

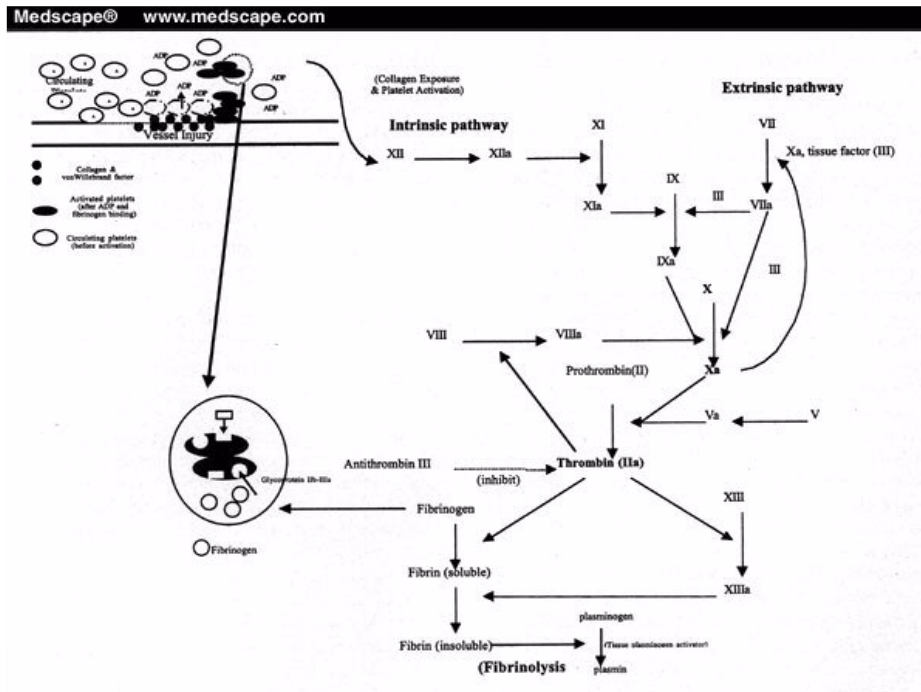
# Blood Clotting

April 21, 2005



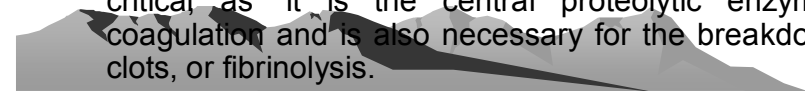
# Blood clotting system

- Precise control of the blood-clotting system is essential for maintenance of the circulation in all higher animals.
- Deficient function of this system can lead to fatal bleeding following even a minor injury.
- Overactivity of this system can produce unwanted blood clots, resulting in blockages to critical blood vessels, as occurs in such diseases as heart attack and stroke.

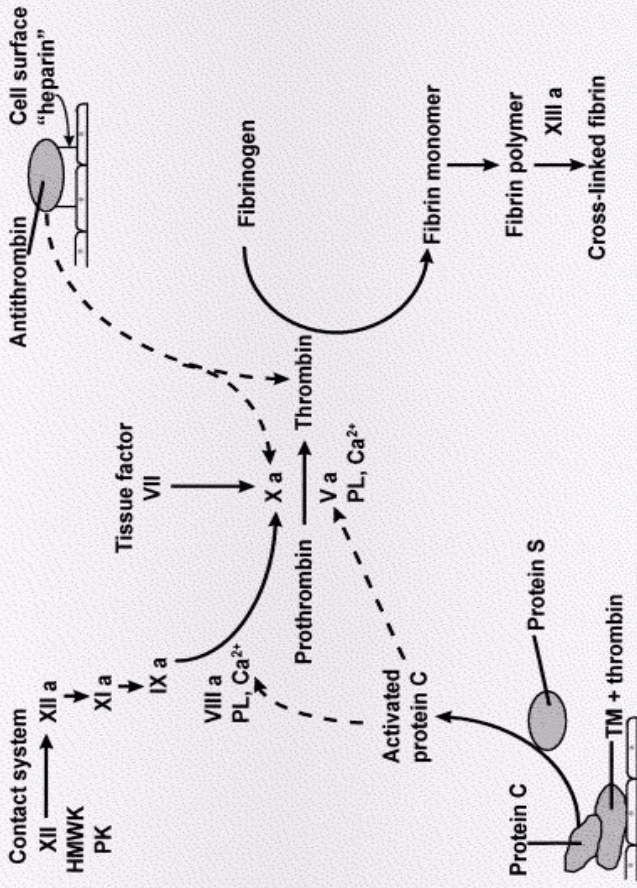
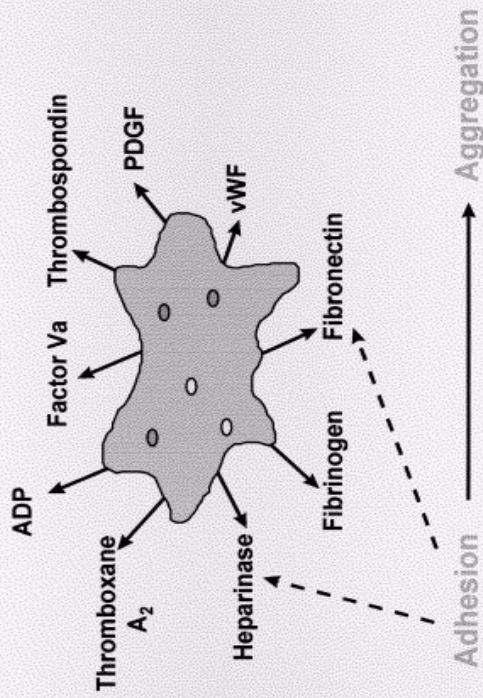


# Hemostasis in Physiological Conditions

- Under homeostatic conditions, the body is maintained in a finely tuned **balance of coagulation and fibrinolysis**. The **activation of the coagulation cascade** yields thrombin that converts fibrinogen to fibrin; the stable fibrin clot being the final product of hemostasis.
- **The fibrinolytic system** then functions to break down fibrinogen and fibrin. Activation of the fibrinolytic system generates plasmin (in the presence of thrombin), which is responsible for the lysis of fibrin clots.
- The breakdown of fibrinogen and fibrin results in polypeptides called **FDPs or fibrin split products (FSPs)**.
- In a state of homeostasis, the presence of thrombin is critical as it is the central proteolytic enzyme of coagulation and is also necessary for the breakdown of clots, or fibrinolysis.

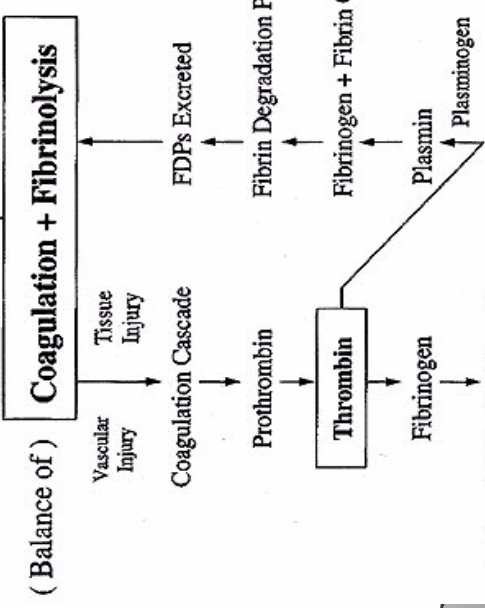


### Activation secretion

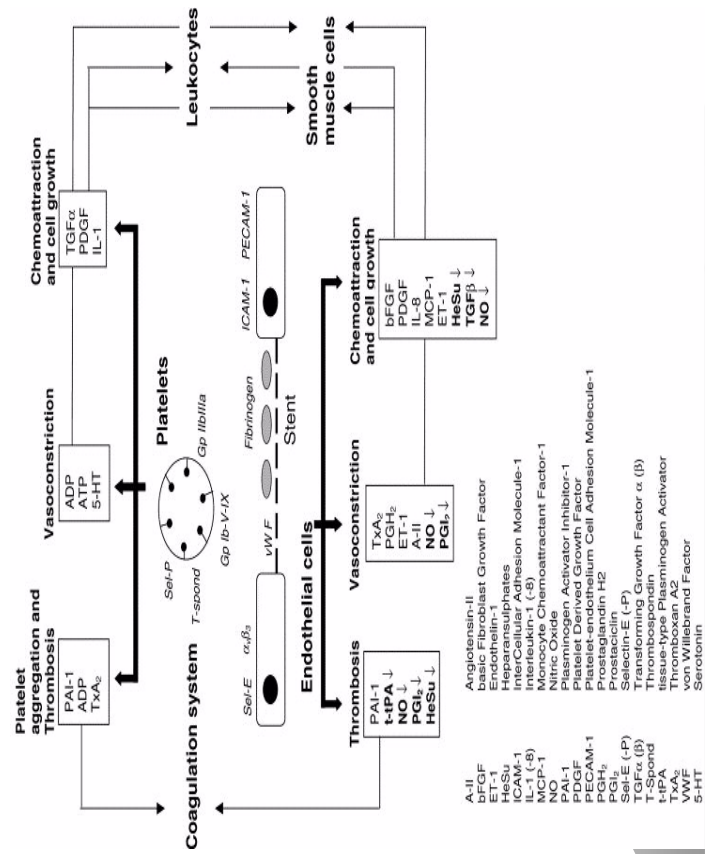


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### Homeostasis



Fibrin ... Stable Clot Formation





# DIC

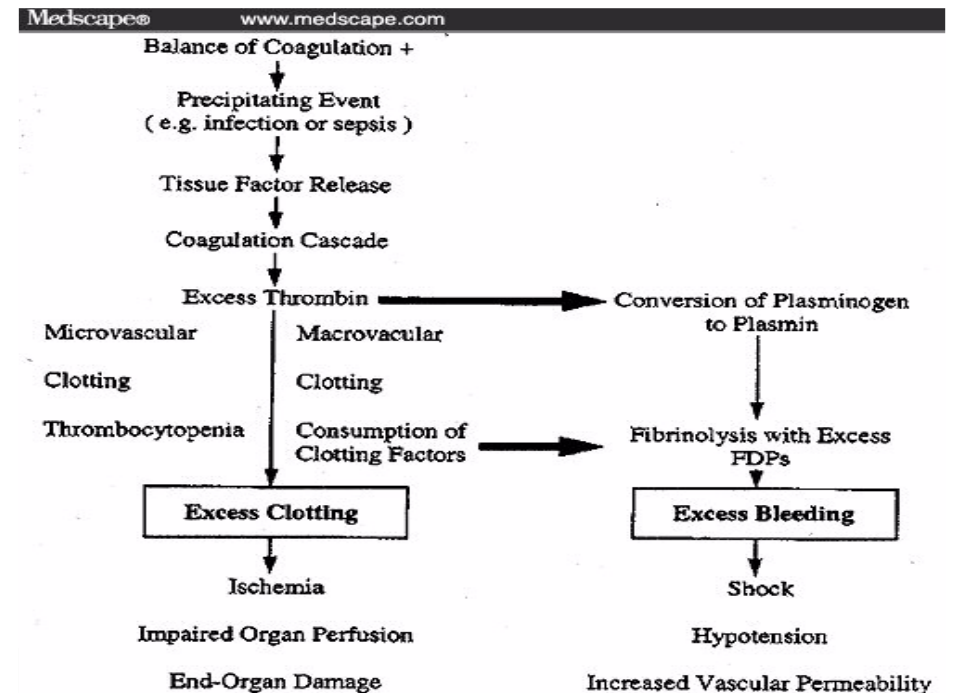
- DIC is a state of hypercoagulation that occurs in a variety of disease states.
- DIC represents an inappropriate overstimulation of normal coagulation in which thrombosis and hemorrhage occur simultaneously.
- Hypercoagulation occurs initially in the process of DIC; multiple small clots are formed in the microcirculation of various organs. This process is followed by fibrinolysis, in which there is consumption of clots and clotting factors, resulting in bleeding.
- Finally, the body is unable to respond to vascular or tissue injury through stable clot formation, and hemorrhage occurs. The hemorrhage associated with DIC can be profound, but it is the diffuse thrombosis (both microvascular and large vessel involvement) that leads to the irreversible morbidity and mortality associated with DIC. It is the thrombosis that leads to ischemia, impairment of blood flow, and end organ damage.

# DIC

- DIC can be acute or chronic in nature.
- Chronic DIC is generally seen in the cancer population and is demonstrated as localized thrombotic events (eg, deep vein thromboses). Chronic DIC is defined as a state of intravascular coagulation, and only minor imbalances in hemostasis exist.
- The acute form of DIC is considered an extreme expression of the intravascular coagulation process with a complete breakdown of the normal homeostatic boundaries. DIC is associated with a poor prognosis and a high mortality rate.

## DIC-pathophysiology

- In DIC, the processes of coagulation and fibrinolysis lose control, and the result is widespread clotting with resultant bleeding.
- Regardless of the triggering event of DIC, once initiated, the pathophysiology of DIC is similar in all conditions.
- One critical mediator of DIC is the release of a transmembrane glycoprotein called **tissue factor (TF)**. TF is present on the surface of many cell types (including endothelial cells, macrophages, and monocytes) and is not normally in contact with the general circulation, but is exposed to the circulation after vascular damage. For example, TF is released in response to exposure to cytokines (particularly interleukin), tumor necrosis factor, and endotoxin. This plays a major role in the development of DIC in septic conditions. TF is also abundant in tissues of the lungs, brain, and placenta. This helps to explain why DIC readily develops in patients with extensive trauma.
- TF binds with coagulation factors that then trigger both the intrinsic and the extrinsic pathways of coagulation.



## DIC

- Excess circulating thrombin results from the excess activation of the coagulation cascade. The excess thrombin cleaves fibrinogen, which ultimately leaves behind multiple fibrin clots in the circulation.
- These excess clots trap platelets to become larger clots, which leads to microvascular and macrovascular thrombosis. This lodging of clots in the microcirculation, in the large vessels, and in the organs is what leads to the ischemia, impaired organ perfusion, and end-organ damage that occurs with DIC.



## DIC

- Simultaneously, excess circulating thrombin assists in the conversion of plasminogen to plasmin, resulting in fibrinolysis. The breakdown of clots results in excess amounts of FDPs, which have powerful anticoagulant properties, contributing to hemorrhage.
- The excess plasmin also activates the complement and kinin systems. Activation of these systems leads to many of the clinical symptoms that patients experiencing DIC exhibit, such as shock, hypotension, and increased vascular permeability.



## DIC

- Coagulation inhibitors are also consumed in this process. Decreased inhibitor levels will permit more clotting so that a feedback system develops in which increased clotting leads to more clotting.
- Thrombocytopenia occurs because of the entrapment of platelets. Clotting factors are consumed in the development of multiple clots, which contributes to the bleeding seen with DIC.

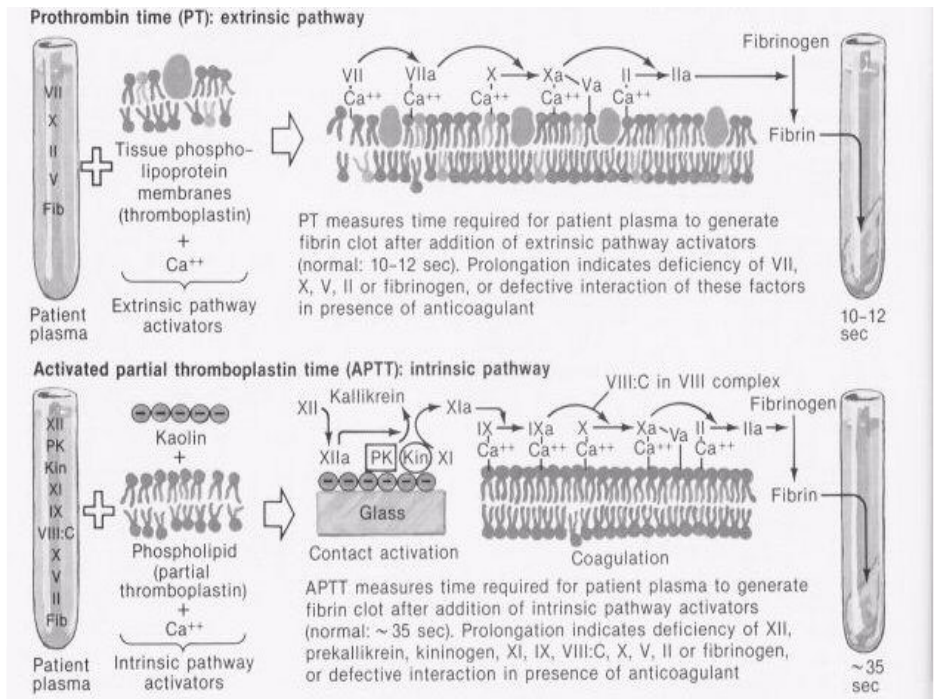


## DIC

- The fibrinolytic system, the body's mechanism for breaking down blood clots, is delicately balanced with the system that forms blood clots. Overactivity of either system results in uncontrolled bleeding or uncontrolled blood clotting. Plasminogen activators are the proteins that turn on the fibrinolytic system. Their activity is controlled by several regulator proteins, including plasminogen activator inhibitor-1 (PAI1) and PAI2.

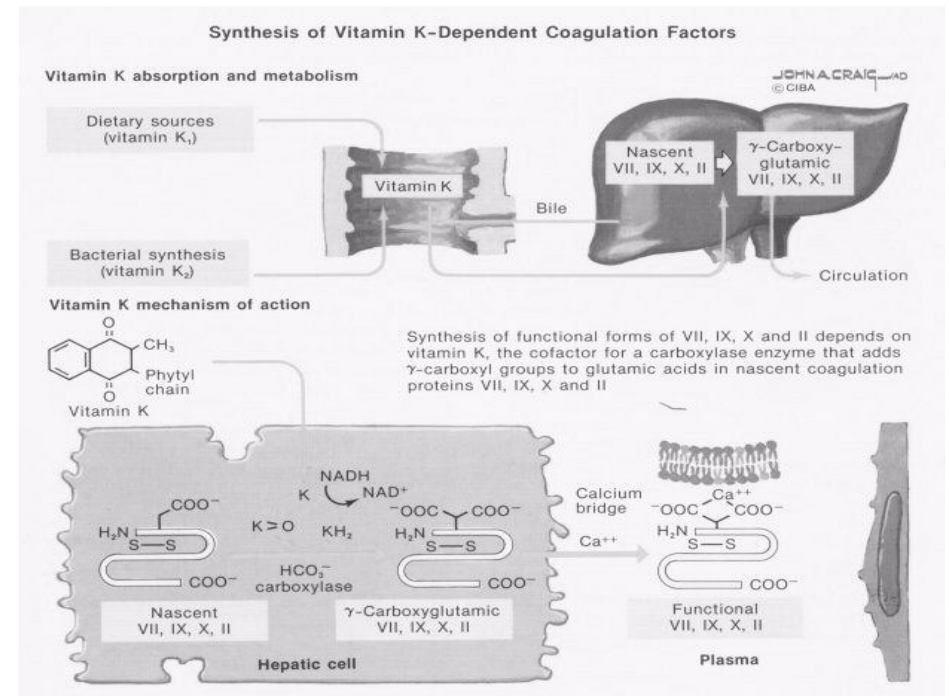


Tests	Results in Disseminated Intravascular Coagulation
General Tests	
Platelet count	Decreased
Fibrinogen level	Decreased
Peripheral blood smear	Schistocytes
D-dimer assay	Elevated
FDP assay	Elevated
Tests to Determine Accelerated Coagulation	
Antithrombin III	Decreased
Fibrinopeptide A	Elevated
Prothrombin activation peptides (F1 & F2)	Elevated
Thrombin-antithrombin complexes	Elevated
<b>Tests used for diagnosis of DIC</b>	
Tests to Determine Accelerated Fibrinolysis	



## Hypocoagulation

- Inherited states
- Acquired states



# Vitamin K Deficiency

- Insufficient amounts of vitamin K in diet
- Inadequate synthesis by gastrointestinal bacteria
- Abnormal absorption from small intestine
- Drugs (antivitamins K-coumadin)



# Clinical Picture of Vitamin K Deficiency

- **Bleeding in:**
- Hospitalized patients on intravenous fluids, with broad-spectrum antibiotics that sterilize the gut
- Newborn (premature) infants with immature liver function
- Abnormalities in fat absorption
- Deficiency in bile salts



# von Willebrand factor

- The blood-clotting protein (VWF) functions as the critical initial bridge connecting blood platelets to the wall of injured blood vessels, thereby helping to control bleeding.
- VWF also serves as the carrier for factor VIII, the substance missing in patients with hemophilia. Abnormalities in VWF result in von Willebrand disease (VWD), the most common human inherited bleeding disorder.



# von Willebrand Factor-genetics

- The molecular basis for the most common variant (type 1) still remains largely a mystery
- In some patients, a mutation inactivating one copy of the *VWF* gene reduces plasma VWF, leading to bleeding; in others, the same change does not result in a significant problem.
- It is now clear that wide variations in disease among individuals with the same or similar defects in the *VWF* gene are due to the action of one or more additional "modifier" genes. Such modifier genes are also likely to contribute to the wide variation in VWF levels observed among normal individuals. Elevated levels of VWF produced in this way may result in an increased risk of blood clotting, in contrast to the bleeding tendency associated with low VWF.



## Trombotic thrombocytopenic purpura (TTP)

- A deficiency of a protein that normally partially breaks down VWF in the blood as the cause of the often catastrophic blood-clotting disease (TTP) was identified.
- Mutations in *ADAMTS13*, the gene for this protein, in nearly all patients with the inherited form of TTP were found.
- Identification of this gene provides new tools for improved diagnosis and should lead to the production of recombinant ADAMTS13 for more effective treatment of TTP.



## Defects Responsible for Hypercoagulability-Inherited

- Activated protein C resistance (factor V Leiden)
- Protein S deficiency
- Protein C deficiency
- Antithrombin deficiency
- Hyperhomocysteinemia
- Prothrombin 20210A allele
- Dysplasminogenemia
- High plasminogen activator inhibitor
- Dysfibrinogenemia
- Elevated factor VIII



## Defects Responsible for Hypercoagulability-Acquired

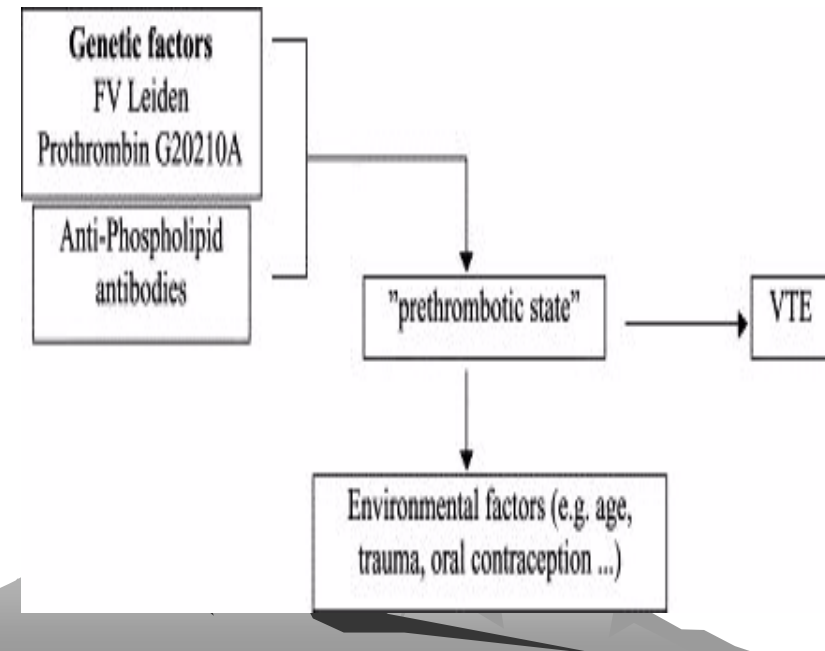
- Antiphospholipid syndrome
- Hyperhomocysteinemia
- Miscellaneous
- Thrombocythemia
- Dysproteinemia
- Heparin-induced thrombocytopenia
- Estrogens
  - Birth control pills
  - Hormone replacement therapy



## Defects Responsible for Hypercoagulability-Noncoagulant factors

- Malignancy
- Pregnancy
- Bed rest
- Surgery
- Trauma





## Coagulation Factor V

- Coagulation factor V is a central regulator in the early phases of blood clot formation. Genetic deficiency of factor V results in a rare bleeding disorder called parahemophilia.
- A subtle change in the factor V gene that increases the function of this protein, called factor V Leiden, is an important cause of an abnormal increase in blood clot formation.
- Factor V Leiden is remarkably common (present in 2–7 percent of the population) and may contribute to up to 50 percent of hospital admissions for blood clot-related illnesses.
- 10 percent of humans with factor V Leiden will develop a serious blood clot during their lifetime from the 90 percent who will remain asymptomatic.

## Combined deficiency of coagulation factor V and coagulation factor VIII

- Combined deficiency of coagulation factor V and coagulation factor VIII is another inherited bleeding disease.
- The molecular basis for this disorder as deficiency of the cellular protein *LMAN1* (also known as *ERGIC53*) was identified.
- Though *LMAN1* gene mutations in many combined deficiency patients were found, the cause of this disorder in approximately 30 percent of these individuals remained unexplained. In these patients, mutations in another gene, termed *MCFD2* was found.
- A complex of *MCFD2* and *LMAN1* appears to serve as a carrier for a subset of proteins, including factors V and VIII, that are destined for export from the cell. These findings provide the first example of such a specific transport pathway within the cells of higher organisms.

## Antithrombin III

- In the final phase of clot formation, thrombin converts fibrinogen to fibrin. Antithrombin (formerly referred to as antithrombin III), named for its action on thrombin, also inhibits the serine proteases of IXa, Xa, XIa, and XIIa.
- Deficiency of antithrombin may be caused by decreased levels or by dysfunctional protein. The "anticoagulant" action of heparin requires the presence of antithrombin; thus, a clinical clue to diagnosis of antithrombin deficiency may be anticoagulation refractoriness to heparin.

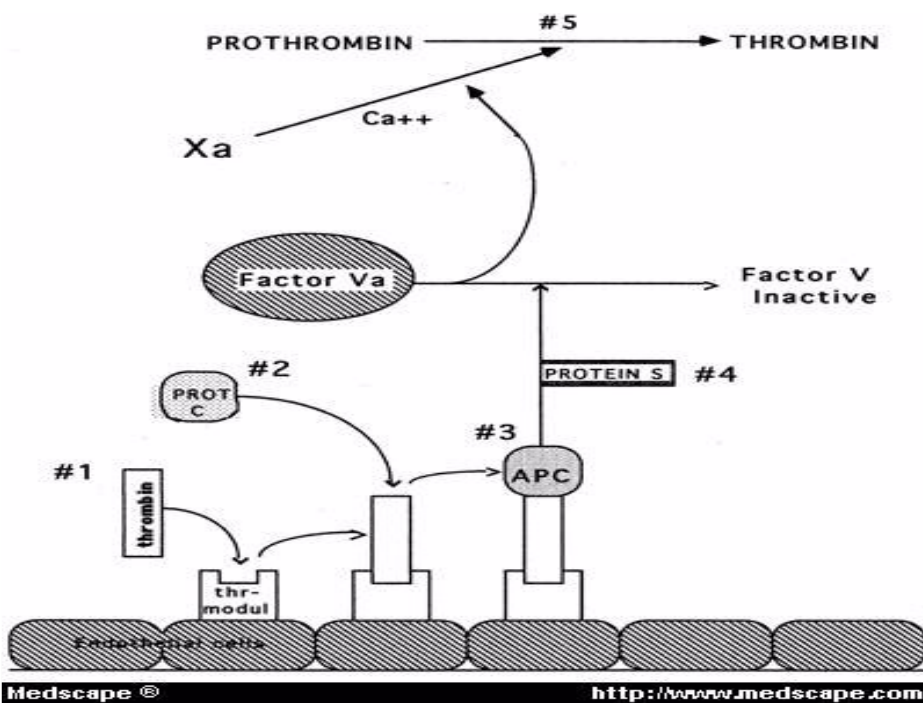


## Heparin-Induced Hypercoagulability

- Heparin associated thrombocytopenia is often seen, but "heparin-induced hypercoagulability" is infrequent.
- IgG antibodies form against platelet-heparin complexes that are sequestered on platelets at platelet Fc receptors and on endothelial cells where they may cause serious vascular occlusive disease and thrombocytopenia.
- Warfarin accelerates this phenomenon by further decreasing proteins of the protein C pathways, thereby enhancing hypercoagulability. The treatment of heparin-induced hypercoagulability, including purpura fulminans, requires immediate discontinuance of heparin administration.
- Low molecular weight heparin (LMWH) is risky because of potential cross reactivity with heparin antibodies.

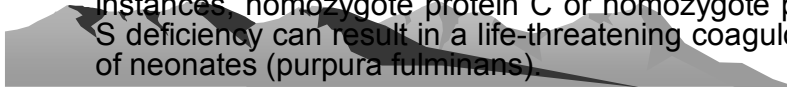
## Activated Protein C Resistance

- The pathophysiologic mechanism of resistance of APC is related to an inherited abnormality of factor V. Investigations have identified a genetic point mutation on chromosome 1 that encodes glutamine at the 506 site rather than arginine (ARG 506 GLN). This modification is responsible for the production of an aberrant factor V that is resistant to the proteolytic destruction by APC.
- Aberrant factor V was originally described in Leiden (Holland) and is more commonly referred to as factor V Leiden. When normal factor V is digested at the arginine 506 site, two other sites cooperate; 70% of the destruction of factor Va occurs at arginine 306, and 30% occurs at arginine 679. Protein S cooperatively acts upon arginine 306; therefore, even in the presence of factor V Leiden, there is a lesser degree of thrombosis when protein S is present. Conversely, when protein S deficiency coexists with factor V Leiden, thrombotic events are more prevalent. Except for other rare genetic abnormalities (factor V Hong Kong and factor V Cambridge, factor V Leiden is synonymous with the term APC resistance.




Reference	Polymorphism	Cases/controls	Endpoints	Effect and OR
Bockholdt et al. [80] <sup>P</sup>	FV R506Q	1302MI/2093 controls	MI	Questionable: OR=1.26 (95% CI: 0.94-1.67)
Juril et al. [79] <sup>P</sup>	FV R506Q	4623MI/12836 controls	MI	Positive: OR=1.20 (95% CI: 1.03-1.39)
Wu and Tsongalis [71] <sup>P</sup>	FV R506Q	2323 MI/3108 controls	CAD	N.S.
Rosendaal et al. [81]	FV R506Q	84 MI/388 controls	Women with MI <45 years of age	Positive: OR=4.0 (95% CI: 1.2-12.1) after adjustment for age and cardiovascular risk factors
Van de Water et al. [82]	FV R506Q	41 MI without coronary stenosis/114 MI with coronary stenosis	Premature MI <50 years without significant coronary stenosis	Positive: OR=4.70 (95% CI: 1.3-17.7)
Mansoura et al. [84]	FV R506Q	107 MI without coronary stenosis/244 MI with coronary stenosis/400 healthy controls	Premature MI <55 years without significant coronary stenosis	Positive: OR=2.93 (95% CI: 1.18-7.31)
Juril et al. [79] <sup>P</sup>	FV R506Q	1501 stroke/10176 controls	Ischemic stroke	N.S.
Wu and Tsongalis [71] <sup>P</sup>	FV R506Q	983 stroke/2416 controls	Cerebrovascular disease	Positive: OR=1.43 (95% CI: 1.03-1.97)
Juril et al. [79] <sup>P</sup>	FV R506Q	453 stroke/1180 controls	Ischemic stroke in children	Positive: OR=4.79 (95% CI: 3.26-7.03)
Doggen et al. [89]	FV HR2 allele	560 MI/646 controls	MI	N.S.


## Protein C and Protein S Deficiencies

- Protein C and protein S are vitamin K-dependent factors that are synthesized in the liver
  - Protein C originates on chromosome 2 and protein S on chromosome 3.
  - Deficiencies of these proteins have been considered autosomal dominant defects, though recent analyses suggest that the defects may be recessive but with a high frequency of concomitant defects of other coagulation proteins.
  - Two types of protein defects cause protein C or protein S deficiency: deficiency of protein content (antigen) or the presence of dysfunctional protein. Protein S deficiency occurs at a slightly greater frequency than does protein C deficiency. Heterozygote protein C and protein S abnormalities cause hypercoagulability; in rare instances, homozygote protein C or homozygote protein S deficiency can result in a life-threatening coagulopathy of neonates (purpura fulminans).
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## Prothrombin 20210A

- Frequency of this abnormality varies from 0.7% to 6.0% among whites, with rare appearances among Africans and Asians, suggesting that the defect may have also appeared after the divergent migrations of the populations.
  - The combination of prothrombin 20210A with other defects such as factor V Leiden, protein S deficiency, protein C deficiency, or antithrombin deficiency has been reported.
  - The mechanism by which prothrombin 20210A allele is responsible for hypercoagulability is uncertain.
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## Other Inherited Disorders

- Other inheritable hypercoagulable diseases such as increased levels of factor VIII have been recently reported.
  - Dysplasminogenemia, hypoplasminogenemia, decreased release of tissue plasminogen activator, and increased concentrations of plasminogen activator inhibitor may occur rarely but are not well established.
  - Dysfibrinogenemias are usually manifested as bleeding disorders because of defective fibrin formation, but thrombotic complications may occur when the defective fibrin is resistant to the lytic effects of plasmin. Recent descriptions of elevated levels in factor VIII suggest an etiologic mechanism for recurrent thromboembolic disease; factor VIII elevations may be increased by inherited and acquired factors.
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## Antiphospholipid Syndrome

- The "lupus anticoagulant" is an acquired biologic abnormality characterized as an "anticoagulant" in vitro but associated with excessive clotting in vivo.
  - This abnormality, also referred to as the antiphospholipid syndrome, should be suspected in young persons with arterial disease such as myocardial infarctions and acute neurologic events (cerebrovascular accidents and transient ischemic attacks).
  - is seen in women with recurrent pregnancy loss or in patients with increased thrombosis especially in unusual locations such as retinal veins, cerebral vessels, and hepatic venous channels (Budd-Chiari syndrome). Patients with antiphospholipid syndrome may have mild thrombocytopenia as well.
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## Other Acquired Disorders

- Malignancy, pregnancy, surgery, connective tissue diseases, lymphoproliferative diseases, myeloproliferative disorders, and dysproteinemias are recognized causes of hypercoagulability, but the mechanisms are unclear and may vary with each situation.
- With malignancy, excessive clotting is allegedly related to thromboplastin-like effects produced by tumor cells or their products. Mucin-producing malignancy has a high association of thrombosis. Excessive clotting with malignancy may also be caused by concomitant infections, effects of chemotherapy, malnutrition and possible folate deficiency with its consequences on homocysteine, and prolonged bed rest. Venous access devices that are commonly used in cancer patients predispose to clotting.

## Other Acquired Disorders

- Cancer patients often receive low-dose warfarin (1 to 2 mg/day) to lessen the frequency of veno-occlusive disease. Cancer patients also are relatively resistant to anticoagulation and have more episodes of recurrent thrombosis.
- Pregnancy associated clotting may relate to excess thromboplastin production; hypercoagulability is more frequent with pregnancy complications, such as abruptio placenta, amniotic fluid embolization, and retained dead fetus. Mechanisms of hypercoagulability with surgery are less clear but may relate to tissue trauma and/or the effects of bed rest. Previous clotting also predisposes to recurrence; some factors include clotting associated abnormalities in the anatomy of the vasculature and associated increased levels of coagulation factors as acute phase reactants.

Factors Responsible for Altered Coagulation Values

Situation	Antithrombin	Protein C	Protein S
Pregnancy	Decrease	Increase	Decrease
Oral contraceptive use	Decrease	Increase	Decrease
Acute deep venous thrombosis	Decrease	Decrease	Decrease
Disseminated intravascular coagulation	Decrease	Decrease	Decrease
Surgery	Decrease	Decrease	Decrease
Liver disease	Decrease	Decrease	Decrease
Inflammation	None	None	Decrease
Heparin	Decrease	None	None/Increase
Oral anticoagulants	Increase	Decrease	Decrease