I. INTRODUCTION

The purpose of blood is to carry numerous substances, including oxygen and nutrients, to various parts of the body. In order to accomplish this task, blood must remain in a liquid state; but we are faced with two questions: How does blood remain fluid? How does blood clot when a blood vessel is injured to prevent escape of the blood from the vessel? The answers lie in a complex process which we will study in the next two lectures.

Normal hemostasis is the complex process by which ruptured vessels undergo changes which prevent blood loss. The major event is the formation of a hemostatic plug that fills the leakage site in the injured vessel.

Hemostasis is dependent upon three major entities:


b. Platelets and platelet release products

c. Coagulation and fibrinolytic systems

* Diseases which effect one or more of these entities will result in abnormal hemostasis. Such pathologic conditions as cancer, sepsis and congenital coagulation defects may lead to bleeding disorders.
II. **NORMAL HEMOSTASIS**

   **A. REGULATION OF HEMOSTASIS**

   * Under normal conditions blood remains fluid and the cellular components of blood (erythrocytes, leukocytes and platelets) are not activated or physiologically altered. Similarly the endothelial cells also remain inert. Only in pathologic conditions is the functional state of these components altered. Endothelial damage, activation of platelets and release of tissue factor from cells results in thrombogenesis. Once a thrombus is formed it can obstruct blood flow and produce an inflammatory response.
B. SUMMARY OF HEMOSTATIC RESPONSES

1. Vasoconstriction occurs immediately and briefly through reflex neurogenic mechanisms. Vasoconstriction may mediated humor factors released from the endothelium such as endothelin. Vasoconstriction serves to reduce blood loss.

Fig. 4-5 A: Robbins and Cotran

- Endothelin Release
  Endothelin is a potent vasoconstricting agent released from the endothelial cells in distress.

- Reflex Vasoconstriction
  Release of various mediators

- Extracellular Matrix
  Contractile fibers

2. Primary hemostasis: Platelet adherence and activation. V injury damages endothelial cells and exposes subendothelial collagen. Platelets quickly adhere to the collagen and become activated. Activation means that the platelets change in shape and release substances such as adenosine diphosphate, thromboxane A2 and serotonin which recruit additional platelets to the site of injury and promote aggregates to form,
resulting in a hemostatic plug.

Fig. 4-5 B: Robbins and Cotran

B. PRIMARY HEMOSTASIS

1. Platelet adhesion
2. Shape change
3. Granule release (ADP, TXA₂)
4. Recruitment
5. Aggregation (hemostatic plug)

a. Platelets adhere to the damaged vessels (GP Ib binding to vWF).
b. Platelets undergo shape change, from discoid to non-discoid formation (extending pseudopods).
c. Light granules (alpha) release PF 4, PDGF and other proteins. Dense granules (beta) release ADP, Ca⁺², histamine, serotonin and epinephrine.
d. Recruitment. Activated platelets recruit other platelets
e. Hemostatic plug formation. Several platelets aggregate and form a plug.

3. Secondary hemostasis: Simultaneously, tissue factor is released at the site of injury from the endothelial cells which combine with platelet factors to initiate the plasma coagulation cascade, ultimately forming thrombin. The coagulation proteins form complexes on the platelet surface utilizing the phospholipids of the platelet membrane.
a. **Tissue factor.** Procoagulant released from various cells. Promotes coagulation.

b. **Phospholipid complex expression.** Surface phospholipids are expressed. Promotes the coagulation process.

c. **Thrombin generation.** By the activation of coagulation cascade, thrombin is generated.

d. **Fibrin polymerization.** The formed fibrin is polymerized by Factor XIIIa.
4. **Formation of platelet-thrombin plug (permanent plug):** Thrombin stimulates recruitment and activation of additional platelets. Thrombin enzymatically converts fibrinogen to fibrin. Fibrin serves to bind (stabilize) and anchor the aggregated platelets. The consolidated platelet-fibrin clot (thrombin) forms a permanent plug which seals the hole in the vessel wall. Erythrocytes and leukocytes become part of the thrombus.

Fig. 4-5 D: Robbins and Cotran

Once the clot is formed, it is subjected to endogenous lysis by fibrinolytic enzymes. Clot size can also be increased due to cellular recruitment.

The composition of the clot depends on the vascular site at which it is formed and the patient’s own pathophysiologic state. The stationary clot (Thrombus) can also break apart and travel to another location in the vasculature (Embolus).
III. COMPONENTS OF THE HEMOSTATIC SYSTEM

A. ROLE OF ENDOTHELIUM

1. Endothelial cells modulate elements of the hemostasis - coagulation sequence. There are two possible pathways depending on the circumstances.
   a. Antithrombotic effect (normal state)
   b. Prothrombotic effect (response to injured endothelium)

2. Antithrombotic effect
   a. Antiplatelet effect: Intact endothelium prevents platelets and coagulation proteins from coming into contact with subendothelial collagen. Furthermore, normal endothelial cells secrete prostacyclin and nitric oxide that prevent platelet aggregation.
   b. Anticoagulant effect: The endothelial cell membrane contains receptors which play an indirect role in anticoagulation. Heparin-like molecules, combine with a naturally occurring anticoagulant protein, antithrombin, to inhibit thrombin and other coagulation factors. Thrombomodulin, binds to thrombin creating a complex that activates protein C, a naturally occurring anticoagulant. The endothelium also secretes protein S which is a cofactor for protein C activation.
   c. Fibrinolytic effect: Endothelial cells also secrete plasminogen activators (t-PA) which promote fibrinolysis. Plasminogen is converted into plasmin and dissolves the clot.
2. **Prothrombotic effect**: Normal endothelial cells inhibit platelet adherence and prevent blood clotting. Injury causes a loss of these anticoagulant mechanisms.
a. Endothelial cells secrete von Willebrand factor (vWF), a protein, which forms a molecular bridge between platelets and subendothelial collagen. Platelet adhesion to endothelial cells occurs.
b. Simultaneously, endothelial cells synthesize and secrete tissue factor, which activates the extrinsic sequence of the coagulation cascade. Cytokines released by injured endothelial cells can stimulate cells to synthesize more tissue factor.
c. Tissue factor promotes the generation of thrombin and formation of a clot. Once the clot is formed it traps other cells such as erythrocytes and leukocytes.
B. PLATELETS

1. DEFINITION: Platelets are discoid, anuclear cells which play a major role in hemostasis.

2. Structure of platelets:
   a. The plasma membrane contains many glycoprotein receptors which play a role in the attachment of platelets to subendothelial proteins, (via von Willebrand factor), inter-adherence between platelets (via fibrinogen) and secretion of substances from intra-cytoplasmic platelet granules.
   
   b. Platelet cytoplasm contains two types of granules which contain substances which play a role in hemostasis.
      i. **Light granules (alpha):** contain fibrinogen, fibronectin, coagulation factors V and VIII, platelet factor 4 (heparin-binding chemokine), and growth factors, PDGF (platelet derived growth factor) and TGFβ (transforming growth factor beta).
      ii. **Dark granules (beta):** contains ADP, ATP, ionized calcium, histamine, serotonin and epinephrine.
   
   c. Platelet receptors
      i. Glycoprotein IIb/IIIa
      ii. Glycoprotein Ib
      iii. Thrombin
      iii. Serotonin
      iv. ADP
3. **Function of platelets:** With vessel injury, circulating platelets are exposed to subendothelial proteins (e.g., collagen, proteoglycans, fibronectin) which causes platelets to undergo three reactions.

   a. **Adhesion:** Platelets attach to the subendothelial collagen through a molecular bridge. The platelet’s plasma membrane, glycoprotein receptor (GP Ib) attaches to the von Willebrand factor which in turn attaches to collagen. Adhesion is a critical reaction because it prevents the blood flow from dislodging the adherent platelets and unplugging the defect in the vessel wall.
b. **Activation and secretion:** Activation of platelets (leading to exposure of platelet membrane phospholipids and secretion of substances within platelet granules) is initiated by molecules binding with platelet membrane GP IIb/IIIa receptors. Platelet-platelet aggregation occurs via the GP IIb/IIIa receptors and a fibrinogen bridge between platelets. Upon activation platelets release granular contents including such substances as coagulation factors, ADP, calcium and thromboxane A2. The phospholipid complex is activated when negatively charged phospholipids become exposed on the platelet surface. This complex serves as a site on which coagulation factors combine with ionized calcium (released from dense granules) to activate the intrinsic pathway of the coagulation cascade to form thrombin.
c. **Aggregation:**

i. The release of ADP and thromboxane A₂ from activated platelet granules initiates a reaction which serves to recruit, activate and aggregate greater numbers of platelets into a primary hemostatic plug (temporary plug). Serotonin and thromboxane A₂, released by platelet granules, vasoconstrict the vessel, decreasing the size of injury, reducing blood flow and the likelihood of the plug detaching from the vessel wall.

ii. Simultaneously, thrombin is formed by the activation of the intrinsic pathway (ADP, calcium, coagulation factors, phospholipid complex). Thrombin, ADP and thromboxane A₂ accelerate platelet aggregation. Thrombin accelerates platelet aggregation and converts fibrinogen to fibrin. Fibrin surrounds and structurally holds platelets in a secondary (irreversible) hemostatic plug.

C. **COAGULATION SYSTEM**

1. The blood coagulation system is comprised of a network of proenzymes, which are activated to their functional form, eventually resulting in the generation of thrombin. Once thrombin is formed, it is capable of converting a soluble, structural plasma protein, namely, fibrinogen into fibrin (clot).

Fig. 4-8: Robbins and Cotran

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THROMBIN(IIa)  FIBRINOGEN  FIBRIN  Xllla  STABILIZED
(SOLUBLE PROTEIN)  (CLOT)  TAFI  FIBRIN
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The fibrin clot is stabilized by a transamidase enzyme (XIIIa) and thrombin activatable fibrinolytic inhibitor (TAFI).
2. **COAGULATION FACTORS**

The coagulation system is composed of a complex network of proenzymes (zymogens), cofactors, activators and inhibitors. Initiation of either the extrinsic or intrinsic pathways results in the formation of thrombin which transforms fibrinogen to fibrin. Factor X plays a central role in the generation of thrombin by the intrinsic (left) and extrinsic (right) pathways.

3. **CLASSIFICATION OF BLOOD COAGULATION**
a. The fibrinogen group
   Factors I, V, VIII, and XIII
b. The prothrombin group
   Factors II, VII, IX, and X
All of these proteins contain $\gamma$-carboxy glutamic acid which is needed for the binding of calcium. All are synthesized in the liver and are vitamin K dependent.
c. The contact group
   Factors XI, XII, Fletcher factor (Prekallikrein), Fitzgerald factor (HMW Kininogen).
d. Other factors
   Protein C, Protein S, Fibronectin

4. INHIBITORS OF THE COAGULATION SYSTEM

These inhibitors are plasma proteins which are capable of inhibiting the formed serine protease enzymes involved in the regulation of the clotting process.

a. Antithrombin (AT)
b. Heparin cofactor II (HC II)
c. Tissue factor pathway inhibitor (TFPI)
d. Inhibitors to clotting factors
e. Lupus anti-coagulant and anti-phospholipid antibodies
f. Antibodies

Antithrombin is a plasma inhibitor which also mediates the anticoagulant actions of heparin. Congenital deficiencies of this inhibitor result in thrombophilia. Heparin cofactor II is a weak inhibitor of thrombin. Tissue factor pathway inhibitor is a potent inhibitor of tissue factor and is responsible for some of the anticoagulant effects of heparin.
D. THE FIBRINOLYTIC SYSTEM

The fibrinolytic system is a network of enzymes that are responsible for the dissolution of a formed clot. Like the coagulation system, this system is comprised of proenzymes which when activated are converted to their enzymatic form and are then capable of digesting the formed clot. When activated these enzymes can also facilitate the digestion of fibrinogen.

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\text{Plasminogen} \xrightarrow{\text{Plasminogen activators (PA)}} \text{Plasmin} \xrightarrow{\text{Fibrin}} \text{Fibrin split products}
\]

Plasminogen (profibrinolysis) is converted by plasminogen activators (PA) into plasmin. Once plasmin is formed it can digest the clot (fibrin). Several physiologic and pharmacological plasminogen activators can convert plasminogen into plasmin. On the other hand, several natural and acquired inhibitors can oppose plasminogen activation.

The important inhibitors of the fibrinolytic system are given below
- Plasminogen activator inhibitor (PAI)
- $\alpha_2$-antiplasmin
- $\alpha_2$–macroglobulin
- Thrombin activatable fibrinolytic inhibitor (TAFI)
IV. THROMBOSIS

A. INTRODUCTION

1. Abnormal hemostasis, thrombosis, is the process by which blood forms a clot within intact blood vessels (vessels which have not ruptured).
2. Abnormal hemostasis is a pathologic process that represents the activation of the clotting system when there are no ruptured vessels.
3. Bleeding occurs when a blood clot ruptures

B. THROMBOSIS

Thrombosis can be defined as a pathologic transition of the state of blood from fluidity to non-fluidity. Initially a stationary thrombus is formed. The stationary clot (thrombus) may progress and eventually break into smaller pieces which when released into the blood circulation are called emboli (embolus). Many factors are responsible for the process of thrombogenesis.

The thrombogenic mechanisms differ in the arterial and venous systems. Such conditions as blood flow, endothelial cell composition, the size of the blood vessel and the degree of oxygenation influences this process.
C. PATHOGENESIS OF THROMBOSIS (VIRCHOW'S TRIAD)

The following three major factors contribute to thrombosis.

1. Injury to endothelium resulting in the release of tissue factor
2. Alterations in blood composition and flow
3. Stasis

D. HYPERCOAGULABLE STATE

Imbalance of the blood coagulation mechanisms leading to thrombotic transitions.

Age, smoking, oral contraception and diet play important roles in contributing to a
hypercoagulable state. Thrombotic stroke, myocardial infarction and peripheral arterial thrombosis result from this syndrome.

1. **MOLECULAR THROMBOPHILIA**
   a. Factor V Leiden (APC resistance)
   b. Prothrombin 20210
   c. Methylene tetrahydrofolate reductase (MTHFR)
   d. Abnormal antithrombin (mutant forms)

2. **ACQUIRED THROMBOPHILIAS**
   a. Sepsis, cancer and trauma
   b. Pregnancy, hyperlipidemia and drugs