of the non-specific inhibitors are fibrin degradation products (FDPs). FDPs are produced when plasmin, the proteolytic enzyme responsible for fibrinolysis, degrades both fibrin and fibrinogen. Normally, FDPs play an important feedback role in the coagulation system. As FDPs accumulate in the blood, they inhibit further clot formation by interfering with fibrin monomer polymerization, a crucial step in the conversion of fibrinogen to fibrin. They also inhibit platelet aggregation. In certain conditions, FDPs may be present in higher than normal concentrations. When this occurs, their enhanced ability to inhibit fibrin formation and platelet aggregation may lead to bleeding.



Two additional inhibitors that are commonly included in this category are lupus anticoagulant (LLA) and anticardiolipin antibody (ACLA). The presence of these inhibitors causes prolongation of the APTT and, to a lesser degree, the PT by interfering with the phospholipid surfaces of the test reagents. However, in vivo, these inhibitors do not inactivate clotting factors and, as such, do not cause bleeding. On the contrary, patients with these inhibitors have a tendency to experience thrombosis. For this reason, they will be covered in depth in the discussion on thrombosis.

Did You Know?

Due to the ability of FDPs to inhibit fibrin formation, the presence of elevated amounts of FDPs in a sample being used for coagulation testing may falsely prolong the results for some of the clot-based coagulation assays. The laboratory technician must be aware of which tests are affected by FDPs, and what amount of FDPs will affect these tests.

THROMBOSIS

I. Definition

II. Arterial thrombosis

- A. Mechanism of thrombus formation
- B. Causes of arterial thrombosis
  - 1. General causes for arterial thrombosis
  - 2. Hyperhomocysteinemia
    - a) Mechanism of thrombosis
    - b) Inherited hyperhomocysteinemia
    - c) Acquired hyperhomocysteinemia
  - 3. C-reactive protein

III. Venous thrombosis

- A. Mechanism of thrombus formation
- B. Causes for venous thrombosis
  1. Deficiencies of biochemical inhibitors
     a. Tissue factor pathway inhibitor
     b. Protein C regulatory system
     c. Antithrombin
     d. Heparin cofactor II
  2. Activated protein C resistance (APCR)
     a. Incidence
     b. Activity of the protein C regulatory system
     c. Defect causing APCR
     d. Laboratory diagnosis of APCR
  3. Prothrombin gene mutation 20210
  4. Antiphospholipid antibody syndromes
     a. Definition
     b. Mechanism of thrombosis
     c. Clinical manifestations
     d. Laboratory diagnosis
        i. APTT
        ii. Dilute Russell's Viper Venom Time (dRVVT)
        iii. Phospholipid neutralization test
        iv. Anticardiolipin antibody test
     e. Treatment
  5. Dysfibrinogenemia
  6. Elevated factor VIII
  7. Defects in the fibrinolytic system

THROMBOSIS

DEFINITION

Thrombosis occurs when a clot, or thrombus (plural = thrombi) forms inappropriately within a blood vessel. Thrombi can develop within arteries, veins, or capillaries. In general, they are composed of platelets, fibrin, and other cellular elements of the blood. However, the exact composition of a thrombus varies depends upon the site and mechanism of formation. A thrombus poses a threat to health and life because it occludes a blood vessel and prevents blood from reaching the tissues normally supplied by the vessel. As a result, tissue death, or **tissue necrosis**, occurs. Another risk is that a portion of the thrombus, referred to as an **embolus**, can break off and travel via the bloodstream to other areas where it can lodge in a smaller vessel and cause additional tissue death. This event is referred to as an **embolism** or **thromboembolism**.

ARTERIAL THROMBOSIS

MECHANISM OF THROMBUS FORMATION

Thrombus formation in an artery begins when the vascular endothelium is damaged and the subendothelial structures are exposed. When platelets and coagulation factors come into contact with the subendothelium, hemostasis begins. Platelets adhere to the subendothelial structures, become activated, and finally aggregate. Simultaneously, coagulation factors are activated and fibrin formation begins. Ultimately, an arterial thrombus, which is composed largely of platelets and fibrin with very few RBCs and WBCs, is formed.

CAUSES OF ARTERIAL THROMBOSIS

GENERAL CAUSES FOR ARTERIAL THROMBOSIS

Damage to the arterial vascular endothelium is most commonly associated with the accumulation of plaques composed of lipids, fibrous connective tissue, macrophages, and excess smooth muscle cells. This condition is called **atherosclerosis**. Therefore, any event that enhances plaque formation, such as a diet rich in cholesterol and fat, increases the risk of arterial thrombosis. Other factors or events that can damage the arterial vascular endothelium and cause thrombosis include:

- Certain viruses.
- Tobacco products.
- Oral contraceptives.
- Hypertension.
- Immune complexes that form in certain diseases.
- Enzymes released by platelets and WBCs in inflammatory states.

The turbulent blood flow within an artery enhances thrombus formation by aggravating endothelial cell damage and forcing platelets onto the exposed subendothelium.

HYPERHOMOCYSTEINEMIA

MECHANISM OF THROMBOSIS

Elevated blood levels of homocysteine cause thrombosis directly and indirectly through a variety of mechanisms, including:

- Injuring arterial endothelial cells.
- Affecting interactions between endothelial cells and platelets.
- Interfering with the expression of thrombomodulin on endothelial cells; results in decreased activation of protein C.
- Causing direct platelet activation.

INHERITED HYPERHOMOCYSTEINEMIA

Homocystinuria is a rare inherited disease caused by the decrease or absence of the enzyme essential to the metabolism of homocysteine. As a result of this enzyme deficiency, homocysteine rises to high levels in the blood.

Extreme elevations of homocysteine, as seen in homozygous cases of homocystinuria, cause diffuse juvenile atherosclerosis and arterial and venous thrombosis.

More moderate elevations of homocysteine, as are seen in the heterozygous form of this disorder, have been linked to coronary artery disease, carotid disease, and peripheral vascular disease in adults. Venous thrombosis and pulmonary embolism may also be prevalent in this group.

ACQUIRED HYPERHOMOCYSTEINEMIA

Because vitamin B12, folic acid, and pyridoxine normally function to help break down homocysteine, deficiencies of these vitamins result in homocysteinemia. Elevated levels are also seen in renal disease and liver disease because of a reduced ability to clear homocysteine from the blood.

C-REACTIVE PROTEIN

There is a clear correlation between high levels of C-reactive protein (CRP) and myocardial infarct and stroke. CRP is an acute-phase protein that increases several hundred-fold during systemic inflammation. In some individuals, inflammation can cause weakening of the artery walls which can suddenly rupture, causing heart attack or stroke. Also, plaque can build up quickly in inflamed arteries, increasing the risk of thrombosis. Testing for CRP levels is an additional way to assess cardiovascular disease risk.

VENOUS THROMBOSIS

MECHANISM OF THROMBUS FORMATION

Blood flow through the veins is much slower and less forceful than blood flow through the arteries. In this type of environment, activation of both platelets and coagulation factors and subsequent thrombus formation can occur. Most venous thrombi that form are lysed by natural processes before causing problems. However, in conditions characterized by prolonged vascular stasis, such as immobilization, obesity, congestive heart failure, or

pregnancy, and in conditions in which the coagulation process is stimulated, such as occurs following surgery, trauma, or childbirth, the incidence of thrombus formation increases dramatically. If the body is unable to halt the clot formation process or is incapable of dissolving and removing the clots that form, life-threatening thrombosis can occur. The term **thrombophlebitis** is used to describe a thrombus that forms in a superficial vein, causing inflammation of the affected vein. Thrombophlebitis is usually benign and self-limiting. The term **deep vein thrombosis (DVT)** describes a symptomatic and clinically significant clot that occurs in a deep (as opposed to a superficial) vein.

The term **thrombophilia** describes any disorder, either inherited or acquired, associated with an increased tendency to venous thromboembolism. Thrombophilia is commonly a multirisk factor disease, meaning that affected individuals usually have more than one or multiple thrombotic disorders occurring simultaneously. Types of hereditary thrombophilia include:

- Hereditary deficiency or defect of biochemical inhibitors, including:
    - Antithrombin (formerly called Antithrombin III)
    - Protein C
    - Protein S
    - Heparin cofactor II
    - Tissue factor pathway inhibitor
- Activated protein C resistance (APCR)
- Prothrombin gene mutation 20210
- Dysfibrinogenemia
- Elevated factor VIII
- Fibrinolytic system defects

Types of acquired thrombophilia include:

- Acquired deficiencies of biochemical inhibitors
- Acquired fibrinolytic system defects
- Antiphospholipid antibody syndromes
    - Lupus anticoagulant
    - Anticardiolipin antibody

CAUSES FOR VENOUS THROMBOSIS

DEFICIENCIES OF BIOCHEMICAL INHIBITORS

The biochemical inhibitors are proteins that have the ability to regulate the enzymatic activities of the activated coagulation factors, thereby slowing and stopping the blood coagulation. The most important inhibitors are tissue factor pathway inhibitor (TFPA), the protein C regulatory system (involves proteins C and S), and the protease inhibitors antithrombin and heparin cofactor II. Deficiencies or defects in any of these inhibitors are associated with thrombotic disorders.

Deficiencies of the biochemical inhibitors can be either inherited or acquired. Most of the cases of hereditary deficiencies are heterozygous in nature, resulting in decreased 4

levels as opposed to a total absence of the affected inhibitor. Acquired deficiencies can occur through a variety of mechanisms.

- Liver disease results in decreased production of these liver synthesized inhibitors.
- Nephrotic syndrome, burns, and enteropathies result in increased loss of these inhibitors from the body.
- Sepsis, disseminated intravascular coagulation, extensive trauma, and surgery result in increased consumption of these inhibitors.

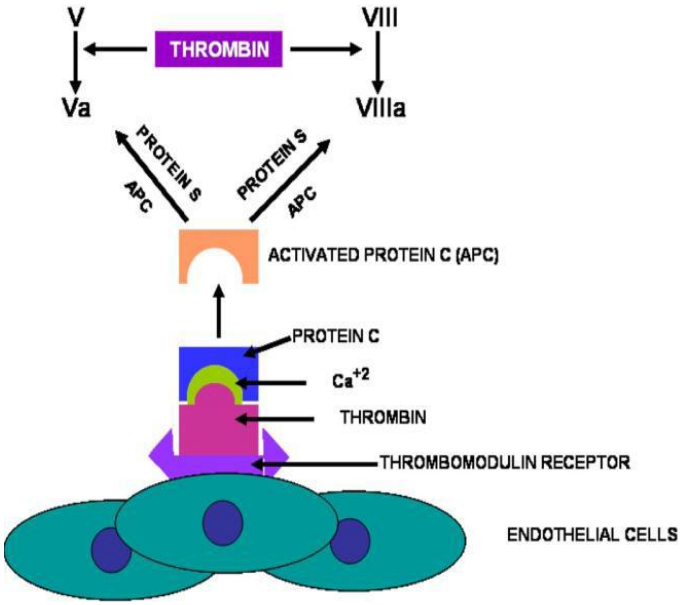
TISSUE FACTOR PATHWAY INHIBITOR (TFPI)

TFPI is produced by several cell lines, including lung, liver, bladder, and endothelial tissue cells. Platelets also produce and secrete TFPI after stimulation with calcium or thrombin. In the initiation phase of coagulation, factor VIIa and tissue factor (TF) form a complex that activates factors IX and X. Factor Xa reacts with the FVIIa/TF complex to bind TFPI. TFPI inactivates factor VIIa, making the FVIIa/TF reaction short liver. Heparin enhances the inhibitory abilities of TFPI by 40-fold.

PROTEIN C REGULATORY SYSTEM

Protein C is activated by thrombin. Once activated, it inhibits the activity of factors Va and VIIIa. In order to be optimally activated, protein C must bind to thrombin (IIa) on the thrombomodulin receptor on the surface of the endothelial cell. Calcium serves as a bridge to bind thrombin and protein C together. Protein S facilitates the activation of protein C by promoting its binding to the thrombomodulin receptor. It also works with activated protein C to accelerate the inactivation of factors Va and VIIIa.

Various assays are available to detect both quantitative and qualitative deficiencies of proteins C and S.

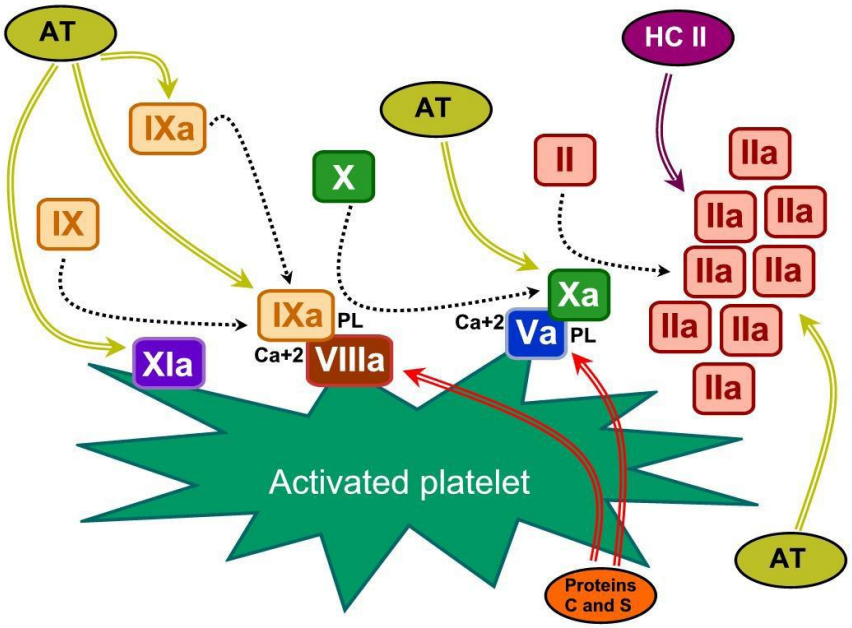


ANTITHROMBIN

Antithrombin (AT) is serine protease that produced in the liver, by endothelial cells, and possibly by megakaryocytes. It is the most important of the protease inhibitors because of its ability to neutralize numerous activated factors, including IIa (thrombin), IXa, Xa, XIa, XIIa, and kallikrein, by forming 1:1 complexes with each of these activated factors. It also neutralizes plasmin, the proteolytic enzyme of the fibrinolytic system that degrades the fibrin clot. Since antithrombin also plays an important role in heparin anticoagulant therapy; patients who are antithrombin deficient will not respond to heparin anticoagulant therapy. Congenital AT deficiency occurs in 1 in 2000 to 5000 persons and accounts for 1% to 2% of recurrent thrombotic episodes. Approximately 90% of the cases are quantitative in nature. The remainder of the cases are caused by a structural abnormalities in the AT 5 molecule. Various assays are available to detect both quantitative and qualitative AT defects.

HEPARIN COFACTOR II

Heparin cofactor II is another serine protease. It inhibits the activity of thrombin only. Heparin enhances the activity of heparin cofactor II but not to the same extent that it enhances antithrombin activity.



Summary of biochemical inhibitors

ACTIVATED PROTEIN C RESISITANCE (APCR)

INCIDENCE

Activated protein C resistance (APCR) is the most common cause for familial (inherited) thrombosis. It occurs in approximately 3% to 8% of Caucasians of northern European decent and is responsible for approximately 20% of the thrombosis cases.

ACTIVITY OF THE PROTEIN C REGULATORY SYSTEM

Proteins C and S are vitamin K dependent, liver synthesized proteins. Activated protein C in conjunction with protein S destroys factors Va and VIIIa by enzymatic cleaving, thereby slowing down the coagulation cascade and preventing excessive clot formation.

DEFECT CAUSING APCR

The thrombosis that occurs in APCR is not due to a defect in protein C but rather to a defect in factor V. This defect involves a single amino acid substitution (glutamine replaces arginine) at the 506^{th} position of the factor V molecule. It is at this exact 6 position that activated protein C normally cleaves factor Va to render it inactive. However, as a result of this amino acid substitution, protein C cannot cleave Va. Factor Va remains active and continues to participate in clot formation. The term **Factor V Leiden** is used to describe the abnormal factor V molecule that is produced by the amino acid substitution. It is designated as $FV:Q_{506}$ or FV:R506Q. 95% to 99% of all cases of APCR are caused by

the same single point mutation. Individuals who are heterozygous for this defect have a 5 to 10 times greater risk of thrombosis while homozygous individuals have a 50 to 100 times increase in thrombosis risk. The thrombosis risk associated with APCR is not as great as that associated with antithrombin deficiency or protein C deficiency alone. However, when APCR occurs in combination with another thrombotic problem (e.g., deficiencies of antithrombin, protein C, or protein S), the risk of thrombosis increases greatly.

LABORATORY DIAGNOSIS OF APCR

The clinical laboratory can diagnose APCR either by using a coagulation-based assay to determine the APCR ratio or by using a DNA-based test for detection of the factor V Leiden mutation. Although both tests can be and are frequently performed, many laboratories choose to perform only the DNA test due to cost considerations.

The coagulation-based assay for APCR is a modified APTT test that utilizes commercially prepared activated protein C (APC) as a reagent. In a normal response, the addition of APC to the test system will substantially prolong the APTT result. This is due to the fact that APC will destroy factors Va and VIIIa in the sample. For the patient with APCR, the APTT will show less of an increase with the addition of APC. This is due to the fact that the APC cannot destroy the patient's defective factor V (factor V Leiden). By comparing the results of the patients APTT values with and without the addition of APC, a preliminary diagnosis can be made.

The procedure is performed as follows:

1. A baseline APTT is performed on the patient's plasma without the addition of activated protein C (APC).
2. A second APTT is performed with APC added to the calcium chloride reagent.
3. A ratio of the two APTT results is calculated. A value of less than two for this ratio indicates activated protein C resistance.

$$\text{APCR ratio} = \frac{\text{APTT with APC}}{\text{Baseline APTT}}$$

In patients with abnormal ratios (less than 2) further analysis by one of several molecular techniques may be performed. The most commonly used method involves the use of the polymerase chain reaction to amplify the segment of the DNA where the mutation is located. Following amplification, a restriction enzyme is used to cut the amplified DNA segments into fragments. Once separated by gel electrophoresis, the DNA fragment pattern is analyzed to determine whether or not the mutation is present.

PROTHROMBIN GENE MUTATION 20210

A defect in the gene that controls prothrombin production is associated with a mild elevation of prothrombin levels (115-130%) and an increased risk of thrombosis. This disorder is the second most common cause of familial thrombosis, representing 18% of cases. It affects approximately 1-2% of Caucasians and is seen infrequently among persons of African descent, Asian Indians, Native Americans, and Koreans. Affected individuals frequently have other genetic risk factors for thrombosis, such as the Factor V Leiden, occurring simultaneously. Molecular testing to identify the genetic mutation can be performed to diagnose the disorder.

ANTIPHOSPHOLIPID ANTIBODY SYNDROMES

DEFINITION

Antiphospholipid antibody syndrome (APS) is the most common acquired thrombotic disorder. One to 2% of members of both sexes and all races and 5% to 15% of individuals with recurrent thrombosis have APS. APS is characterized by the presence of autoantibodies directed against protein-phospholipid complexes. APS was first described in the 1950s when it was noted that patients with system lupus erythematosus (SLE) often had prolonged activated partial thromboplastin times (APTT). The elevation of the APTT was not accompanied by hemorrhagic complications but, rather, by a greater incidence of both venous and arterial thrombosis.

The antiphospholipid (APL) antibodies are immunoglobulins (IgG, IgM or IgA) that bind protein-phospholipid complexes. Although their name implies that they bind phospholipids directly, their targets are actually the proteins assembled on phospholipid surfaces. **Lupus anticoagulant (LA)** and **anticardiolipin antibody (ACA)** are the most widely studied APL antibodies. 60% of patients with APS have both of these antibodies. Other less well-characterized APL antibodies distinct from LA and ACA have also been identified. Like LA and ACA, they are capable of promoting abnormal coagulation. Most APL antibodies arise in response to infection (bacterial, viral, fungal or parasitic) or numerous drug regimens and are transient in nature, usually disappearing within 12 weeks and have no clinical consequences. Of the APL antibodies that persist, 30% are associated with arterial and venous thrombosis.

MECHANISM OF THROMBOSIS

The mechanism by which APL antibodies cause thrombosis is not clearly understood, but appears to be multifaceted, affecting both humoral and cellular components involved in hemostasis. One aspect involves the interaction of the antibody with the platelet phospholipid present at many points in the hemostasis system. This interaction causes inappropriate activation of the coagulation system, resulting in thrombus formation. In laboratory tests, however, the effect of the antibody is quite different. Phospholipids are used in coagulation tests such as the PT and APTT to provide a surface for the coagulation reaction to occur. In vivo, platelets provide this surface. In vitro, however, phospholipid must be added as a reagent to substitute for the platelets that are removed from the patient sample by centrifugation. The APL antibodies in the patient plasma sample bind to the phospholipid and interfere with the coagulation reaction, thereby prolonging the clotting time. The APTT is more affected than the PT by the antibody presence.

Other mechanisms by which APL antibodies cause thrombosis include inhibition of endothelial cell activation of protein C and inhibition of endothelial cell release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation.

CLINICAL MANIFESTATIONS

The majority of persons who have APS are otherwise healthy and have no underlying medical conditions. This is referred to as **primary APS**. APS is also seen in association with other diseases. This is called **secondary APS**. Secondary APS is seen most commonly is association with systemic lupus erythematosus (SLE) and other autoimmune conditions. It

can also occur in response to exposure to various drugs, following certain infections, and in certain malignancies.

APS is associated with a variety of clinical manifestations. The best described manifestations include:

- Venous thrombosis (e.g., deep vein thrombosis and pulmonary embolus).
- Arterial thrombosis.
- Neurological disease, including strokes, early onset dementia, ocular events, and migraines (caused by the presence of microthrombi).
- Recurrent fetal loss (caused by microthrombosis in the placenta).
- Thrombocytopenia (caused by destruction of platelets by the APL antibody).

LABORATORY DIAGNOSIS

APTT:

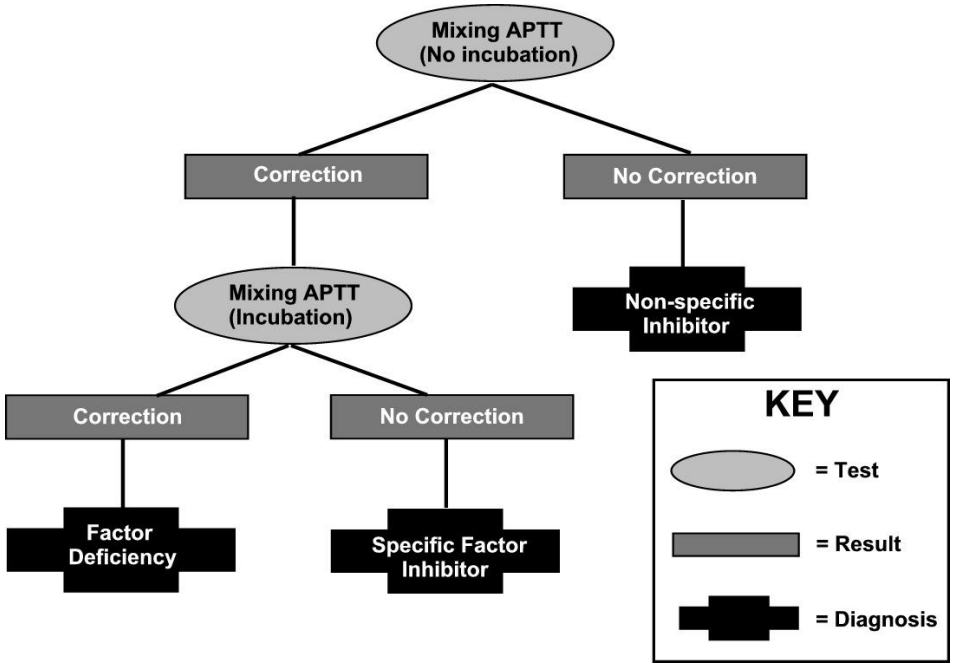
One of the most commonly performed coagulation tests used in the clinical laboratory is the APTT. Phospholipids are used in this test to provide a surface for the coagulation reaction to occur. In vivo, platelets provide this surface. In vitro, however, phospholipid must be added as a reagent to substitute for the platelets which were removed from the sample by centrifugation. The APL antibodies in the patient plasma sample bind to the phospholipid reagent and interfere with the coagulation reaction, thereby prolonging the clotting time.

A variety of conditions can cause an elevated APTT value, including heparin, factor deficiencies, specific factor inhibitors (e.g., antibody to factor VIII), and non-specific inhibitors such as APL antibody. Once an elevated APTT is found in the patient suspected of having APS, further testing must be performed to confirm the presence of an APL antibody. This can be accomplished by performing an **APTT mixing study**.

The procedure for performing APTT mixing studies is as follows:

1. Perform a thrombin clotting time (TCT) to determine if heparin is the cause of the prolonged APTT. A normal value for the TCT indicates that no heparin is present and additional testing must be performed. Continue to step 2.
2. Mix equal parts of the patient's plasma and pooled normal plasma. Perform an APTT on the dilution. If the APTT of the mixture is within 10% of the APTT of the pooled normal plasma, a correction has occurred. This indicates that a factor deficiency may exist; the pooled plasma, which contains sufficient amounts of all factors, has provided the missing factor(s) to correct the APTT. Before testing to isolate the factor deficiency is performed, however, more testing to rule out the presence of a specific factor inhibitor is necessary; proceed to step 3. If the initial mixture is not corrected by the pooled normal plasma, lupus anticoagulant is suspected; proceed to step 4.
3. Mix a second aliquot of patient plasma with an equal amount of pooled normal plasma and incubate for 1 to 2 hours at 37^{o}C. The activity of factor specific inhibitors such as anti-factor VIII are enhanced by incubation at 37^{o}C. If, after the incubation period, the APTT is corrected, the presence of factor deficiency is supported. If, on the other hand, the APTT is prolonged (uncorrected), a specific factor inhibitor is indicated. The **Bethesda titer** is used to confirm the presence of specific factor inhibitors.
4. If the APTT on the initial mixture (step 1) is not corrected, a non-specific inhibitor such as lupus anticoagulant is suspected. An APTT is performed on a new aliquot of patient plasma

using a kit with high-concentration phospholipid (contains phospholipid in excess of what the lupus anticoagulant will neutralize). Shortening of the high-phospholipid APTT assay by at least 8 seconds compared with the original APTT confirms the presence of lupus anticoagulant.



The following chart summarizes the APTT values for each of the diagnostic considerations. Note that the APTT for normal plasma alone will be normal prior to and after incubation at 37°C while the APTT values for patient plasma alone will always be prolonged.

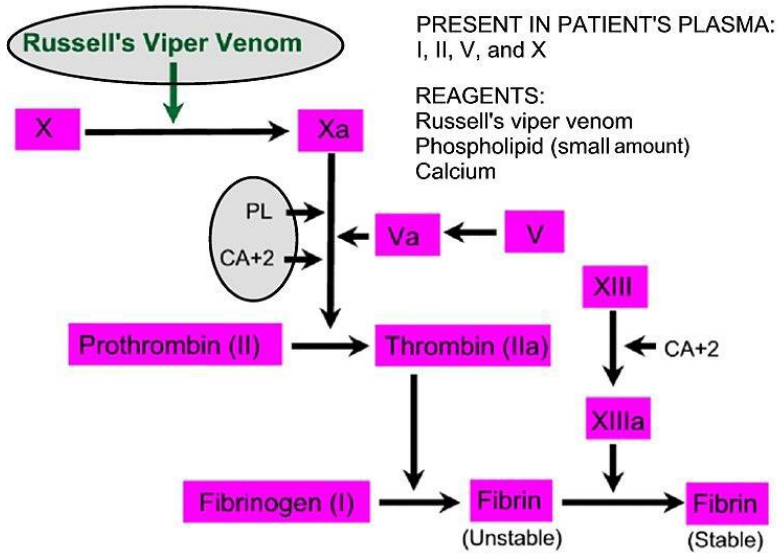
Sample	Examples of APTT values	
	Immediate (no incubation)	Following incubation at 37°C
Normal plasma	30 seconds	33 seconds
Patient plasma	50 seconds	54 seconds
50:50 Mix: Factor Deficiency	30 seconds	33 seconds
50:50 Mix: Specific Factor Inhibitor	30 seconds	40 seconds
50:50 Mix: Non-specific Factor Inhibitor	45 seconds	46 seconds

Although many laboratories have used the APTT as a screening test for APL antibody, it is not a reliable screening test because the sensitivity of the APTT to the presence or absence of the antibody is highly dependent upon the sensitivity of reagents used. In fact, only 30% of patients with APS will have a prolonged APTT value. Therefore, one cannot exclude a diagnosis of APS based upon normal APTT results.

Dilute Russell's Viper Venom Time (dRVVT)

The dilute Russell's viper venom time (dRVVT) is more sensitive to the presence of APL antibody, specifically the lupus anticoagulant, than is the APTT. Russell's viper venom is snake venom that, in the presence of calcium ions, is able to convert factor X to its activated form Xa. The Xa, together with phospholipid and factor V, converts prothrombin to thrombin.

The dilute Russell's viper venom time (dRVVT) is made sensitive to the presence of lupus anticoagulant by adding minimal amounts of phospholipid. The presence of even small amounts of antibody in the patient sample will neutralize the phospholipid, resulting in prolongation of the clotting time. Since the Russell's viper venom activates factor X directly, the dRVVT will not be prolonged in deficiencies of or with inhibitors to factors VIII, IX, XI, XII, and VII. However, it will be affected by deficiencies of factors I (fibrinogen), II (prothrombin), V and X.



Phospholipid Neutralization Test

If the dRVVT is prolonged, a phospholipid neutralization test can be performed to differentiate between a factor deficiency and LA. In this test, excess phospholipid is added and the dRVVT is repeated. If, in the presence of excess phospholipid, the dRVVT shortens, a LA is indicated. This is due to the fact that the phospholipid was added in such a great amounts that, even after the antibody was saturated, additional phospholipid needed to complete clot formation remained. If, on the other hand, the dRVVT remains prolonged in the presence of excess phospholipid, a factor deficiency is indicated, and the specific factor deficiency must be identified with other laboratory tests.

Anticardiolipin Antibody Test

An enzyme-linked immunosorbent assay (ELISA) is the method of choice for confirming the diagnosis of anticardiolipin antibody.

TREATMENT

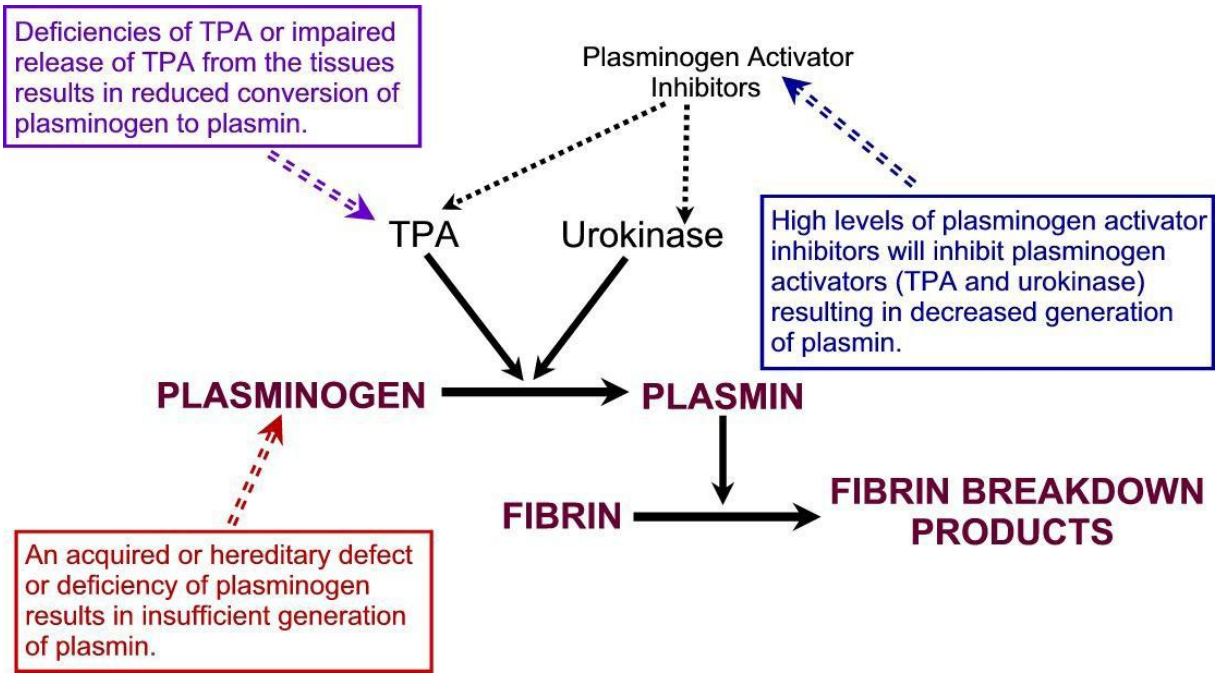
Despite the fact that APS is an autoimmune disorder, immunosuppression does not prevent recurrent thrombosis, fetal loss, or neurological syndromes, and should not play a role in the therapy of thrombotic APS. Low intensity anticoagulation with courmarin is effective in most patients. If more aggressive anticoagulation is needed, higher doses of coumarin or heparin therapy can be administered. To prevent miscarriages in pregnant women with APS, low molecular weight heparin therapy is recommended.

DYSFIBRINOGENEMIA In some cases, the presence of defective fibrinogen results in thrombosis rather than bleeding. The mechanism of thrombosis may involve abnormal resistance of fibrinogen to lysis by plasmin.

ELEVATED FACTOR VIII Factor VIII levels in excess of 150% are associated with increased risk of thrombosis. Although this disorder appears to be hereditary in nature, no specific mutation of the factor VIII gene has been identified.

DEFECTS IN THE FIBRINOLYTIC SYSTEM

The major function of the fibrinolytic system is to break down fibrin clots. Failure to accomplish this will result in thrombosis. Plasmin, the proteolytic enzyme that degrades the fibrin clot, is derived from plasminogen through the action of various plasminogen activators, including kallikrein and tPA. There are also various plasminogen activator inhibitors that serve as a feedback mechanism to prevent over activation of plasminogen and excessive fibrinolysis. Various defects in the fibrinolytic system can result in thrombosis as shown in the diagram below.



INTRODUCTION TO COAGULATION TESTING

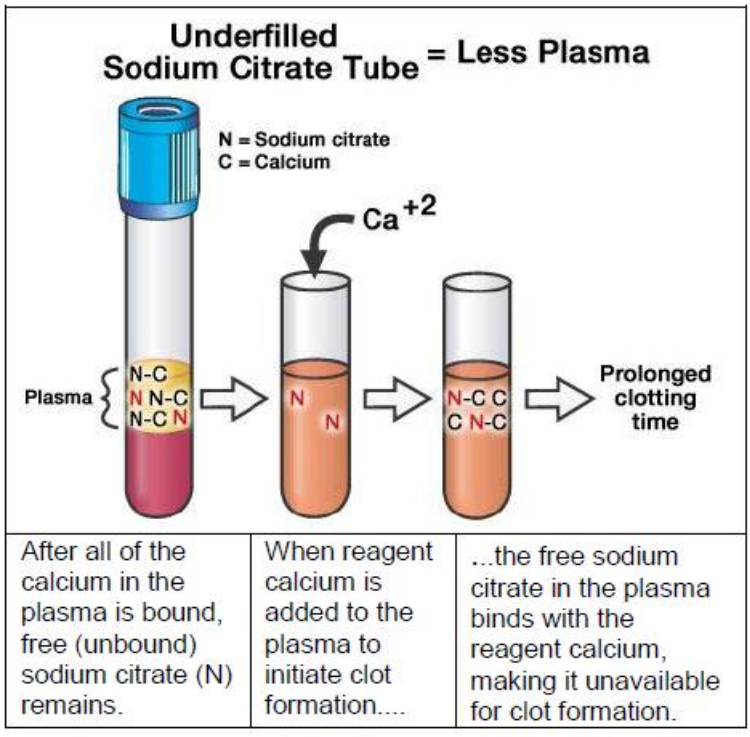
I. Collection of the specimen
II. Preparation of the plasma sample
III. Performing coagulation tests
    A. Testing methods
        1. Mechanical clot detection
            a. Electromechanical
            b. Viscocity detection system
        2. Photo-optical clot detection
            a. Turbidimetry
            b. Nephelometry
        3. Chromogenic end point detection
        4. Immunologic end point detection
    B. Preanalytical variables

INTRODUCTION TO COAGULATION TESTING

COLLECTION OF THE SPECIMEN

1. Specimens should be collected in tubes containing 0.109M (3.2%) sodium citrate. The ratio of blood to anticoagulant should be 9 parts to 1 part sodium citrate. Blood collection tubes that have less than 90% expected fill are unacceptable, and the specimen must be redrawn.

   CLSI recommends 3.2% sodium citrate as opposed to other concentrations because most ISIs used for INR calculation are based upon a sodium citrate concentration of 3.2%. INRs tend to be more accurate when 3.2% sodium citrate is used for collection of prothrombin time specimens. Sodium citrate binds calcium to form a soluble complex, making the calcium unavailable for the blood coagulation. If there is too much sodium citrate in the blood collection tube, there will be excess free (unbound) sodium citrate remaining after all of the calcium in the sample has been consumed. When calcium is added as a reagent in the test system, the free sodium citrate remaining in the sample will bind some of the reagent calcium, making it unavailable for testing and falsely prolonging test results.



2. When at all possible, use a 19 to 21 gauge needle when collecting coagulation specimens. A 23 gauge needle is acceptable for pediatric collections and small size samples.

   *There is **less** of a chance of lysing red blood cells when using larger gauge needles.*

3. A clean, non-traumatic venipuncture is imperative to ensure a specimen that is free of procoagulants (e.g, tissue factor that is released from the surrounding tissue), hemolysis, or partial clotting. When collecting multiple tubes from a patient, the coagulation tube should be collected first or immediately after a non-additive tube. It should not be collected immediately following tubes containing heparin, EDTA, sodium fluoride, clot-promoting additives, or serum separator materials.

   Trauma during specimen collection could cause the release of procoagulants (e.g, tissue factor) from the surrounding and possible activation of the tissue factor pathway. Hemolysis of red blood cells releases procoagulants from the red blood cell membranes that may initiate the clotting mechanism and falsely shorten test results. Therefore, specimens with any amount of hemolysis should be recollected. Collecting the coagulation tube after an additive tube could result in the additives being transferred to the coagulation tube on the stopper needle. This would invalidate the coagulation test results.

4. Specimens should be immediately inverted 6 times to mix the blood with the anticoagulant and prevent clotting. Avoid vigorous mixing or excessive agitation.

   The presence of even very small clots makes the specimen useless for coagulation testing. Excessive agitation of the specimen may cause red blood cell lysis, procoagulant activation, and platelet activation.

5. When a winged infusion (“butterfly”) needle system is used in combination with evacuated tubes, the phlebotomist must compensate for the blood volume which remains behind in the tubing (approximately 0.5 mL). To accomplish this, a non-additive discard tube should be collected before the coagulation specimen.

   This ensures that the needle set’s tubing if filled before the coagulation specimen is collected, allowing for sufficient blood to be delivered and the proper blood- to- anticoagulant ratio to be maintained.

6. Following collection of a coagulation specimen with a syringe and winged-infusion (“butterfly”) needle set, place the syringe on a clean surface and clamp the tubing near the syringe hub. Remove the tubing and place a 19 gauge needle on the syringe. Gently push the needle through the stopper of the evacuated coagulation tube and allow blood to flow gently down the side of the tube until it is filled completely. Do not force the blood into the tube by pushing the syringe plunger; this may cause red blood cell lysis. In order to reduce the chance of needle stick while performing this procedure, the tube should be placed in a rack, not hand held, when filling it with the syringe and needle. When full, gently invert the tube six times.

7. The following protocol should be followed when collecting blood from a venous access device (e.g., indwelling catheter).
   a. Flush the venous access device line with 5 mL of sterile saline to clear heparin from the line.
   b. Attach a syringe to the line and aspirate 5 mL (or a volume that is six times the device’s line volume) of blood. Discard this blood.

c. Collect the necessary amount of blood into a second syringe and transfer it to an evacuated tube. Use a one-handed technique when performing this maneuver to avoid a needle stick.
d. Allow the evacuated tube to fill on its own; do not depress the syringe plunger to force blood into the tube.
e. Indicate on the specimens label that it is a catheter line-draw.

8. When collecting a coagulation specimen, the tourniquet must be released within 1 minute of its application.

Prolonged tourniquet application results in vascular stasis, which causes local concentration of the factor VIII-vWF complex and may result in false shortening of clot-based coagulation tests

9. Specimens should be collected in containers with nonwettable surfaces (i.e., glass tube coated with silicone or plastic tube) to reduce the effect of glass contact activation.

Contact activation of factor XII initiates the intrinsic coagulation pathway. While factor XII is not involved in in vivo blood coagulation, it is involved in in vitro blood coagulation and will impact the results of laboratory tests that monitor the intrinsic coagulation pathway.

PREPARATION OF THE PLASMA SAMPLE

1. Specimens should **not** be placed on ice after collection.

2. If at all possible, coagulation specimens should be centrifuged within 1 hour of collection.

3. Before centrifugation, the blood should be inverted gently and inspected for the presence of clots. Specimens with even very small clots must be recollected.

4. Centrifuge specimens at 2500 x g for 15 minutes to ensure platelet free plasma.

Platelets contribute various factors to blood coagulation. Since most coagulation tests routinely performed in the laboratory are designed to monitor the levels of coagulation factors, the variables introduced by platelets are eliminated by removing platelets from the sample by centrifugation.

5. Upon removing the specimen from the centrifuge, inspect the plasma for hemolysis. Hemolyzed specimens should be recollected.

Hemolysis releases substances from the red blood cell membranes that act as procoagulants and can activate the clotting mechanism.

6. Grossly lipemic or icteric specimens should not be tested on instruments using photo-optical detection systems.

Lipemia or icterus may increase the amount of light scatter off the sample and, as a result, reduce light transmission through the sample, thereby affecting the end point determination.

7. Plasma may remain on the packed cells in the collection tube if testing is to be done immediately. If testing is delayed for more than 2 hours, remove the plasma sample to a plastic snap top tube using a plastic transfer pipette.

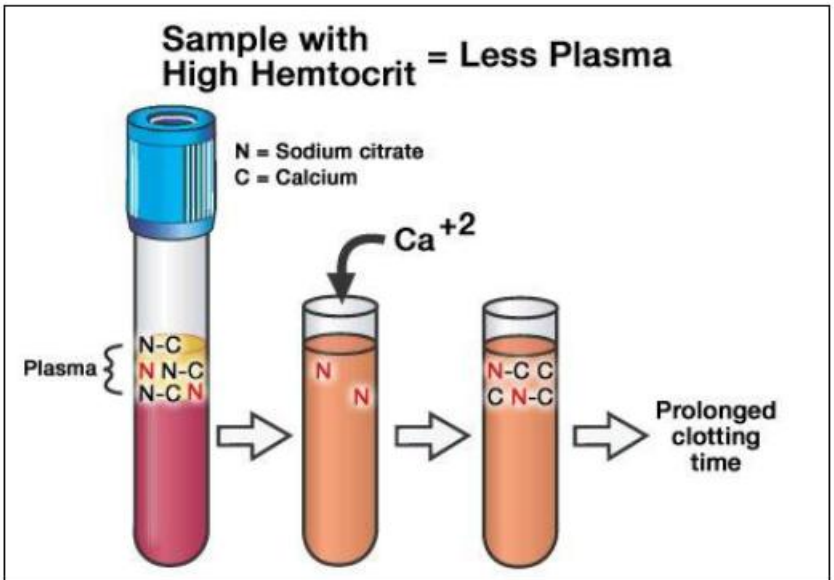
8. Specimen tubes should remain stoppered and kept in an upright position when not being used.

   Prolonged exposure of the plasma to air results in a pH change, which may result in invalidly prolonged clotting times.

9. Specimens with high or low hematocrit values cause the blood-to-anticoagulant ratio to be incorrect. Therefore, whenever a patient's hematocrit is greater than 55% or less than 20%, the specimen must be recollected using the proper amount of anticoagulant calculated as follows.

$$\text{Volume of Na citrate} = \frac{100 - \text{Hct}}{595 - \text{Hct}} \times \text{mL of blood used}$$

A high hematocrit (>55%) has the same effect as an underfilled sodium citrate tube; there is too little plasma for the amount of sodium citrate in the collection tube. As a result, there will be excess free (unbound) sodium citrate remaining after all of the calcium in the sample has been consumed. When calcium is added as a reagent in the test system, the free sodium citrate remaining in the sample will bind some of the reagent calcium, making it unavailable for testing and falsely prolonging test results.



The reverse situation can occur if the patient is very anemic. If a sample has a hematocrit of less than 20%, the amount of sodium citrate in the collection tube is insufficient for the amount of plasma. This will result in excess calcium remaining in the sample after all of the sodium citrate is consumed, and clot formation may occur. The following procedure would be used to prepare a special collection tube when the hematocrit value is <20% or >55%.

a. Determine how much blood needs to be collected to perform all of the required coagulation tests.

b. Calculate the appropriate amount of sodium citrate to use for the amount of blood required.

Example: Given a hematocrit of 61%, determine the amount of sodium citrate to use if 5 mL of blood is to be collected.

$$\text{Volume of NaCirate} = \frac{100-61}{595-61} \times 5\text{ mL}$$

$$= \frac{39}{534} \times 5\text{ mL}$$

$$= 0.37\text{ mL} = \boxed{0.4\text{ mL}}$$

c. Add the appropriate amount of sodium citrate to a plastic tube or glass tube with non-wettable interior.

d. Label the tube with the appropriate information for collecting the specimen.

Example: Add 5 mL whole blood and mix well.

e. Collect the blood specimen with a syringe and add the proper amount to the specially prepared sodium citrate tube. Blood in excess (approximately 1 mL) of the amount required should be collected into the syringe. The blood drawn first into the syringe, which may contain procoagulants such as tissue factor, should not be used for testing.

10. Coagulation testing should always be performed as soon as possible. However, if necessary, specimens for prothrombin time testing may be held at 2^{o}C to 4^{o}C or 18^{o}C to 24^{o}C for up to 24 hours. Specimens collected for APTT testing may also be stored at 2^{o}C to 4^{o}C or 18^{o}C to 24^{o}C for up to 4 hours. If testing is to be delayed for longer than the specified time interval, samples must be quick frozen and can be stored at –20^{o}C for up to 2 weeks or -70^{o}C for up to 6 months. Frozen plasma samples must be thawed quickly at 37^{o}C. Samples can be tested immediately after thawing or can be held at 2^{o}C to 4^{o}C for a maximum of 2 hours.

Did You Know?

Many laboratory procedures require the use of a centrifuge, either for preparation of the sample or as a step in the test method. In any given laboratory, most of the procedures will specify the speed at which centrifugation must occur in terms of rpm, or revolutions per minute. However, the speed of centrifugation in rpm does not accurately describe the amount of force required to separate two phases in a centrifuge. This force is more accurately described in terms of relative centrifugal force (RCF). RCF units are expressed as the number of times greater than gravity (e.g., 5000 X g). While generic laboratory procedures, such as those proposed by CLSI or those found in textbooks, specify centrifugation requirements in terms of RCF (X g), individual laboratories will specify centrifugation requirements as rpm when they implement these procedures for their own use.

Refer to the document entitled *Centrifugation: Converting RCF to RPM*.

PERFORMING COAGULATION TESTS

1. Most coagulation studies are carried out at 37°C. All reagents and samples should be warmed to 37°C prior to testing. Heating blocks and incubators should be monitored closely, and should not fluctuate more than +1°C from 37°C.

   Most activated coagulation factors are proteolytic enzymes that function optimally at 37°C. Therefore, temperatures below 37°C cause falsely prolonged clotting times. Overheating or prolonged heating at 37°C may also cause prolonged clotting times due to deterioration of some labile coagulation factors.

2. Timing of many coagulation tests is very important. Accurate timing devices should be used, and procedures closely followed.

3. The following rules apply to coagulation reagents:

   a. Always follow the manufacturer's instructions when preparing reagents.
   b. Date, time, and initial reagents when prepared.
   c. Prepared reagents should not remain at room temperature for extended periods of time. Follow the manufacturer's instructions for storage and stability.
   d. When using reagents, vial caps should not be interchanged. Care must be taken to avoid cross contamination of reagents.

Did You Know?

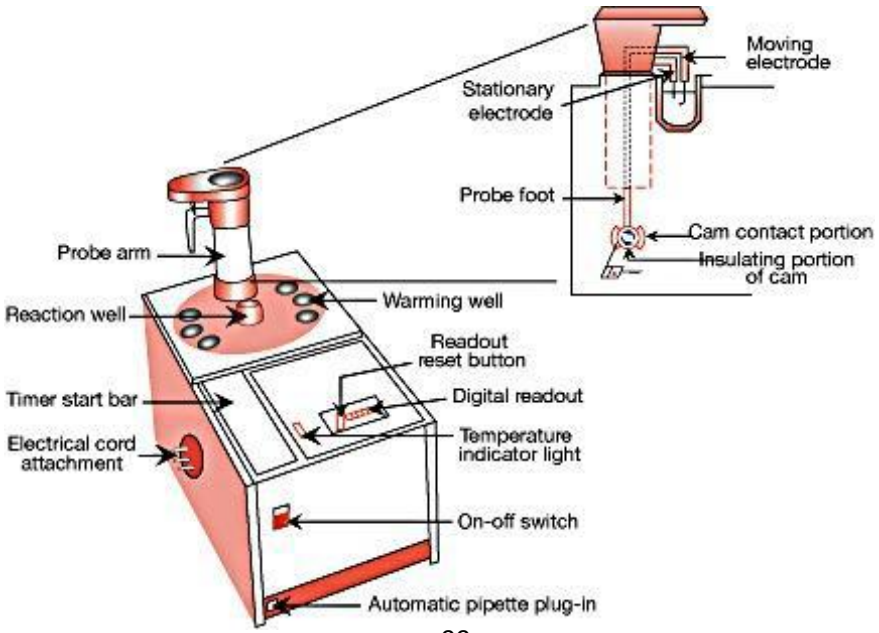
There are two types of expiration dates that apply to reagents. The date that is stamped on the side of the reagent container by the manufacturer is the **closed vial expiration date**. This means that if the unopened reagent is stored under the appropriate conditions (i.e., temperature, humidity, etc.) as recommended by the manufacturer, it will remain stable for the indicated period of time. Once the reagent container is opened or the reagent is prepared, a different expiration date applies. The period of time during which the prepared or opened reagent is stable is called the **open vial stability**. In order for opened or prepared reagents to remain stable for the specified period of time, they must be handled properly (i.e., proper storage temperature). Upon opening or preparing a reagent, the technician must label the vial or container with the date and time plus his/her initials.

TESTING METHODS

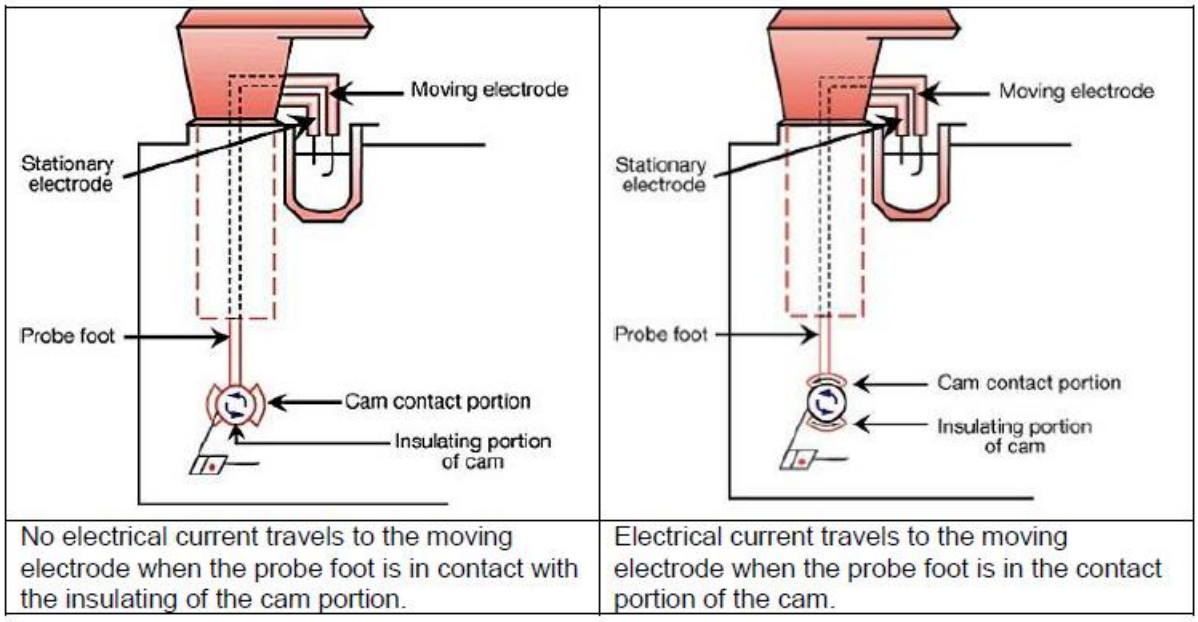
There are numerous instruments available for performing coagulation tests. These instruments can be categorized based upon the end point detection method employed as mechanical, viscocity detection, photo-optical, chromogenic, or immunologic.

MECHANICAL CLOT DETECTION

In the laboratory, you will be using a semi-automated mechanical instrument called the Fibrometer. It is based upon the principle that a fibrin clot will conduct electric current. The term **electromechanical**, a more descriptive term for this type of mechanical clot detection, is commonly used to describe the fibrometer. The components of the Fibrometer are shown below.

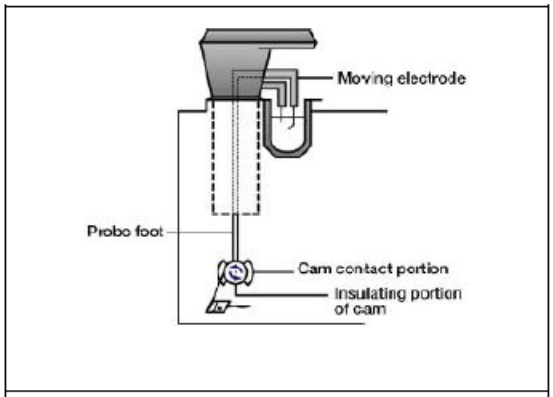


The probe arm has two electrodes: a **stationary electrode** and a **moving electrode**. The moving electrode is attached to the **probe foot**. When seated in the instrument during operation, the probe foot is in contact with a **rotating cam**. The cam has two sections; a raised, **insulated section** and a **conducting section**. As the cam rotates, the probe foot goes up and down, causing the moving electrode to move up and down as well. When the moving electrode is in the down position, the probe foot is in contact with the insulating portion of the cam. This means that electrical current is not conducted from the base of the instrument through the probe foot and into the moving electrode. However, when the moving electrode is in the up position, the probe foot is in contact with the conducting part of the cam, and current is able to flow from the base of the instrument to the moving electrode.

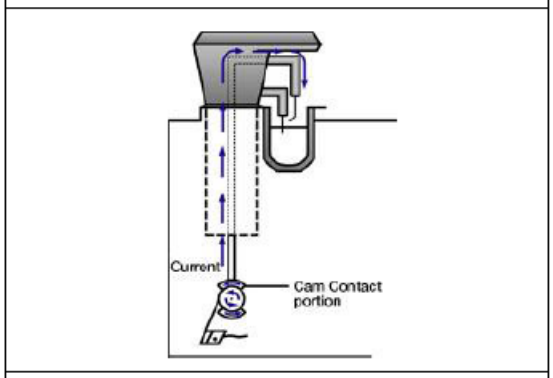


No electrical current travels to the moving electrode when the probe foot is in contact with the insulating of the cam portion.	Electrical current travels to the moving electrode when the probe foot is in the contact portion of the cam.

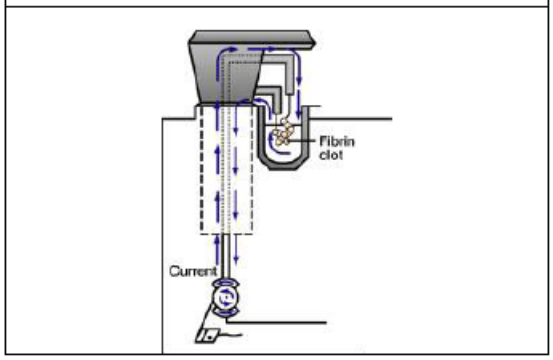
When performing a test using the Fibrometer, reagents and sample are added to a sample cup in the reaction well and the timer is started. During clot detection, the moving electrode moves up and down. When it is in the down position, the probe foot is in contact with the insulating portion of the cam, and, therefore, no current is flowing to the moving electrode. When the moving electrode is in the up position, the probe foot is in contact with the conducting portion of the cam and current flows to the moving electrode but cannot pass to the stationary electrode because the two electrodes are two far apart. As the clot forms, it bridges the gap between the two electrodes, which results in completion of the electric circuit. When the instrument senses that the circuit is complete and electrical current is flowing between the electrodes, the timer stops.



When the moving electrode is in the down position, the probe foot is in contact with the insulating portion of the cam, and, therefore, no current is flowing to the moving electrode.

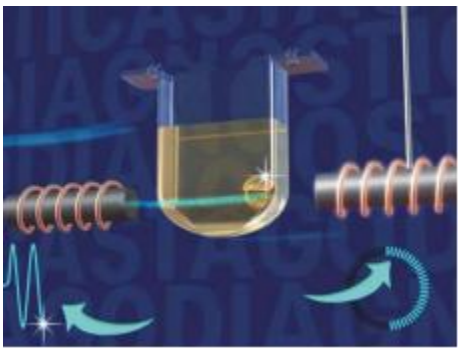


When the moving electrode is in the up position, the probe foot is in contact with the conducting portion of the cam and current flows to the moving electrode but cannot pass to the stationary electrode because the two electrodes are too far apart.



As the clot forms, it bridges the gap between the two electrodes and results in completion of the electric circuit. When the instrument senses that the circuit is complete and electrical current is flowing between the electrodes, the timer stops.

An alternate method of mechanical clot detection involves the **Viscosity Detection System.** This method of mechanical clot detection involves the magnetic movement of a steel ball. Electromagnetic fields alternatively on each side of the cuvette maintain a swinging motion. The principle consists in measuring the variation of the ball's oscillation amplitude. A decrease of the amplitude corresponds to an increase of the medium viscosity, i.e. to the phenomenon of coagulation. As the clot forms, the slowing of the ball is detected by a sensor and the endpoint is triggered as seen below:

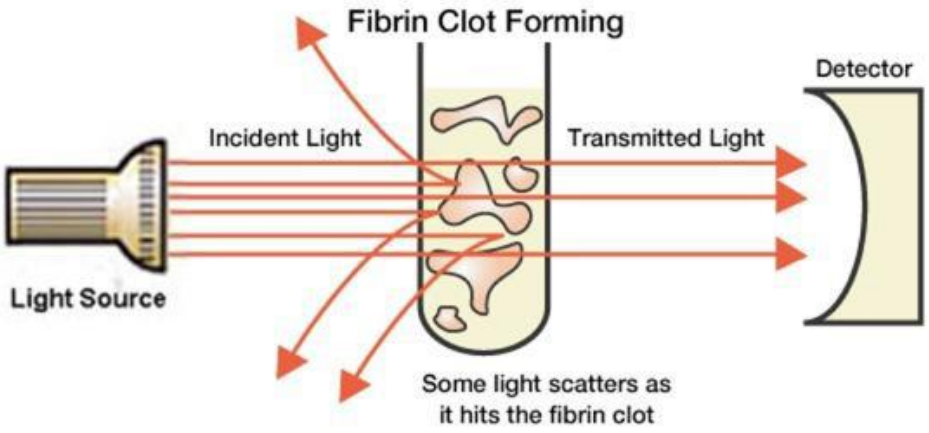


This method greatly reduces the interference from lipemia, icterus or hemolysis - maximizing accuracy and precision. Local infra-red lighting allows light to penetrate the medium and minimizes the interference of outside light. The motion of the balls is captured by a camera.

PHOTO-OPTICAL CLOT DETECTION

There are numerous automated coagulation instruments that utilize photo-optical clot detection methods. Some of these instruments utilize the principle of turbidimetry while others are based upon the principle of nephelometry. Yet another method is a photo-optical detection method using magnetic fields called viscosity detection system.

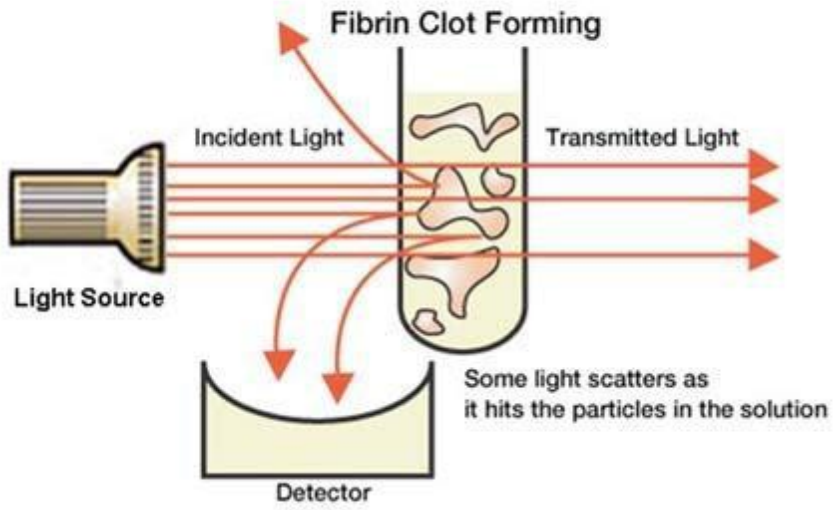
Turbidimetry is based upon the fact that, as the clot forms, less light is able to pass through, or be transmitted through, the sample. A decrease is light transmittance, therefore, indicates clot formation and triggers the endpoint of the test. The instrument used to perform turbidimetric analysis is basically a spectrophotometer, with a light source that provides the light that will shine upon the sample (incident light), a reaction well where the clot forms, and a detector that determines the amount of light that is transmitted through the sample. The components of the instrument are arranged in a linear fashion.



The procedure for performing a turbidimetric coagulation test is summarized as follows:

1. The sample and test reagents are mixed together in the reaction well.
2. The instrument takes a baseline reading of the amount of light being transmitted through the sample.
3. As the clot forms, the sample becomes turbid and less light is able to pass through to reach the detector.
4. The endpoint is detected when the amount of transmitted light decreases by a certain amount.

Nephelometry is based upon the fact that, as the clot forms, it scatters light. The components of the instrument used to perform nephelometry are similar to those of the instrument used to perform turbidimetry. However, the arrangement of the components varies in that the detector is located at a 90° angle to the reaction well in order to detect scattered light rather than transmitted light.



The procedure for performing a nephelometric coagulation tests is summarized as follows:

1. The sample and test reagents are mixed together in the reaction well.
2. The instrument takes a baseline reading of the amount of light being scattered by the sample.
3. As the clot forms, the sample becomes turbid and the amount of light being scattered increases.
4. The endpoint is detected when the amount of scattered light increases by a certain amount.

CHROMOGENIC END POINT DETECTION

Some of the more sophisticated coagulation instruments available today combine the traditional clot-based detection principles of nephelometry or turbidimetry with chromogenic analysis. Chromogenic analysis involves chemical reactions that produce color changes. Since the activated coagulation factors are proteolytic enzymes, they can catalyze chemical reactions, producing colored end products. The amount of color, which is determined spectrophotometrically, is related to the amount of end product produced, which is directly proportional to the amount of enzyme (activated coagulation factor).

IMMUNOLOGIC END POINT DETECTION

Immunologic end point detection systems are based upon antigen-antibody reactions. Tiny latex particles are coated with antibody directed against the analyte in question, which functions as the antigen in the reaction. A monochromatic light shines upon the test well as the reaction occurs. A detector is positioned to determine the amount of light that is transmitted through the sample. As the antibody-coated beads bind with the antigen, agglutination occurs, resulting in the formation of larger particles. These larger particles absorb more light, causing a decrease in light transmission. The amount of light absorbed is proportional to the size of particles formed, which, in turn, is proportional to the amount of antigen (analyte).

PREANALYTICAL VARIABLES

Coagulation assays are highly vulnerable to preanalytical variability for the following reasons:

- Biochemical and cellular reactions are complex.
- Several of the coagulation proteins are labile.
- Prothrombin time and APTT reactions are dependent upon calcium.
- Platelets are highly excitable and central to the assay reaction.

The following patient variables may affect coagulation test results:

- Many coagulation test results vary with patient age. Therefore, age-specific reference ranges are critical for interpretation.
- Patients with group O blood type have less vWF:FVIII activity than other blood groups.
- Platelet activation is highest in the morning, which correlates with a higher incidence of myocardial infarction in the morning.
- Cold weather is associated with increased coagulation activation, which correlates with a higher incidence of myocardial infarction in cold weather.
- Diet and alcohol intake affect coagulation activation in diabetics.
- Smoking elevates plasma fibrinogen, vWF, and thrombin generation.
- Oral contraceptives, pregnancy, and monthly fluctuations in hormone levels are associated with increased coagulation for women.
- Vigorous physical exercise promotes coagulation. For this reason, patients who have been exercising should rest for 15 to 30 minutes before coagulation specimens are collected.
- Psychological stress changes both coagulation and fibrinolysis.
- Diseases leading to too little or too much red blood cell production alter the coagulation test results because of the change in plasma amount affects anticoagulant ratios.
    - Anemia may falsely shorten coagulation clot times.
    - Polycythemia falsely prolongs coagulation clot times.

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 1

I. Evaluation of bleeding disorders
    A. Tests to monitor the vascular system and platelets
        1. Capillary fragility test
            a. Principle
            b. Procedure
            c. Interpretation of results
        2. Bleeding time
            a. Principle
            b. Procedure
            c. Interpretation of results
        3. Platelet count
        4. Platelet aggregation studies
            a. Principle and procedure
            b. Interpretation of results

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 1

EVALUATION OF BLEEDING DISORDERS

Whenever a patient presents with excessive bruising, petechiae, or bleeding, a variety of laboratory tests will be performed to isolate the cause. Which tests are performed will be determined in part by the patient's clinical history and symptoms. Laboratory tests are available to monitor all aspects of the coagulation process, including the vascular system, platelets, coagulation factors, and the fibrinolytic system.

TESTS TO MONITOR THE VASCULAR SYSTEM AND PLATELETS

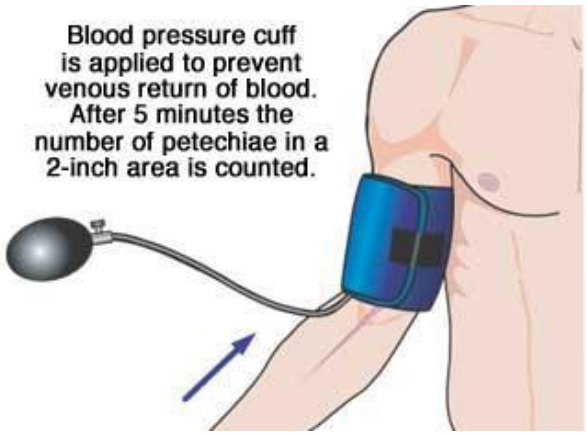
CAPILLARY FRAGILITY TEST

PRINCIPLE

A blood pressure cuff is applied to the arm and inflated with enough pressure to prevent venous return of blood. The resulting increased capillary pressure may cause capillary rupture, which manifests as petechiae. The capillary fragility test is largely dependent upon vascular integrity and stability. Platelet number and function play a less important role.

PROCEDURE

1. A blood pressure cuff is applied to the arm and inflated to between 80 mmHg and 100 mmHg.
2. After 5 minutes, the pressure is released and the number of petechiae in a 2-inch diameter area is counted.



INTERPRETATION OF RESULTS

A score of 1+ in the test area is considered normal. Test results of 2+ to 4+ are abnormal, indicating a vascular defect (capillary weakness), thrombocytopenia, or abnormal platelet function.

BLEEDING TIME

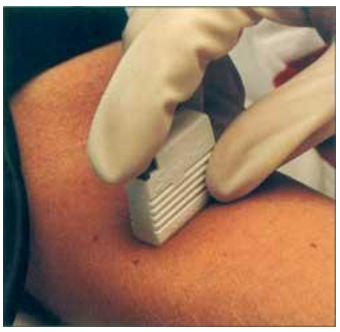
PRINCIPLE The bleeding time measures the amount of time required to stop bleeding from a standardized skin wound. It is largely dependent upon the ability of platelets to form a hemostatic plug. As such, it is an indirect measure of platelet number and function. Vascular contractility plays a secondary role in the bleeding time.

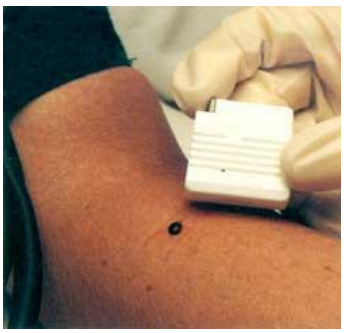
PROCEDURE

1. A blood pressure cuff is placed on the upper arm and inflated to 40 mm Hg.

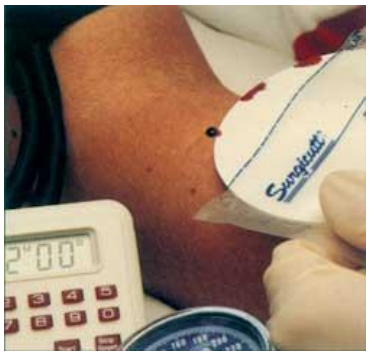
2. The forearm is cleansed in an area free of veins, scars, and bruises approximately 5 cm below the antecubital crease.

3. A spring loaded bleeding time lancet that produces a standardized incision 5 mm long and 1 mm deep is placed firmly on the site parallel to the long axis of the arm and triggered. A stopwatch is started at the moment the lancet is triggered.



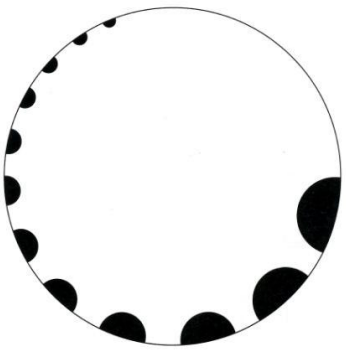


4. At 30-second intervals, the edge of a piece of filter paper is used to blot the blood until the bleeding stops.



NOTE: When blotting the blood with the filter paper, care must be taken to touch only the blood and not disrupt the platelet plug that is forming. Disrupting the platelet plug with falsely prolong the bleeding time results.

5. The length of time it takes for the bleeding time to stop is recorded as the bleeding time.



For the example on the right, each spot of blood represents 30 seconds. Therefore, the recorded bleeding time for this example would be 6 minutes because there are 12 spots.

6. Remove the blood pressure cuff. Carefully cleanse the incision site and apply a pressure bandage.

INTERPRETATION OF RESULTS

Reference intervals for bleeding time vary with the method used; each laboratory should establish its own reference interval. The general reference interval is between 2 minute and 9 minutes. Prolonged bleeding time results may be due to thrombocytopenia, defects in platelet function, or vascular defects. Factor deficiencies will not produce prolonged bleeding time results if the test is properly performed. A single dose of aspirin causes a measurable prolongation of results in approximately 50% of normal individuals. Many other drugs may cause prolonged results.

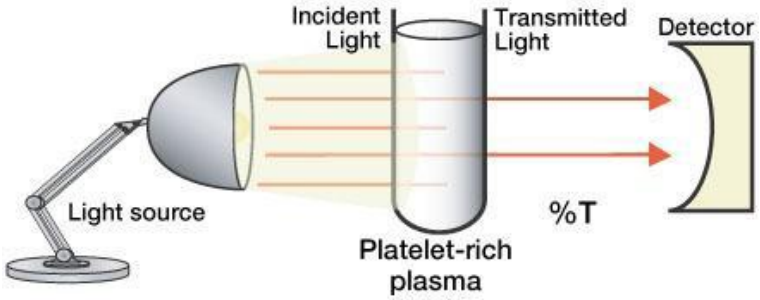
PLATELET COUNT

A platelet count is performed to determine if there are sufficient platelets to form an adequate platelet plug. A low platelet count can occur for two reasons; either there is inadequate production of platelets by the bone marrow or platelets are being produced in adequate numbers but are being consumed or destroyed at an increased rate after being released from the bone marrow. In order to determine which of these two conditions exists, a bone marrow exam may be required. An inadequate number of megakaryocytes in the bone marrow indicates decreased platelet production. An adequate or increased number of bone marrow megakaryocytes in conjunction with a decreased peripheral blood platelet count, on the other hand, indicates that platelets are being produced in sufficient numbers but are being prematurely destroyed in the peripheral circulation.

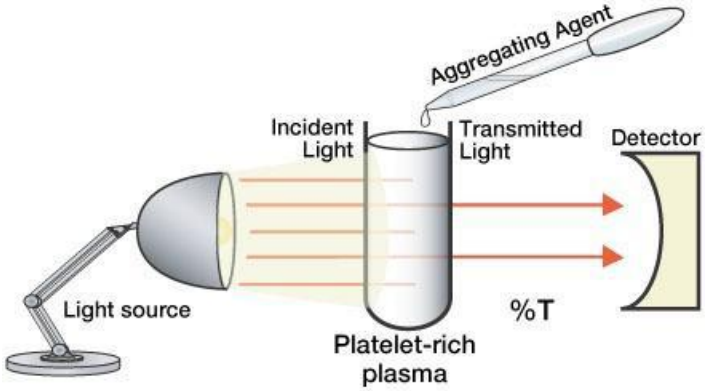
PLATELET AGGREGATION STUDIES

PRINCIPLE AND PROCEDURE

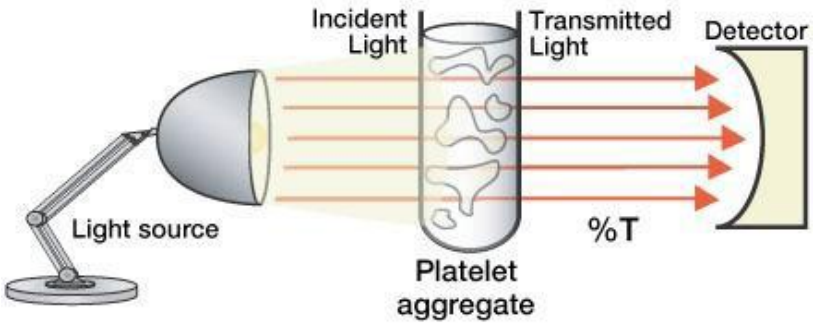
Platelet aggregation studies measure the ability of platelets to stick to one another to form a platelet plug. They are performed using a platelet aggregometer, a photo-optical instrument connected to a chart recorder. Platelet-rich plasma, which is cloudy in appearance, is added to a cuvette along with a plasticized stir bar and warmed to 37oC. The stirring and recorded devices are started. Light transmittance through the platelet-rich plasma is recorded. An aggregating agent (agonist) is added to the cuvette. Platelets begin to aggregate in response to the aggregating agent, resulting in the formation of larger platelet aggregates and a corresponding clearing in the platelet-rich plasma. As the plasma clears, the instrument records the increase in light transmittance.



Platelet rich plasma is cloudy, allowing little light to be transmitted. A baseline transmittance reading (%T) is recorded.



An aggregating agent is added to the platelet-rich plasma.



As platelets aggregate, there is partial clearing of the platelet solution. More light is able to pass through and %T increases.

INTERPRETATION OF RESULTS

Platelet aggregation studies assess all aspects of platelet plug formation, including adhesion, aggregation, and secretion. Aggregometry occurs in five phases: baseline at 0% aggregation, shape change after addition of the agonist, primary aggregation, ADP and ATP release from platelet granules (secretion), and second-wave aggregation that forms large platelet aggregates.

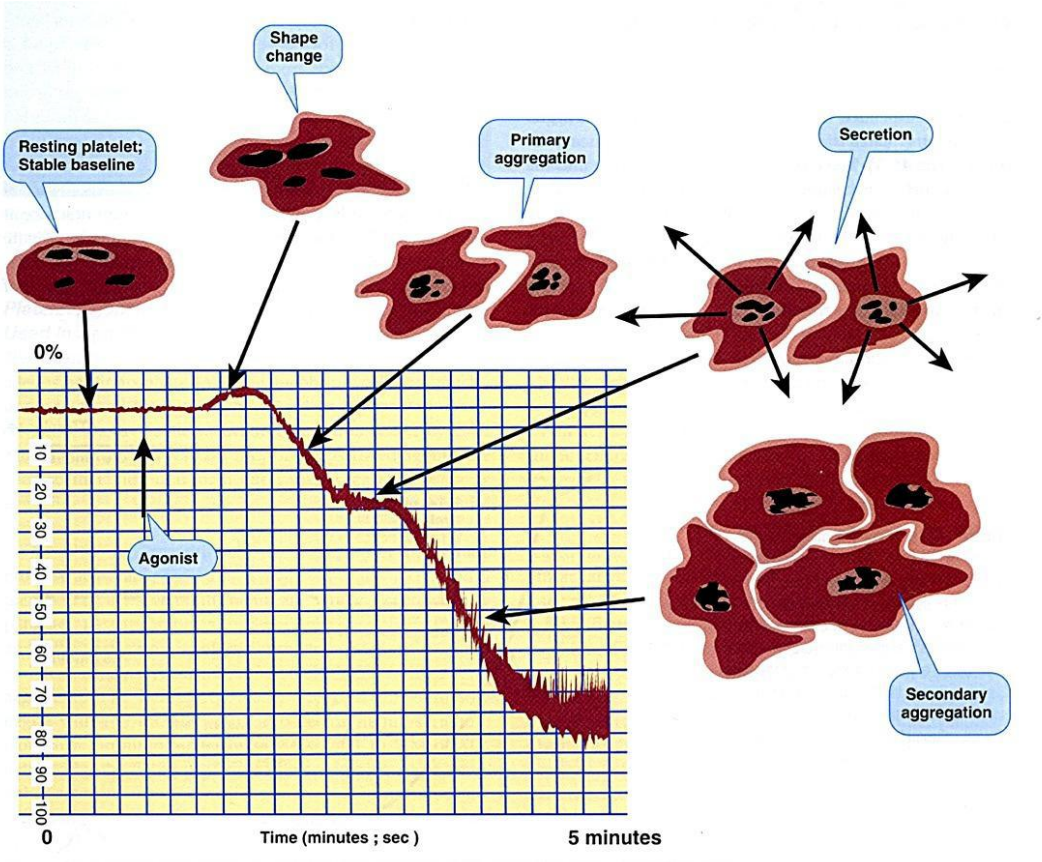
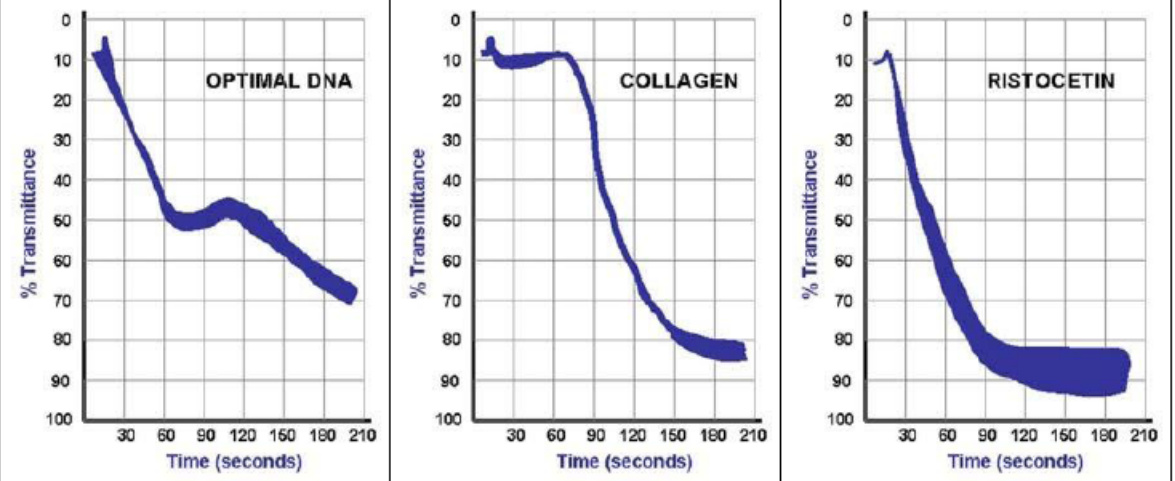


Diagram from Hematology: Clinical Principles and Applications by Rodak, et al

Various aggregating agents (agonists) are used, including ADP, thrombin, collagen, ristocetin, epinephrine, and arachidonic acid. Depending upon the agent used, aggregation occurs in either one or two waves. The primary wave represents the direct response of the platelets to the aggregating agent. The secondary wave represents complete aggregation, which occurs in response to agonist release from platelet granules during primary aggregation. Ristocetin is unique among aggregating agents because its action is dependent upon the interaction of vonWillebrand factor and the glycoprotein Ib/IX/V platelet receptor. Technically, the reaction with ristocetin represents platelet agglutination rather than aggregation.

Normal Response Patterns of Aggregating Agents				
Collagen	**ADP**	**Epinephrine**	**Risotcetin**	**Arachidonic Acid**
Monophasic wave	Biphasic curve with optimal DNA amount	Biphasic curve	Monophasic curve	Monophasic wave: Evaluates the thromboxane A_2 synthesis pathway

Examples of Normal Platelet Aggregation Patterns



Biphasic curve with optimal amount of DNA (2×10^{-5} M)	Monophasic curve with collagen	Monophasic curve with ristocetin

The pattern of responses to the various aggregating agents helps to isolate the platelet abnormality. The table on the following page summarizes the typical response to aggregating reagents in various qualitative platelet disorders.

Disorder	Nature of Defect	Aggregating Agent				
		Collagen	ADP	Epinephrine	Ristocetin	Arachidonic Acid
vonWillebrand disease	Deficiency of vWF	Normal	Normal	Normal	No response	**
Bernard-Soulier syndrome	Platelets lack GB Ib/IX/V receptors	Normal	Normal	Normal	No response	**
Glanzman's thrombasthenia	Platelets lack GP IIb/IIIa receptors	No response	No response	No response	Normal	**
Storage pool disease	Defect or deficiencies of platelet granules	No response	Primary wave only	Primary wave only	Normal	Normal
Aspirin ingestion	Interferes with thromboxane A_2 synthesis	No response	Primary wave only	Primary wave only	Normal	Suppressed response

**Not applicable. Arachidonic acid evaluates the thromboxane A_2 synthesis pathway.

To differentiate between von Willebrand disease and Bernard-Soulier syndrome, a **ristocetin induced platelet aggregation (RIPA) test** can be performed. In this test, exogenous vWF from normal plasma is added to the ristocetin aggregation study. The vWF restores the ristocetin aggregation reaction for all cases of von Willebrand disease except for those classified as subtype 2b. In patients with Bernard-Soulier syndrome, the added vWF does not improve aggregation with ristocetin because the platelets in this disorder lack the glycoprotein Ib/IX/V receptor that binds vWF.

Platelets in vWD subtype 2b aggregate in response to less amount of ristocetin than normal platelets due to mutations in the vWF receptor binding site that raise the affinity for the glycoprotein 1b/V/IX receptor. To subtype vWF 2, a multimer analysis requiring electrophoresis is performed.

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 2

I. Tests to monitor the coagulation factors
    A. Prothrombin Time
        1. Principle
        2. Procedure
        3. Interpretation of results
        4. International normalized ratio (INR)
    B. Activated Partial Thromboplastin Time
        1. Principle
        2. Procedure
        3. Interpretation of results
            a. Anti-factor Xa assay
            b. Activated clotting time
    C. APTT mixing studies for lupus anticoagulant and factor inhibitors
    D. Fibrinogen assay
        1. Principle and procedure
        2. Interpretation of results
    E. Thrombin Clotting Time
        1. Principle and procedure
        2. Interpretation of results
    F. Reptilase Time
    G. Factor Assay
        1. Principle
        2. Procedure
        3. Interpretation of results
    H. Urea Solubility Test
        1. Procedure
        2. Interpretation of results

II. Tests to monitor the fibrinolytic system
    A. Fibrin Degradation Products
        1. Principle
        2. Procedure
        3. Interpretation of results
    B. D-dimer
    C. Euglobulin Clot Lysis Test

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 2

TESTS TO MONITOR THE COAGULATION FACTORS

Tests performed to monitor the coagulation factors are clot-based assays. These assays, which are performed on platelet-poor plasma, measure the time interval from initiation of coagulation to visible clot formation. A prolonged clot-based test result indicates a deficiency or defect in one or more of the coagulation factors.

PROTHROMBIN TIME (PT)

PRINCIPLE

The prothrombin time (PT) is the time required to form a fibrin clot when plasma is added to a tissue factor-calcium-phospholipid reagent. When mixed with citrated platelet-poor plasma, the reagent triggers activation of factor VII. Factor VIIa in the presence of calcium and phospholipid, forms a complex with tissue factor to begin the initiation phase of coagulation, which ultimately progresses to the propagation phase and culminates in fibrin formation. Although the coagulation scheme implies that the PT would be prolonged with deficiencies of fibrinogen (I), prothrombin (II), and factors V, VII, VIII, IX and X, this is not truly the case. The PT is most sensitive to factor VII, is moderately sensitive to factors V and X, sensitive to severe deficiencies of fibrinogen and prothrombin, and insensitive to factors VIII and IX. Traditionally, it has been said that the PT measures factors involved in the extrinsic coagulation pathway, including VII, which is unique to the extrinsic pathway, and factors I, II, V, and X, which represent the common pathway. Based upon the PT's sensitivity to the various coagulation factors as stated above, this is actually true despite new theories of coagulation that put less emphasis on the coagulation cascade.

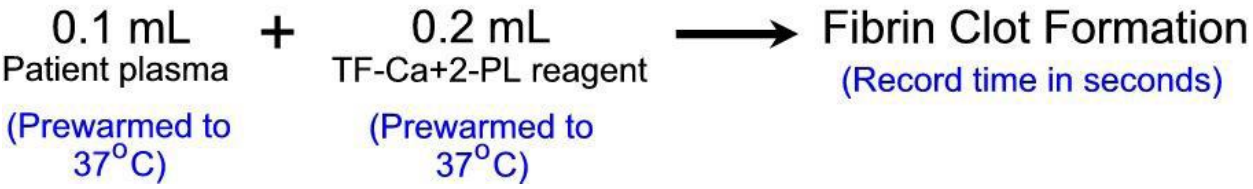
The PT can be used to screen for acquired or inherited deficiencies fibrinogen (I), prothrombin (II) and factors V, VII and X. The most frequent clinical application of the PT, however, is to monitor the course of warfarin (coumarin) oral anticoagulant therapy. Warfarin functions as a therapeutic anticoagulant by interfering with the carboxylation of the vitamin-K dependent plasma factors (II, VII, IX, X) in the liver by interrupting the enzymatic phase of this reaction. This results in the production of non-functional factors II, VII, IX and X. These non-functional factors are referred to as ***p***roteins ***i***nduced by ***v***itamin ***K a***ntagonist (PIVKAs), or noncarboxylated K-dependent factors. The PT is the test of choice for monitoring warfarin therapy because factor VII, a factor measured by this test, has a very short half-life and is the first factor affected by warfarin therapy. In addition, two other factors that are affected by warfarin therapy (II and X) are measured by the PT.

PROCEDURE

The only reagent used when performing the PT is tissue factor-calcium-phospholipid reagent, which was previously called thromboplastin-C and now goes by various names depending on the manufacturer. The tissue factor is needed to initiate the tissue factor 2 pathway (extrinsic pathway) by activating factor VII. In vivo, factor VII is exposed to on the surface of subendothelial cells following vessel injury. Calcium and phospholipid are needed at various points of the coagulation pathway. The calcium in the original sample is bound by

sodium citrate to prevent the sample from clotting while the phospholipid, which is provided in vivo by platelet, is removed by centrifugation and preparation of a platelet poor plasma sample. As such, both of these components are not present in the plasma sample and must be added as reagents in order for fibrin clot formation to occur.

The PT procedure is summarized as follows.



INTERPRETATION OF RESULTS

The normal range for the PT varies with test reagents and methods, but is approximately 11.0 seconds to 14.0 seconds. Patients on warfarin oral anticoagulant therapy or patients with hereditary or acquired deficiencies of factors I, II, V, VII, and/or X will have prolonged PT values. However, the PT is not very sensitive to these factor deficiencies. As a general rule, only a 30% to 40% concentration of factors II, V, VII and X, and a fibrinogen (factor I) concentration of greater than 100 mg/dL are needed to produce a normal value for the PT.

INTERNATIONAL NORMALIZED RATIO (INR)

The INR is used to standardize PT results from institution to institution. The PT results on a given sample will vary depending upon the test method and sensitivity of reagent used. (The more sensitive the thromboplastin reagent, the more prolonged the PT results on plasma from patients receiving coumarin.) Reported INR values are independent of the reagents and test methods used, and therefore, provide a more standardized, consistent approach to warfarin dosing. The INR is calculated from the PT value as follows:

$$INR = R^{ISI} \quad \text{where } R = \frac{\text{Patient PT value}}{\text{Mean of PT normal range}}$$

The ISI value is supplied by the manufacturer and varies with type of reagent, lot number of reagent, and test method.

Two categories of treatment levels are recommended based upon INR values:

- For patients being treated for pulmonary emboli, venous thrombosis, acute myocardial infarction, atrial fibrillation, hereditary deficiencies of the biochemical coagulation inhibitors, (i.e., AT), presurgical prophylaxis, or tissue heart valves, the recommended range for INR is 2.0 - 3.0 with corresponding PT values of approximately 14 seconds to 17 seconds.

- For patients with mechanical prosthetic heart valves or recurrent systemic embolism, the treatment should be more intensive, resulting in INR values of 2.5 to 3.5 and corresponding PT values of 17 seconds to 22 seconds.

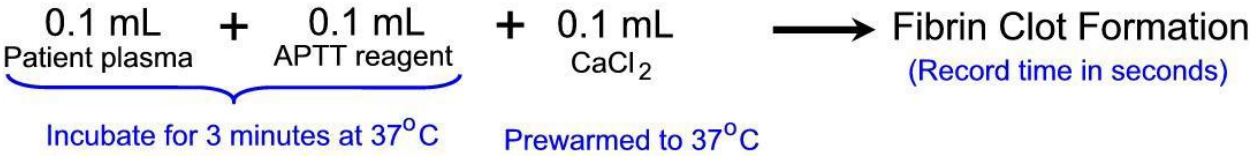
ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

PRINCIPLE

The APTT is a screening test used to measure factors in the intrinsic (VIII, IX, XI, XII) and common (I, II, V, X) pathways. The APTT can be used to screen for acquired or hereditary deficiencies of these factors. The most frequent clinical application of the APTT, however, is to monitor standard (unfractionated) heparin anticoagulant therapy. Heparin functions as a therapeutic anticoagulant by enhancing the effect of anti-thrombin (AT). AT is a biochemical coagulation inhibitor that deactivates factors IIa (thrombin), IXa, Xa, XIa, XIIa, and kallikrein.

PROCEDURE

Plasma is incubated with APTT reagent (previously called activated cephaloplastin or cephalin), which contains phospholipid and a negatively charged particulate activator such as kaolin, ellegic acid, or Celite. The phospholipid serves as a substitute for platelet phospholipid that is removed when platelets are eliminated from the plasma sample by centrifugation. The particulate activator provides a surface for contact activation of factor XII to begin the intrinsic coagulation pathway. At the end of the incubation period, calcium chloride is added and the time required to form a fibrin clot is measured. (The calcium in the original sample is bound by sodium citrate to prevent the sample from clotting and is no longer available for the coagulation cascade. Therefore, calcium must be added.)



INTERPRETATION OF RESULTS

The normal range for the APTT varies with test reagents and methods, but is approximately 22.0 seconds to 34.0 seconds. Patients on standard (unfractionated) heparin therapy or patients with hereditary or acquired deficiencies of factors I, II, V, VIII, IX, X, IX, and/or XII will have prolonged APTT values. However, the APTT is not very sensitive to these factor deficiencies. As a general rule, only a 30% to 40% concentration of factors II, V, VIII, IX, X, XI, and XII and a fibrinogen (factor I) concentration of greater than 100 mg/dL are needed to produce a normal value for the APTT. The APTT value will also be prolonged in the presence of lupus anticoagulant, an immunoglobulin with an affinity for phospholipid bound proteins, and anti-factor VIII antibody.

NOTE: Although factor XII does not play a significant role in *in vivo* blood coagulation, it plays an important role *in vitro* coagulation; contact activation of factor XII begins the intrinsic pathway. Therefore, individuals with a factor XII deficiency do not experience bleeding but will have prolonged APTT values.

There are two ways in which to use the APTT is adjust standard heparin dosage.

- Adjust the heparin dose so that the APTT value following heparin therapy is about 1.5 times to 2.5 times the patient's baseline APTT (the APTT value before any heparin is administered). This corresponds to a heparin dose of 0.2 to 0.4 units/mL.
- Utilize a heparin response curve to determine how the APTT values respond to various heparin doses.

The APTT is not a valid test to monitor heparin therapy using low molecular weight heparin (LMWH). If monitoring is deemed necessary when administering LMWH, the **chromogenic anti-factor Xa heparin assay** is the test of choice. A standard curve must be prepared to interpret test results and adjust the heparin dose.

The **activated clotting time (ACT)** may be used to monitor heparin therapy in settings where the APTT cannot be performed, such as in a clinic, at the patient's bedside, or in the surgical suite. To perform the ACT, 2 mL. of blood in collected into an evacuated tube containing diatomaceous earth, a particulate clot activator. As soon as the specimen in collected, a timer is started and the tube is inverted a few times to disperse the clot activator. The phlebotomist continues to invert the tube until a clot forms. The median normal ACT is approximately 98 seconds. Sufficient heparin is administered to achieve ACT results of 180 to 240 seconds for patients with deep vein thrombosis or 400 seconds for patients undergoing coronary bypass surgery. Various instruments that perform the ACT are also available.

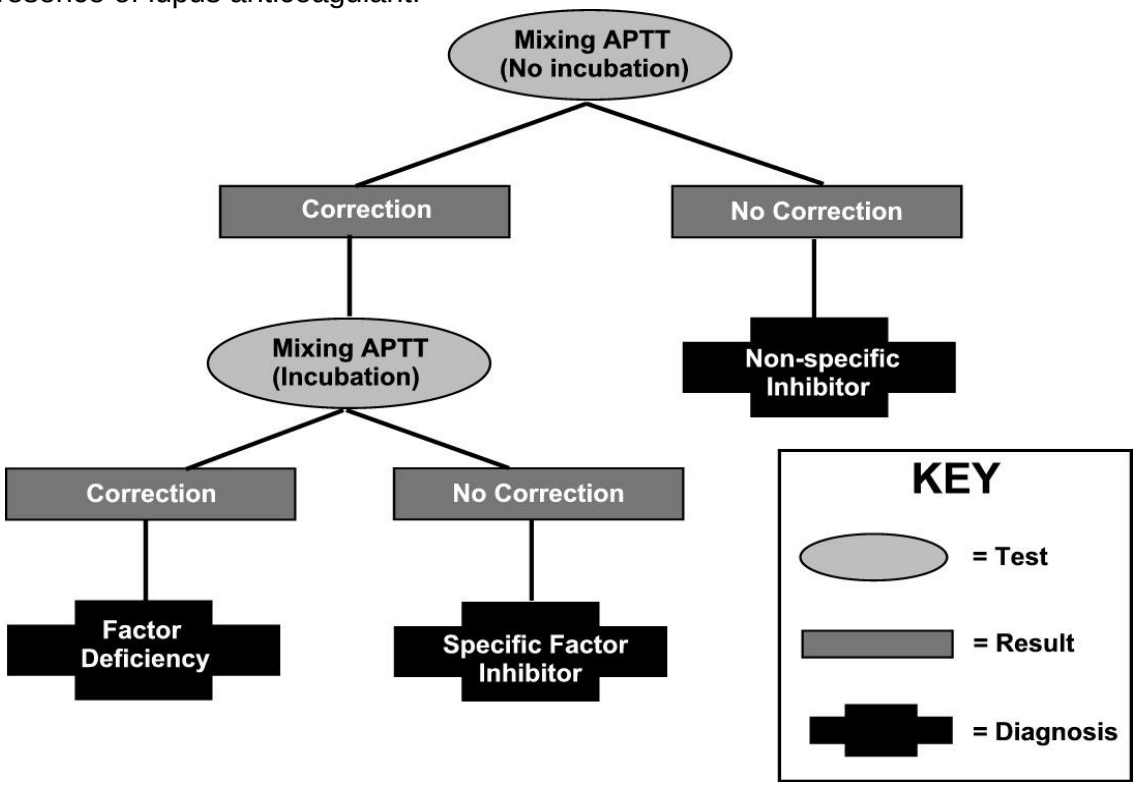
ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT) MIXING STUDIES FOR LUPUS ANTICOAGULANT AND FACTOR INHIBITORS

APTT mixing studies may be performed to screen for the presence of lupus anticoagulant or specific factor inhibitors. **Lupus anticoagulant (LA)** is an IgG immunoglobulin directed against several phospholipid-protein complexes. In the test tube, LA attacks the phospholipid reagent, rendering it incapable of participating in fibrin formation and causing the APTT and other phospholipid-dependent coagulation assays to be prolonged. Clinically, the patient with LA has an increased risk of thrombosis. Various mechanisms have been proposed to explain the increased risk of thrombosis, including inhibition of endothelial activation of protein C and inhibition of endothelial cell production and/or release of prostacyclin. Because LA has a variety of targets, it is called a **nonspecific inhibitor**. Patients with LA have thrombotic tendencies rather than bleeding problems.

Specific factor inhibitors are IgG immunoglobulins directed against specific coagulation factors, such as factors VIII and IX. They may arise in response to factor concentrate treatment in severe hemophilia and, as such, represent an immune response to the factor concentrate. Inhibitors to most coagulation factors have been described. Anti-factor VIII, the most common of the specific inhibitors, is detected in 10% to 20% of severe hemophiliacs, while anti-factor IX is detected in 1% to 3% of factor IX deficient patients.

Lupus anticoagulant, factor deficiencies, and specific factor inhibitors may cause an unexpected prolonged APPT result. APTT mixing studies are necessary to distinguish between these three conditions. The procedure for performing APTT mixing studies is as follows:

1. Perform a thrombin clotting time (TCT) to determine if heparin is the cause of the prolonged APTT. A normal value for the TCT indicates that no heparin is present and additional testing must be performed. Continue to step 2.
2. Mix equal parts of the patient's plasma and pooled normal plasma. Perform an APTT on the dilution. If the APTT of the mixture is within 10% of the APTT of the pooled normal plasma, a correction has occurred. This indicates that a factor deficiency may exist; the pooled plasma, which contains sufficient amounts of all factors, has provided the missing factor(s) to correct the APTT. Before testing to isolate the factor deficiency is performed, however, more testing to rule out the presence of a specific factor inhibitor is necessary; proceed to step 3. If the initial mixture is not corrected by the pooled normal plasma, lupus anticoagulant is suspected; proceed to step 4.
3. Mix a second aliquot of patient plasma with an equal amount of pooled normal plasma and incubate for 1 to 2 hours at 37^{o}C. The activity of factor specific inhibitors such as anti-factor VIII are enhanced by incubation at 37^{o}C. If, after the incubation period, the APTT is corrected, the presence of factor deficiency is supported. If, on the other hand, the APTT is prolonged (uncorrected), a specific factor inhibitor is indicated. The **Bethesda titer** is used to confirm the presence of specific factor inhibitors.
4. If the APTT on the initial mixture (step 1) is not corrected, a non-specific inhibitor such as lupus anticoagulant is suspected. An APTT is performed on a new aliquot of patient plasma using a kit with high-concentration phospholipid (contains phospholipid in excess of what the lupus anticoagulant will neutralize). Shortening of the high-phospholipid APTT assay by at least 8 seconds compared with the original APTT confirms the presence of lupus anticoagulant.



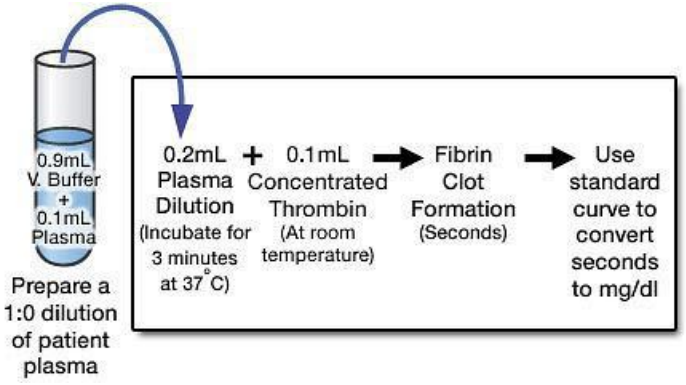
FIBRINOGEN ASSAY

PRINCIPLE AND PROCEDURE

The fibrinogen assay is performed to quantify the amount of fibrinogen (factor I) in plasma. In this procedure, an excess amount of thrombin is added to a specimen of diluted plasma and the time required to form a fibrin clot is measured. This result is compared with clotting times of plasmas containing known amounts of fibrinogen using a standard curve (calibration curve).

Reagents used to perform this procedure include:

- Veronal buffer: An isotonic barbital buffer (pH 7.35) that is used to dilute the sample. Without dilution, plasma clotting times as determined by this procedure would be so short that they would be difficult to measure.
- Thrombin: A commercially prepared lyophilized preparation of bovine thrombin that is highly concentrated (50 NIH units/mL).



INTERPRETATION OF RESULTS

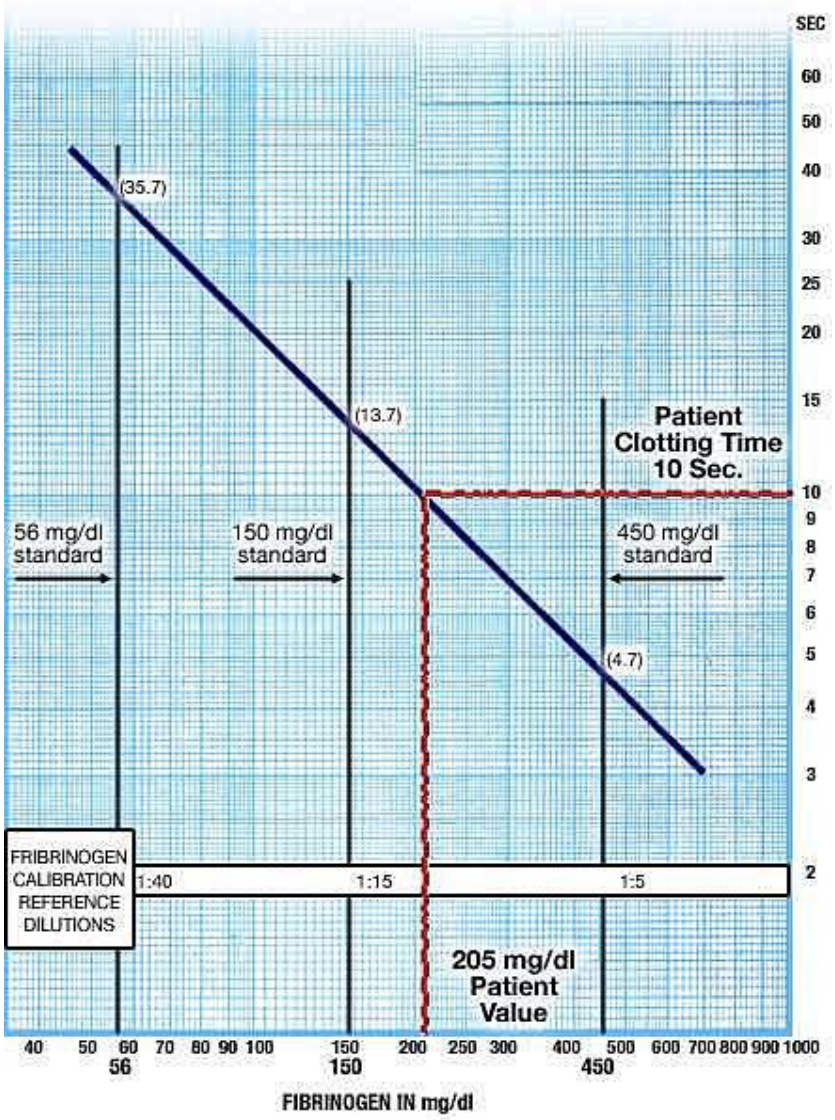
The clotting time in seconds in converted to mg/dL using a standard curve. The reference interval for fibrinogen is 150 mg/dL to 350 mg/dL.

The fibrinogen assay is performed to evaluate hereditary and acquired deficiencies of fibrinogen.

Hereditary deficiencies include:
Afibrinogenemia: No measurable fibrinogen
Hypofibrinogenemia: Fibrinogen levels of less than 100 mg/dL

Acquired fibrinogen deficiencies are seen in liver disease, disseminated intravascular coagulation (DIC), and fibrinolysis.

FIBRINOGEN STANDARD CURVE



THROMBIN CLOTTING TIME

PRINCIPLE AND PROCEDURE

The thrombin clotting time is the time required to convert fibrinogen to fibrin. In this respect, it is similar to the fibrinogen determination. However, since the thrombin reagent used to perform the thrombin time is much less concentrated than the thrombin reagent used to perform a fibrinogen determination (2 NIH units/mL as opposed to 50 NIH units/ mL), it makes the test much more sensitive to thrombin inhibitors (e.g., heparin), fibrin degradation products, and dysfunctional fibrinogen molecules.

INTERPRETATION OF RESULTS

The thrombin time is reported in seconds. The reference interval is 15 seconds to 20 seconds. Prolonged results will occur as a result of low fibrinogen levels (usually less than 100 mg/dL), the presence of thrombin inhibitors (e.g., heparin), increased FDPs, and dysfunctional fibrinogen molecules. The thrombin time is reported in seconds. The reference interval is 15 seconds to 20 seconds. Prolonged results will occur as a result of low fibrinogen levels (usually less than 100 mg/dL), the presence of thrombin inhibitors (e.g., heparin), increased FDPs, and dysfunctional fibrinogen molecules.

REPTILASE TIME

The reptilase time is another procedure that monitors the time it takes to convert fibrinogen to fibrin. However, this procedure utilizes reptilase rather than thrombin. Reptilase is a proteolytic enzyme found in the venom of the Bothrops atrox snake that is capable of converting fibrinogen to fibrin. Because it has a different mechanism of hydrolyzing fibrinogen, the enzyme forms a more fragile clot than the one formed with thrombin. The advantage of the reptilase time is that it is not affected by heparin and is minimally affected by FDPs. Therefore, it serves as a test to detect hypofibrinogenemia in patients receiving heparin therapy. Normal values are approximately 15 seconds to 20 seconds.

Comparison of TCT and Reptilase Time Results		
TCT	**Reptilase Time**	**Defect**
Infinitely prolonged	Infinitely prolonged	Dysfibrinogenemia
Infinitely prolonged	Infinitely prolonged	Afibrinogenemia
Prolonged	Equally prolonged	Hypofibrinogenemia
Prolonged	Normal	Heparin
Prolonged	Slightly to moderately prolonged	FDPs

FACTOR ASSAY

PRINCIPLE

A factor assay is performed to quantitate the level of activity of a single deficient factor. Analyzing the results of other coagulation tests, including the PT, APTT, and mixing studies, isolates the deficient factor. The factor assay involves mixing the patient sample with commercially prepared plasma that lacks the deficient factor but contains all other factors that are necessary for clot formation. For example, when performing a factor VIII assay on a hemophilia A patient, the patient's plasma is mixed with factor VIII deficient plasma (plasma that lacks only factor VIII). Coagulation tests are then performed on the patient plasma-factor deficient plasma mixture to determine how much the patient's plasma is able to correct the specific factor deficient plasma. The amount of correction is totally dependent upon the amount of factor VIII present in the patient's plasma. Further explanation is needed to clarify the concept of the factor assay. Let's look at the case of a patient with hemophilia A. A patient with hemophilia A has a hereditary deficiency of factor VIII. As a result, he will have a prolonged APTT value in combination with a normal PT value. (Remember, the APTT measures factors I, II, V, VIII, IX, X, XI, and XII, while the PT measures factors I, II, V, VII, and X.) The level of factor VIII activity present in hemophilia A can range from 0% to 20%. To determine the level of factor VIII activity, a factor assay must be performed. To do this, the patient's plasma is combined with factor VIII deficient plasma (plasma that lacks only factor VIII but has all other factors needed for clot formation). If an APTT were performed on the factor VIII deficient plasma alone, the results would be infinitely long. In other words, since there is no factor VIII, fibrin clot formation would not occur. If we mix this factor VIII deficient plasma with normal plasma that contains 100% factor VIII and repeat the APTT, the results of the APTT would be within the normal range. This is because the normal plasma provides sufficient factor VIII to compensate for (or correct for) the total lack of VIII in the factor deficient plasma. (Remember, it requires only 30% to 40% of factor VIII activity to produce a normal APTT value.) If we mix the factor VIII deficient plasma with plasma that has only 50% of normal factor VIII activity and repeat the APTT, clot formation would occur, but the value for the APTT would be prolonged. In other words, the patient plasma has enough factor VIII to partially, but not fully, correct the APTT of the factor VIII deficient plasma. If we mix the factor VIII deficient plasma with plasma from a hemophilia A patient that has no factor VIII, the plasma mixture would have no factor VIII and the APTT values would be infinitely prolonged. In other words, the patient's plasma provides no correction of the factor deficient plasma's APTT. In order to determine how the APTT value (which is reported in seconds) correlates to percent factor activity, a standard curve must be prepared. When preparing a standard curve, a series of dilutions of normal plasma (which contain 100% factor activity) and factor deficient plasma are prepared. Each dilution represents a different standard with a different level of factor activity. An APTT is performed on each of the dilutions (standards). The time in seconds for each dilution (standard) is plotted against the concentration of factor to prepare the standard curve.

When the unknown patient sample is mixed with factor deficient plasma, the APTT value of the mixture (reported in seconds) is converted to percent activity using the standard curve. The APTT is performed when assaying for factor VIII, IX, XI, or XII, while the PT is performed when assaying for factor II, V, VII, or X. Factor assay results are reported in percent activity. A fibrinogen determination is performed to quantify levels of fibrinogen (factor I). The unit for reporting fibrinogen is mg/dL.

PROCEDURE

Prepare the standard curve:

1. Prepare serial dilutions of normal plasma with buffered saline as shown in the table below.

Tube #	Amount of Normal Plasma	Amount of Saline	Dilution	% Factor Activity
1	0.1 mL	0.9 mL	1:10	100.00
2	0.5 mL of tube #1	0.5 mL	1:20	50.00
3	0.5 mL of tube #2	0.5 mL	1:40	25.00
4	0.5 mL of tube #3	0.5 mL	1:80	12.50
5	0.5 mL of tube #4	0.5 mL	1:160	6.26
6	0.5 mL of tube #5	0.5 mL	1:320	3.13
7	0.5 mL of tube #6	0.5 mL	1:640	1.56
8	0.5 mL of tube #7	0.5 mL	1:1280	0.78

2. Mix 0.1 mL of specific factor deficient plasma with 0.1 mL of each of the dilutions of normal plasma.
3. Perform either a PT (for factors II, V, VII, or X) or an APTT (for factors VIII, IX, XI, or XII) on each of the dilutions. The dilutions would be analyzed like any other patient or control sample.
4. Plot results on semi-log graph paper, with percent factor activity on the x-axis and seconds on the y-axis. Draw a best-fit line between the points.

Test the patient sample:

1. Prepare a 1:10 dilution of the patient plasma with buffered saline.
2. Mix 0.1 mL of specific factor deficient plasma with 0.1 mL of the patient dilution.
3. Perform either a PT (for factors II, V, VII, or X) or an APTT (for factors VIII, IX, XI, or XII) on the patient-factor deficient plasma dilution. The dilution would be analyzed like any other patient or control sample.
4. Read the percent activity directly from the standard curve.

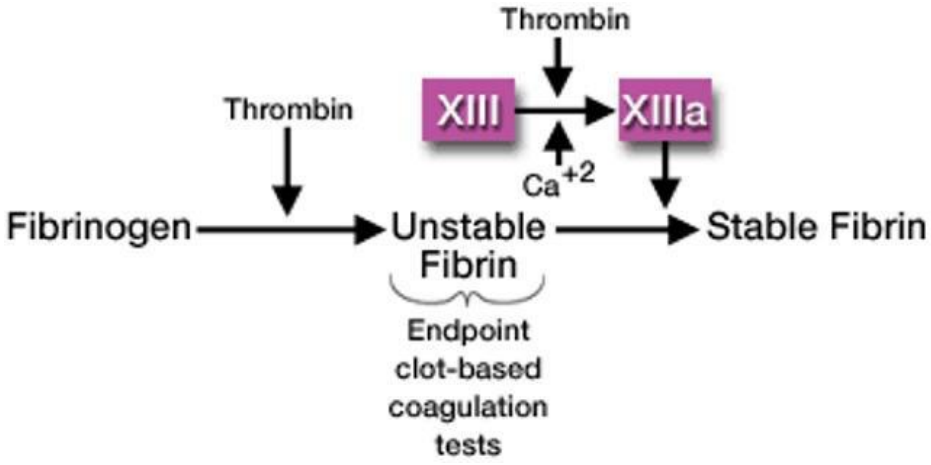
INTERPRETATION OF RESULTS

An approximate range of 50% to 150% factor activity is considered normal.

UREA SOLUBILITY TEST

PRINCIPLE

The urea solubility test is a screening test for factor XIII activity. Factor XIII, which is also known as fibrin stabilizing factor, stabilizes the fibrin clot by converting the hydrogen bonds to covalent bonds. This test is based upon the principle that a fibrin clot that has not been stabilized by the action of factor XIII will dissolve within 24 hours when suspended in the 5M urea solution. Fibrin clots that have been stabilized by factor XIII will not dissolve within 24 hours. A deficiency of factor XIII is not detected by other clot-based coagulation tests since the endpoint of these tests occurs at the first formation of unstabilized fibrin. Therefore, a urea solubility test should be performed when a patient exhibits bleeding tendencies and other coagulation tests are normal.



PROCEDURE

A sample of patient plasma is clotted using thrombin. The fibrin clot is then suspended in a 5M urea solution and allowed to incubate at room temperature for 24 hours.

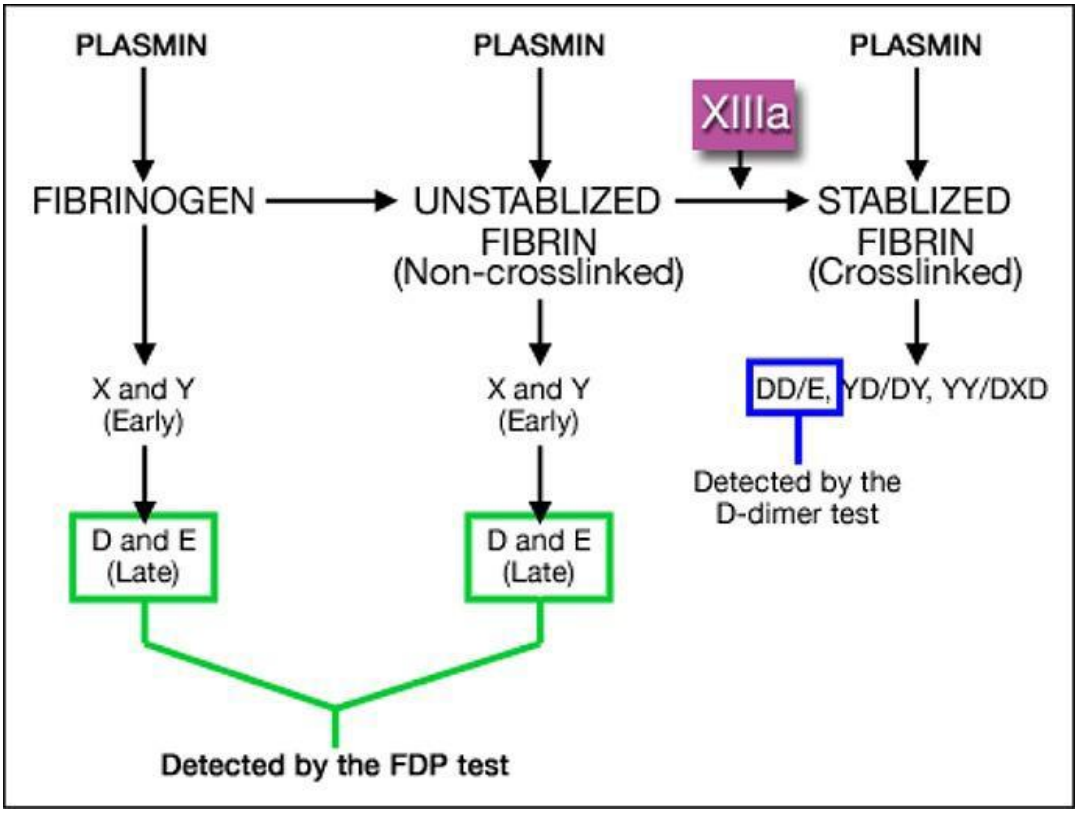
INTERPRETATION OF RESULTS

At the end of 24 hours, the presence of a formed clot indicates sufficient factor XIII concentration. Normally, only 5% of factor XIII concentration is required to adequately perform fibrin stabilization. Only patients with a homozygous inherited factor XIII deficiency will have factor XIII concentrations below this level and will experience bleeding tendencies. I If the urea solubility test indicates decreased levels of factor XIII, the amount of factor XIII activity can be quantitated by a chromogenic assay.

TESTS TO MONITOR THE FIBRINOLYTIC SYSTEM

Plasmin enzymatically degrades fibrinogen, non-crosslinked fibrin (before stabilization by factor XIII), and crosslinked fibrin (after stabilization by factor XIII). The initial fragments produced from degradation of fibrinogen and non-crosslinked fibrin are X and Y (early degradation products). X and Y are further degraded by plasmin to fragments D and E (late degradation products). The degradation of crosslinked (stabilized) fibrin, on 12
the other hand, produces 3 types of crosslinked fragments (DD/E, YD/DY and YY/DXD).

There are two tests that detect fragments of fibrin and fibrinogen degradation; the fibrin degradation products (FDPs) test and the D-dimer test. The FDP test detects the presence of fragments D and E, while the D-dimer test detects the presence of the DD portion of the DD/E fragment. The DD portion of the DD/E fragment is known as D-dimer.



FIBRIN DEGRADATION PRODUCTS

PRINCIPLE

The FDP test is used to detect and semiquantitate degradation products of in vivo digestion of both fibrinogen and fibrin (specifically non-cross linked fibrin) by plasmin. The test is a direct latex agglutination slide test that utilizes latex particles that have been coated with antibody to human fibrinogen fragments D and E (fragments produced when plasmin degrades fibrinogen and non-crosslinked fibrin). The presence of FDPs in serum will cause the the latex particles to clump, yielding macroscopic agglutination. This is considered a positive result. An approximate concentration of FDPs in the sample can be determined by testing the sample at different dilutions.

PROCEDURE

Collection of the specimen:

The blood sample is collected in a special sample collection tube that contains:

- Thrombin to ensure complete clotting and removal of fibrinogen from the sample.
- A proteolytic inhibitor (soybean trypsin) to prevent in vitro activation of the fibrinolytic system.

Once thoroughly clotted, the sample in centrifuged and the serum is remove for testing.

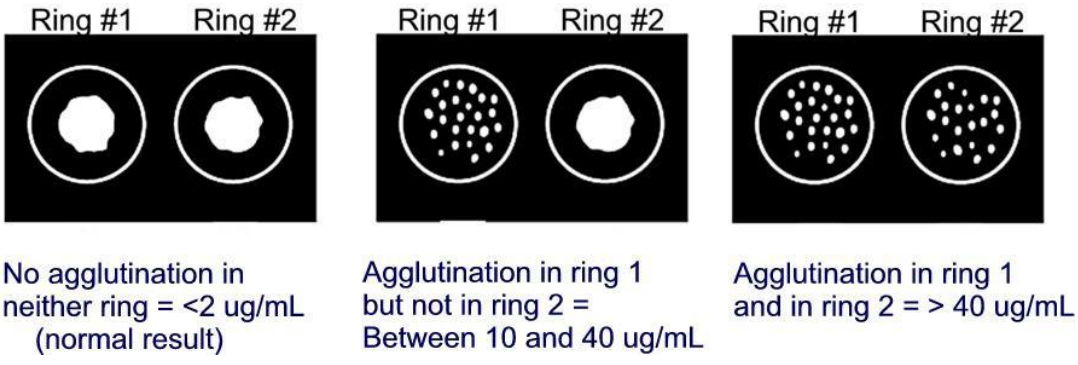
Testing of the specimen:

1. Two dilutions (1:5 and 1:20) are prepared using a buffered diluent.
2. A drop of each dilution is transferred to a ring on a special glass slide. The 1:5 dilution in transferred to ring #1 and the 1:20 dilution is transferred to ring #2.
3. A drop of the latex suspension is added to the dilutions in rings #1 and #2. The serum dilution-latex mixture in each ring is mixed thoroughly.
4. The glass slide is rotated gently for 2 minutes and observed for agglutination in both rings.

INTERPRETATION OF RESULTS

The test is sensitive to 2ug of FDPs per milliliter. Agglutination in neither ring #1 or ring #2 represents an FDP concentration of less than 2ug/mL; this is a normal result. The presence of agglutination in ring #1 (which is the 1:5 dilution) and no agglutination in ring #2 (which is the 1:20 dilution) indicates an FDP concentration between 10ug/mL and 40ug/mL. Agglutination in both rings indicates an FDP concentration of greater than 40ug/mL.

Since the test cannot distinguish between fibrinogenolysis (breakdown of fibrinogen) and fibrinolysis (breakdown of fibrin), its utility in monitoring thrombolytic therapy or following the course of a suspected case of DIC is questionable.



D-DIMER

This test is used to detect the fibrin breakdown products that are seen only as a result of the degradation of a stable fibrin clot, and does not detect fragments that result from the degradation of fibrinogen or the unstabilized fibrin clot (before stabilization). Like the FDP test, this test is a direct latex agglutination slide test. However, this test utilizes latex particles that have been coated with antibody to human D-dimer fragments (fragments produced when plasmin degrades crosslinked fibrin) as opposed to fragments D and E.

The D-dimer test is considered more valuable than the FDP test for monitoring thrombolytic therapy and for following the course of a suspected case of DIC. On the other hand, the FDP is considered more valuable in conditions, such as cirrhosis of the liver, in which fibrinogen lysis may be more abundant than fibrin lysis. In these cases, the clinician needs to accurately quantify FDPs in order to evaluate the risk of hemorrhage due to the effect of FDPs on fibrin monomer polymerization and platelet aggregation. When cost is not a factor, it is suggested that both the FDP test and D-dimer test be performed.

EUGLOBULIN CLOT LYSIS TEST

The euglobulin clot lysis test is used to detect increased fibrinolytic activity. In this procedure, the euglobulin fraction of plasma, which contains fibrinogen, plasminogen and plasminogen activators, is precipitated with 1% acetic acid and refrigeration. The sample is centrifuged and the supernatant is decanted. The precipitated euglobulin fraction is dissolved in buffered saline and thrombin is added to clot the euglobulins. The resulting clot serves as a substrate for plasmin, which is generated from plasminogen by the plasminogen activators. Lysis of the clot in less than 2 hours indicates increased fibrinolytic activity. The euglobulin clot lysis may be used to differentiate primary fibrinolysis from secondary fibrinolysis due to DIC. The test can also be used to monitor streptokinase or urokinase thrombolytic therapy in patients with acute myocardial infarct (heart attack).

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 3

I. Evaluation of thrombotic disorders

A. Thrombotic problems related to increased clot formation

1. Decreased levels of biochemical inhibitors
2. Activated protein C resistance
3. Antiphospholipid thrombosis syndromes

a) Dilute Russel's Viper Venom Time

b) Phospholipid Neutralization Test

c) Enzyme-linked immunosorbent assay

LABORATORY EVALUATION OF COAGULATION DISORDERS – PART 3

EVALUATION OF THROMBOTIC DISORDERS

Thrombosis can be due to either increased clot formation or decreased clot breakdown (decreased fibrinolytic activity).

THROMBOTIC PROBLEMS RELATED TO INCREASED CLOT FORMATION

DECREASED LEVELS OF BIOCHEMICAL INHIBITORS

The biochemical inhibitors are proteins that have the ability to regulate the enzymatic activities of the activated coagulation factors, thereby slowing and stopping the coagulation cascade. The most important inhibitors are tissue factor pathway inhibitor, the protein C regulatory system (proteins C and S), and the protease inhibitors, mainly antithrombin and heparin cofactor 2. Defects in these inhibitors can be either qualitative or quantitative in nature. Quantitative defects are those in which the amount of the protein is decreased. Qualitative defects, on the other hand, are those in which the protein is present in sufficient amount but does not function properly, or, in other words, has decreased activity. Assays are available for determining both the amount and functional status of each of these inhibitors.

ACTIVATED PROTEIN C RESISTANCE

Activated protein C resistance (APCR) is characterized by the presence of an abnormal factor V molecule, which is referred to as the **factor V Leiden**. The factor V Leiden is resistant to deactivation by activated protein C and, as a result, the activity of activated factor V proceeds unchecked. This leads to an increased incidence of thrombosis. The clinical laboratory can diagnose APCR either by using a coagulation-based assay to determine the APCR ratio or by using a DNA-based test for detection of the factor V Leiden mutation.

The coagulation-based assay for APCR is a modified APTT test that utilizes commercially prepared activated protein C (APC) as a reagent. In a normal response, the addition of APC to the test system will substantially prolong the APTT result. This is due to the fact that APC will destroy factors Va and VIIIa in the sample. For the patient with APCR, the APTT will show less of an increase with the addition of APC. This is due to the fact that the APC cannot destroy the patient's defective factor V (factor V Leiden). By comparing the results of the patients APTT values with and without the addition of APC, a preliminary diagnosis can be made.

The procedure is performed as follows.

1. A baseline APTT is performed on the patient's plasma without the addition of activated protein C (APC).
2. A second APTT is performed with APC added to the calcium chloride reagent.

3. A ratio of the two APTT results is calculated. A value of less than two for this ratio indicates activated protein C resistance.

$$\text{APCR Ratio} = \frac{\text{APTT with APC}}{\text{Baseline APTT}}$$

A value of less than 2 indicates APCR.

For patients with abnormal ratios (less than 2) further analysis by one of several molecular techniques may be performed. The most commonly used method involves the use of the polymerase chain reaction to amplify the segment of the DNA where the mutation is located. Following amplification, a restriction enzyme is used to cut the amplified DNA segments into fragments. Once separated by gel electrophoresis, the DNA fragment pattern is used to determine whether or not the mutation is present.

ANTIPHOSPHOLIPID THROMBOSIS SYNDROMES

The antiphospholipid antibodies are immunoglobulins (IgG or IgM) that react with phospholipids, nucleic acids, and glycosaminoglycans. Lupus anticoagulant (LA) and anticardiolipin antibody (ACA) are the most widely studied. The mechanism by which antiphospholipid antibodies (APL antibodies) cause thrombosis is not clearly understood, but appears to be multifaceted, affecting both humoral and cellular components involved in hemostasis. ACL antibodies are capable of reacting with the phospholipid reagents used to perform clot based coagulation assays such as the APTT, resulting in prolongation of the results. Therefore, the **APTT** has been used as a screening test for APL antibodies, but it is not very reliable when used in this capacity. In fact, only 30% of patients with antiphopholipid antibody sydrome will have prolonged APTT values. As a result, one cannot exclude a diagnosis of antiphospholipid antibody syndrome based upon normal APTT results.

DILUTE RUSSEL'S VIPER VENOM TIME (dRVVT)

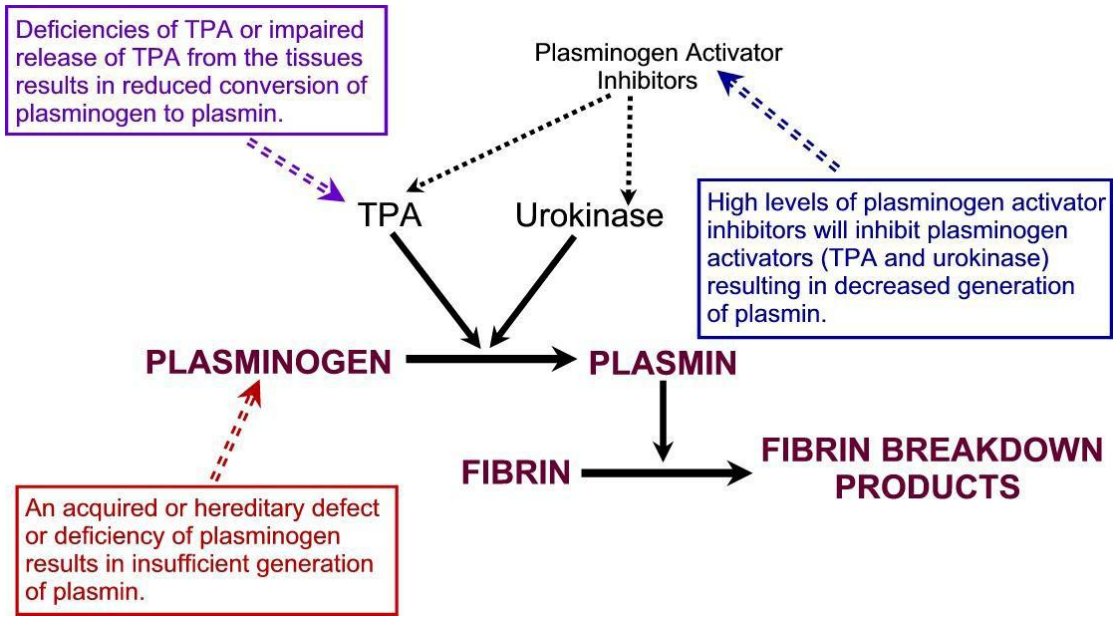
The **dilute Russell's viper venom time (dRVVT)** is more sensitive to the presence of APL antibody, specifically the lupus anticoagulant, than is the APTT. Russell's viper venom is snake venom that, in the presence of calcium ions, is able to convert factor X to its activated form Xa. The Xa, together with phospholipid and factor Va, converts prothrombin to thrombin. The dilute Russell's viper venom time (dRVVT) is actually a modified prothrombin time (PT), which has been made sensitive to the presence of lupus anticoagulant by adding minimal amounts of phospholipid. The presence of even small amounts of antibody in the patient sample will neutralize the phospholipid, resulting in prolongation of the clotting time. Since the Russell's viper venom activates factor X directly, the dRVVT will not be prolonged with deficiencies of, or with inhibitors to, factors VIII, IX, XI, XII, and VII. However, it will be affected by deficiencies of factors I (fibrinogen), II (prothrombin), V and X.

PHOSPHOLIPID NEUTRALIZATION TEST
If the dRVVT is prolonged, a **phospholipid neutralization test** can be performed to differentiate between a factor deficiency and LA. In this test, excess phospholipid is added and the dRVVT is repeated. If, in the presence of excess phospholipid, the dRVVT shortens, an LA is indicated. This is due to the fact that the phospholipid is added in such a great amount that, even after the antibody is saturated, additional phospholipid needed to complete clot formation remains. If, on the other hand, the dRVVT remains prolonged in the presence of excess phospholipid, a factor deficiency is indicated.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)
An **enzyme-linked immunosorbent assay (ELISA)** is the method of choice for confirming the diagnosis of anticardiolipin antibody.

THROMBOTIC PROBLEMS RELATED TO FIBRINOLYTIC SYTEM DISORDERS (DECREASED CLOT BREAKDOWN)

The major function of the fibrinolytic system is to break down fibrin clots. Failure to accomplish this will result in thrombosis. The proteolytic enzyme that is responsible for clot breakdown is plasmin. The diagram below illustrates deficiencies or defects that could impair plasmin generation.



ANTICOAGULANT THERAPY AND THROMBOLYTIC THERAPY

I. Anticoagulant therapy
    A. Heparin therapy
        1. Standard heparin
        2. Factor Xa inhibitors
            a) Low molecular weight heparin
            b) Oral factor Xa inhibitors
    B. Direct thrombin inhibitors
    C. Oral anticoagulant therapy
        1. INR
    D. Combined heparin and oral anticoagulant therapy
    E. Antiplatelet drugs
    F. Ideal anticoagulant therapy
    G. Thrombolytic therapy

ANTICOAGULANT THERAPY AND THROMBOLYTIC THERAPY

Patients with thrombotic disease or in whom prevention is warranted are treated with drugs that either prevent thrombosis (anticoagulants) or induce removal of clots that have formed (thrombolytic agents).

ANTICOAGULANT THERAPY

Anticoagulant therapy is administered to prevent inappropriate clot formation in the patient with thrombotic disease. Clots can form in either the veins (venous thrombosis) or arteries (arterial thrombosis). Venous thrombi result from either the inappropriate activation of the coagulation system or the inability of the body to slow down and stop the system once it is activated. Since the clotting process involved in venous thrombosis is dominated by the clotting factors, the thrombi that result are composed largely of fibrin and red blood cells. Arterial thrombi, on the other hand, start with platelet plug formation followed by deposition of fibrin. Therefore, arterial thrombi are composed largely of platelets and fibrin.

HEPARIN THERAPY

There are two forms of heparin that are available for the treatment of venous thrombosis: standard heparin and low-molecular-weight heparin (LMWH). Heparin does not have a direct effect on blood coagulation but acts indirectly by enhancing the activity of antithrombin (AT). AT is one of the serine protease inhibitors that have the ability to regulate the enzymatic activities of the activated coagulation factors, thereby slowing and stopping the coagulation cascade. Specifically, AT neutralizes factors IIa (thrombin), IXa, Xa, XIa, XIIa, and kallikrein, by forming 1:1 complexes with each of these activated factors. Heparin binds with AT causing a conformational change that greatly increases some aspects of AT activity. Thrombin and factor Xa are the most sensitive to inactivation by the heparin-AT complex.

It is important to remember that heparin, either standard heparin or LMWH, in and of itself does not anticoagulate the blood. Rather, it is heparin's effect of AT that produces the anticoagulant effect. Therefore, individuals who are AT deficient will not respond to heparin therapy. Heparin also enhances the activity of heparin cofactor II but not to the same extent that it enhances AT activity. Heparin cofactor II inhibits the activity of thrombin only.

STANDARD HEPARIN

Standard heparin, which is also referred to as unfractionaed heparin, mainly affects the ability of AT to deactivate thrombin. In fact, the heparin-induced change enhances the rate with which AT binds with and deactivates thrombin by a factor of 1000 times. Commercial sources of standard heparin are porcine intestinal mucosa and bovine lung.

Standard heparin can be administered intravenously or subcutaneously. Low dose heparin therapy is usually administered every 8 to 12 hours by subcutaneous injection. Moderate doses are administered every 4 to 6 hours by subcutaneous or intravenous injection, or can be given through continuous IV drip. High doses are routinely administered by continuous

IV drip. When administered intravenously, heparin has an immediate anticoagulant effect. When administered subcutaneously, the anticoagulant effect peaks within 2 to 4 hours from the time of injection.

Standard heparin therapy is most commonly monitored with the activated partial thromboplastin time (APTT). In the past, heparin dose was adjusted to produce an APTT value of 1.5 to 2.5 times the patient's baseline APPT value. However, the general use of a fixed APTT range for adjusting dosage is no longer recommended due to the fact that different commercial APTT reagents vary in their responsiveness to heparin. In other words, a 0.2 unit/mL dose of heparin may produce a 20 second increase in a patient's APTT value when using one type of APTT reagent and a 40 second increase when using another type of APTT reagent. For this reason, each laboratory should establish a therapeutic APTT range for management of unfractionated heparin therapy.

The recommended protocol for establishing a therapeutic APTT range is as follows:
1. Blood is collected from at least 50 patients who are receiving heparin at all levels of anticoagulation.
2. APTTs are performed on all specimens.
3. Chromogenic anti-factor Xa assays are performed on all specimens.
4. APTT and anti-factor Xa values for each specimen are plotted on linear graph paper.
5. The APTT range in seconds that correlates with the chromogenic anti-Xa range of 0.3 to 0.7 u/mL is the therapeutic range.

- Standard heparin therapy has several limitations and side effects. The complicated manner is which standard heparin is cleared from the body results in substantial variation in anticoagulant response from patient to patient. This can result in hemorrhage, if the dose is too high, or an increased risk of thrombosis, if the dose is too low.

- Standard heparin therapy requires careful and frequent laboratory monitoring. The APTT is used for this purpose.

- Standard heparin can cause thrombocytopenia and increase the risk of hemorrhage. This phenomenon is referred to as HIT (heparin induced thrombocytopenia). Approximately 25% of patients being treated with full-dose therapy develop mild thrombocytopenia approximately 2 days to 15 days after the first dose. The mechanism by which this type of thrombocytopenia develops is not clearly understood. In approximately 5% to 6% of patients, a more severe thrombocytopenia develops. This occurs when the body produces an anti-heparin antibody; one end of the antibody attaches to the heparin molecule and the other end of the antibody attaches to the platelet. The antibody coated platelet is destroyed by the spleen. The platelet count in affected individuals may drop as low at 50 X 10^3/uL.

- Long-term therapy (greater than 4-5 months) with standard heparin has been associated with osteoporosis. The mechanism by which osteoporosis develops is poorly understood. It has been suggested that heparin could cause an increase in bone reabsorption by increasing the number of osteoclasts, and by enhancing the activity of individual osteoclasts.

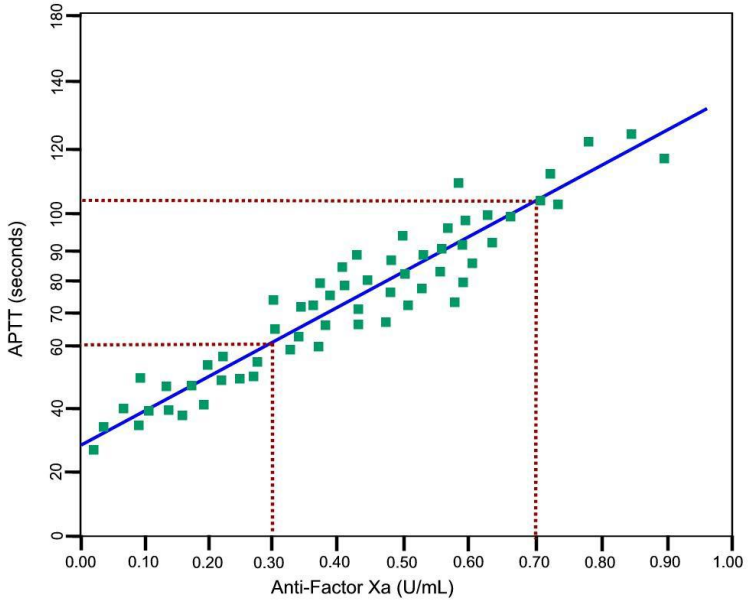
FACTOR Xa INHIBITORS

LOW MOLECULAR WEIGHT HEPARIN

The use of low-molecular-weight heparin (LMWH), which is also called fractionated heparin, is increasing because it has a more predictable anticoagulant effect, does not need to be administered as frequently, does not cause thrombocytopenia, and has a lower risk of osteoporosis. LMWH is prepared from standard heparin using chemical or enzymatic fractionation. Fractionation yields a product with a molecular weight approximately one-third that of standard (unfractionated) heparin. It is administered once or twice a day through subcutaneous injection and produces its anticoagulant effect mainly by enhancing the ability of AT to inhibit factor Xa rather than thrombin (factor IIa). Because the anticoagulant effect of LMWH is much more predictable than that of standard heparin, laboratory monitoring need not be done as frequently. When monitoring is indicated to prevent excessive or insufficient anticoagulation in certain patients, a **chromogenic anti-factor Xa assay** is performed rather than an APTT.

To perform the chromogenic anti-factor Xa assay, patient plasma is added to a known amount of factor Xa with excess antithrombin. If heparin is present in the patient plasma, it will bind to antithrombin and inhibit factor Xa. The amount of residual factor Xa is inversely proportional to the amount of heparin in the plasma. The amount of residual factor Xa is detected by adding a chromogenic substrate that resembles the natural substrate of factor Xa. Factor Xa cleaves the chromogenic substrate, releasing a colored compound that can be detected by a spectrophotometer. Results are reported as anticoagulant concentration in anti-factor Xa units/mL, such that high anti-factor Xa values indicate high levels of anticoagulation and low anti-factor Xa values indicate low levels of anticoagulation. The range of APTT values in seconds that correspond to 0.3 to 0.7 anti-factor Xa units per milliliter (U/mL) is the therapeutic range.

For this example of an anti-factor Xa assay, the range of APTT values that corresponds to 0.3 to 0.7 anti-factor Xa U/dl is 60 seconds to 104 seconds. This is the therapeutic APTT range.



ORAL FACTOR Xa INHIBITORS

A derivative of the well-known intravenous heparin anticoagulant which can be taken orally is **rivaroxiban** (Xarelto). Outcomes of studies comparing this drug to enoxaparin (LMWH) are favorable, but there is no standardized laboratory test for monitoring. Its benefits include rapid action and few drug interactions, but cannot be used in patients with renal insufficiency. Use of this drug will have to balance the need for increased compliance over injectable anticoagulants with the inability to monitor by laboratory testing.

DIRECT THROMBIN INHIBITORS

Various drugs that directly inhibit thrombin offer alternatives to heparin therapy. These drugs deactivate thrombin directly and do not require antithrombin for activity as does heparin. Also, they are not associated with the development of thrombocytopenia. They are administered intravenously and their anticoagulant effect is immediate. The APTT is used to monitor patient response to these drugs, with the therapeutic range being approximately 1.5 to 3 times the mean of the laboratory's reference interval for APTT. Examples are **Lepuriden** which is a recombinant hirudan (derived from saliva of medicinal leeches) and **Argatroban** which does not require renal adjustment, but costs about $2000/day.

Fondaparinux is a derivative of heparin which is administered by subcutaneous injection. Since it does NOT have a side effect of thrombocytopenia, it is used for patients who develop heparin induced thrombocytopenia following heparin usage. It can be monitored by an anti-chromogenic Xa assay specific for fondaparinux.

Dabigatran (Pradaxa) is an oral direct thrombin inhibitor which has immediate action and few interactions, but has increased gastrointestinal bleeding as compared to coumadin. Its elimination is dependent upon renal function. There is no available antidote, nor is there a laboratory test for monitoring.

ORAL ANTICOAGULANT THERAPY

Oral anticoagulants are administered in pill form by mouth. The most commonly used oral anticoagulants belong to the coumadin family. The most popular of these drugs is Warfarin sodium (coumadin sodium). Coumadin functions as an anticoagulant by impairing liver synthesis of the vitamin K dependent factors (II, VII, IX, and X) by interrupting an enzymatic step required to enable vitamin K to carboxylate the factors. This results in the production of nonfunctional factors called PIVKAs (**P**roteins **I**nduced by **V**itamin **K** **A**ntagonist) or noncarboxylated K-dependent factors. The anticoagulant effect of the drug is not fully apparent until 3 to 4 days after administration. This is due to the fact that the effect of the drug is delayed until the normal clotting factors are cleared from circulation. Factor VII, with a half-life of only 6 to 7 hours, is the first to be cleared. Levels of factors II, IX, and X, however, do not drop until after 72 to 96 hours. The prothrombin time (PT) is the test of choice for monitoring coumadin therapy. Factor VII, a factor measured by the PT, has a very short half-life and is the first factor affected by coumadin therapy. In addition, two other factors (II and X) are measured by the PT. In the past, the dosage of coumadin was adjusted based upon the PT response to therapy: a 1.5 times to 2 times increase in the patient's baseline PT value was equated with proper dosage. Today, the International Normalized Ratio (INR) is used to determine proper dosage.

INTERNATIONAL NORMALIZED RATIO (INR)

The INR is used to standardize PT results from institution to institution. The PT results on a given sample will vary depending upon the test method and sensitivity of reagent used. (The more sensitive the reagent, the more prolonged the PT results on plasma from patients receiving coumadin). Reported INR values are independent of the reagents and test methods used. The INR is calculated as follows:

$$INR = R^{ISI} \quad \text{where } R = \frac{\text{Patient PT value}}{\text{Mean of PT normal range}}$$

The ISI value is supplied by the manufacturer and varies with type of reagent, lot number of reagent, and test method.

Two categories of treatment levels are recommended based upon INR values:
For patients being treated for pulmonary emboli, venous thrombosis, acute myocardial infarction, atrial fibrillation, hereditary deficiencies of the biochemical coagulation inhibitors, (i.e., AT), presurgical prophylaxis, or tissue heart valves, the recommended range for INR is 2.0 - 3.0 with corresponding PT values of approximately 14 seconds to 17 seconds.
For patients with mechanical prosthetic heart valves or recurrent systemic embolism, the treatment should be more intensive, resulting in INR values of 2.5 to 3.5 and corresponding PT values of 17 seconds to 22 seconds.

The INR is specifically intended for assessing only those patients who are stabilized on long-term (greater than 6 weeks) oral anticoagulant therapy. It should **not** be used in conjunction with the PT for evaluating the induction phase of oral anticoagulant therapy, for evaluating the hemostatic system in presurgical patients, or for evaluating hemostasis in patients with liver disease or factor deficiencies. In reality, however, it is reported with every PT value regardless of patient clinical history.

COMBINED HEPARIN AND ORAL ANTICOAGULANT THERAPY

The hospitalized patient with thrombosis (e.g., deep vein thrombosis or pulmonary emboli) is first treated with heparin because it has an immediate anticoagulant effect. Due to the fact that heparin is administered intravenously or subcutaneously, it is an inconvenient form of treatment for the patient who is returning home. For this purpose, oral anticoagulant therapy is preferred. However, oral coumadin requires approximately 3 to 4 days to be fully effective. Therefore, they must be administered in advance of the patient leaving the hospital. It is common practice to overlap the heparin and coumadin therapy for a period of 4 days to 5 days. As the patient is being stabilized on coumadin, the heparin dosage is slowly reduced until it is completely eliminated.

ANTIPLATELET DRUGS

A number of drugs that impair platelet function are available for use alone or in combination with other anticoagulants for the prevention of arterial thrombosis. The most commonly used antiplatelet agent is aspirin. It impairs platelet function by inhibiting the synthesis of thromboxane A_2, one of the agonists that promotes secondary platelet aggregation. Once ingested, aspirin will affect thromboxane A_2 production for the entire lifespan of the platelet (9 to 12 days). It may also affect megakaryocytes in the bone marrow. Therefore, platelet aggregation and platelet plug formation will be impaired until new platelets from unaffected megakaryocytes are produced.

Other antiplatelet agents affect different aspects of platelet function. One drug binds specifically with the ADP receptor on the surface of the platelet, thereby preventing activation of the platelet. Another drug binds with the IIb /IIIa receptor on the surface of the activated platelet, thereby preventing fibrinogen binding and subsequent aggregation.

IDEAL ANTICOAGULANT THERAPY

The ideal anticoagulant attributes include:

- Oral administration for better compliance
- Quick acting so no bridging therapy is needed
- Few interactions with food or drugs
- Ability to monitor using laboratory testing, but not necessarily requiring monitoring
- Ability to rapid reversibility with or without antidote
- Not requiring renal adjustment
- Low cost

Choice of an anticoagulant agent takes all of these factors into consideration.

THROMBOLYTIC THERAPY

Anticoagulant therapy is administered to prevent further clot formation in the patient with thrombosis. However, in the event that the clot poses a threat to life by nature of its size or location, a thrombolytic drug may also be administered to help dissolve the clot (lyse the thrombus) more quickly. Thrombolytic drugs, which are injected intravenously, do not directly dissolve the clot. Instead, they function by activating plasminogen to plasmin, which in turn lyses the fibrin clot. With the advent of DNA technology, these drugs have become easier to produce and, as a result, are more widely used. All of these drugs, with the exception of streptokinase, are naturally occurring components of the human hemostatic system. The thrombolytic drugs include:

- Streptokinase: A bacterial enzyme derived from group C-beta hemolytic streptococci.
- Acylated plasminogen streptokinase activator complex (APSAC): A complex of streptokinase and plasminogen.
- Urokinase: Commercially produced from human kidney cells or from urine.
- Pro-urokinase: A modification of the original urokinase.
- Tissue-type plasminogen activator (tPA): Derived from recombinant DNA techniques; various forms are available, including Alteplase, Reteplase, and Tenecteplase.

Thrombolytic drug therapy has quite a profound affect on coagulation test results as shown in the table below.

Laboratory Test	Result	Explanation
Prothrombin time (PT)	Increased	Decreased levels of fibrinogen and factor V due to destruction of these factors by plasmin
Activated partial thromboplastin time (APTT)	Increased	Decreased levels of fibrinogen and factors V, VIII, IX, XI and XII due to destruction of these factors by plasmin
Fibrinogen	Decreased	Increased lysis of fibrinogen by plasmin
Plasminogen	Decreased	Increased conversion of plasminogen to plasmin by the thrombolytic drug
Fibrin degradation products (FDPs)	Increased	Increased breakdown of both fibrinogen and fibrin (stabilized and unstabilized) by plasmin
Plasmin	Increased	Increased conversion of plasminogen to plasmin by the thrombolytic drug
$Alpha_2$-antiplasmin	Decreased	Increased amounts of this plasmin inhibitor are consumed in response to increased amounts of plasmin being produced

While each of the thrombolytic drugs has different advantages and disadvantages, a disadvantage that is common to all is bleeding. This is due to the fact that the plasmin that is generated in response to therapy destroys fibrinogen and other coagulation factors as well as fibrin.

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