HAEMOSTASIS

- **normal haemostasis = the sequence of events that follows vascular injury to rapidly produce a localised plug to prevent blood loss**

- normal haemostatic mechanisms are also responsible for maintaining blood in a clot-free fluid state

- haemostasis depends on three components:

  - **blood vessel wall**
  - **platelets**
  - **coagulation (clotting) factors**

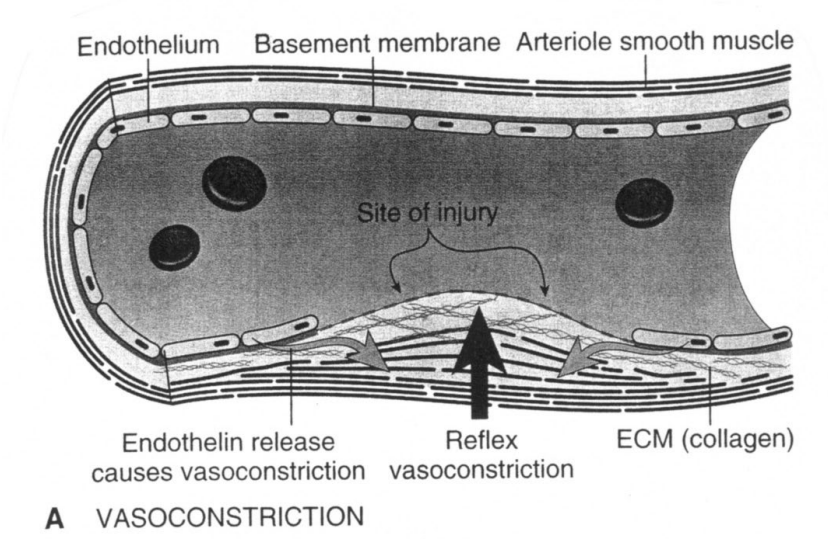
- following injury to a blood vessel, the pressure of blood accumulating extravascularly also contributes to limiting further haemorrhage

EVENTS IN NORMAL HAEMOSTASIS

PHASE 1 – VASOCONSTRICTION

- after vascular injury, there is a brief period of vasoconstriction (Figure 1)
- this response occurs immediately after vessel wall injury and is largely attributable to reflex neurogenic mechanisms, augmented by local secretion of factors such as **endothelin**, a potent vasoconstrictor released by endothelium
- vasoconstriction is effective in reducing local blood flow but it lasts only a few seconds
- bleeding will resume if there is not subsequent activation of platelets and the coagulation cascade

Figure 1

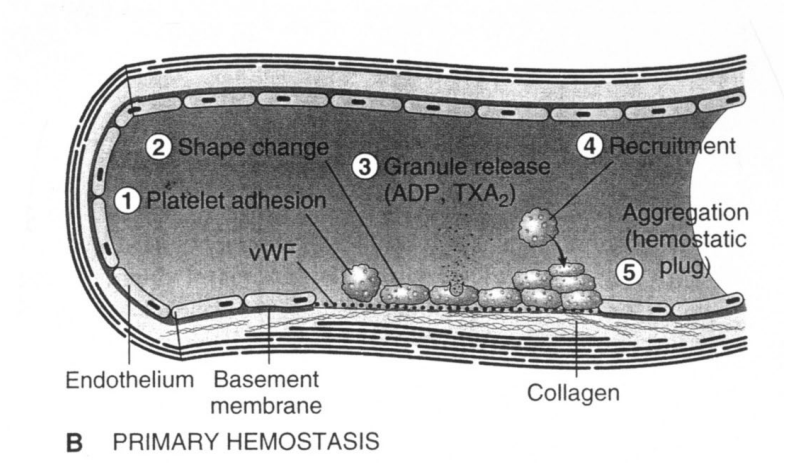


Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

PHASE 2 – PRIMARY HAEMOSTASIS

- endothelial injury exposes the highly thrombogenic (procoagulant) subendothelial extracellular matrix, causing platelets to adhere within seconds of vessel injury
- the adherent platelets become activated and release products that cause recruitment of additional platelets
- the platelets form a **primary haemostatic plug** at the site of vessel injury (Figure 2)
- the primary haemostatic plug is unstable and short-lived (up to a few minutes) but it is sufficient to control bleeding from tiny injuries to small vessels

Figure 2



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- platelets are derived from bone marrow megakaryocytes
- platelets circulate as anucleate, membrane-bound discs with integrin glycoprotein receptors on their surfaces

- platelets contain two types of granules:

- **alpha granules** – contain fibrinogen, fibronectin, clotting factor V, von Willebrand factor, platelet factor 4 (which binds heparin), thrombospondin, platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β)
- **dense granules** – ADP and ATP, ionised calcium, histamine, serotonin and adrenalin

- following vascular injury, circulating platelets encounter subendothelial extracellular matrix components that promote platelet adhesion, e.g. collagen (types I to IV), elastin, fibroblasts, smooth muscle cells, basement membrane, fibronectin etc
- **collagen is the only matrix protein that promotes both platelet adhesion and activation; it also expresses and is capable of binding von Willebrand factor**

Platelet Adhesion

- **platelet adhesion = adhesion of platelets to a non-platelet surface**

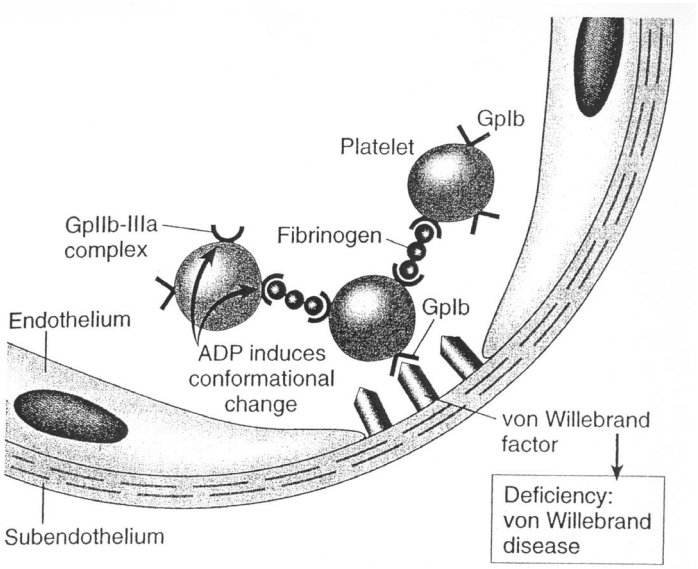
- under conditions of low shear stress, platelet adhesion to subendothelial collagen is

mediated by platelet membrane glycoprotein receptors, **GpIa/IIa** (also known as integrin $\alpha_2\beta_1$) and **GpVI**
- in blood vessels with high shear rates (e.g. arteries and arterioles), both **GpIa/IIa** and **von Willebrand factor** (vWf) are essential for platelet adhesion to exposed collagen

- vWf is a large glycoprotein that is expressed by subendothelial tissues
- it is also synthesised by endothelial cells and megakaryocytes and is present in plasma and, in some species, within alpha granules of platelets
- within plasma, vWf circulates as a complex with factor VIII which it stabilises
- within endothelial cells, vWf is stored within granules called Weibel-Palade bodies; injured endothelium releases vWf so that it can bind to exposed collagen

- platelets bind to vWf by means of their surface **glycoprotein Ib (GpIb) receptor** (Figures 3 and 4)
- once tethered by vWf, the platelets can become anchored by their surface **GpIa/IIa receptor** to the collagen
- without vWf, platelets will be dislodged from collagen by the high shearing forces of flowing blood in arteries and arterioles

Figure 3



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- platelet adhesion requires divalent calcium (Ca^{2+}) (factor IV) and is stimulated by ADP (released by damaged endothelium and by activated platelets), adrenalin, thrombin, thromboxane and platelet activating factor (PAF)

Platelet Activation and Secretion (Platelet Release Reaction)

- following adhesion to collagen, platelets undergo **activation**, an event that leads to a change in cell shape and release of the contents of alpha and dense granules
- these steps are regulated by alterations in the intracellular concentrations of cyclic

nucleotides, influx of calcium ions, hydrolysis of membrane phospholipids and phosphorylation of key intracellular proteins

- the cascade of signalling events that causes activation and secretion is initiated by binding of agonists (e.g. vWf, collagen, fibrinogen, ADP, thrombin, thromboxane) to platelet surface receptors → activation of membrane phospholipase A_2 and phospholipase C → hydrolysis of membrane phosphatidylinositol and phosphotidylcholine → release of arachidonic acid

- a small quantity of the arachidonic acid is metabolised via the cyclo-oxygenase pathway to **thromboxane A_2** (TXA_2)
- TXA_2 is a potent stimulant of platelet adhesion, activation and aggregation and of vasoconstriction; locally intense vasoconstriction assists in the control of bleeding
- the action of TXA_2 is opposed by that of **prostaglandin I_2** (PGI_2 or **prostacyclin**) produced by endothelial cells; PGI_2 is a vasodilator and inhibits platelet activation and aggregation

- phospholipase C leads via a series of steps to release of Ca^{2+} from the smooth endoplasmic reticulum into the cytoplasm → rearrangement of actin filaments → change in cell shape
- the platelets become swollen and spherical with protruding spiky pseudopods

- the change in cell shape promotes release of the contents of the alpha and dense granules and of lysosomes
- release of dense granule contents is especially important because **Ca^{2+}** is required in the coagulation cascade (see later) and **ADP** is a potent mediator of platelet adhesion, activation and aggregation
- ADP promotes clustering of **GpIIb-IIIa** receptors for **fibrinogen** on the platelet plasma membrane
- ADP induces approximately 40,000-50,000 binding sites for fibrinogen on a single platelet
- ADP also augments further ADP release from other platelets

- platelet activation and shape change also lead to exposure of a negatively charged **phospholipid complex** on the platelet membrane surface
- this complex acts as a critical nucleation site for Ca^{2+} and coagulation factors in at least two steps in the ensuing coagulation cascade: activation of factor VIII and cleavage of prothrombin (factor II) to thrombin (see later and Figure 6)

- activated platelets also release **platelet factor 4** which binds heparin (an anticoagulant) and promotes polymerisation of fibrin (see later)

- activated platelets also release **platelet activating factor** (PAF) which promotes platelet adhesion and aggregation

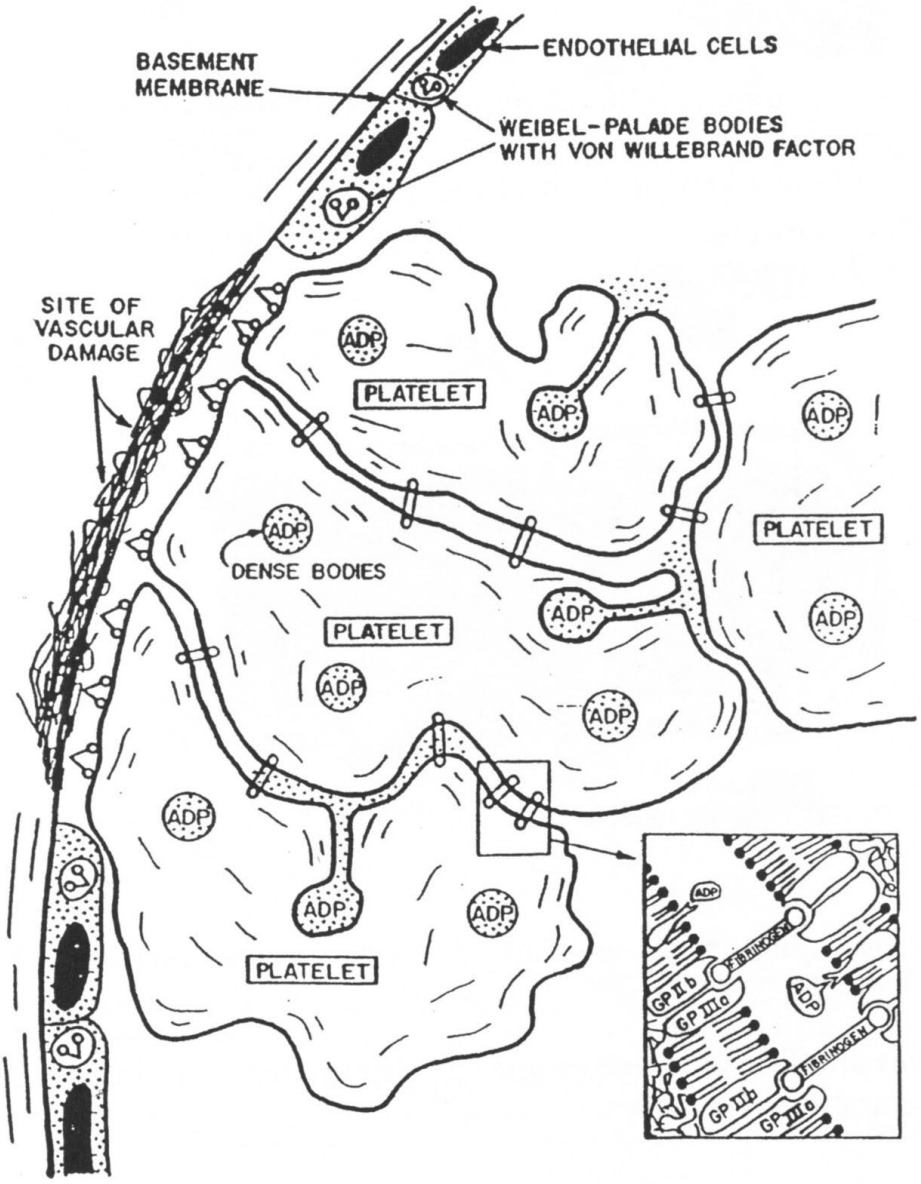
Platelet Aggregation

- see Figures 3 and 4
- **platelet aggregation = sticking of platelets to each other**

- platelet aggregation follows adhesion and secretion
- **ADP** and **thromboxane A_2** are the most potent triggers of platelet aggregation

- **fibrinogen** (factor I) and Ca^{2+} are essential cofactors for platelet aggregation

Figure 4



Reference: "Mechanisms of Disease – A Textbook of Comparative General Pathology" – D.O. Slauson and B.J. Cooper, 2nd edition, Williams and Wilkins, Baltimore, 1990

- ADP promotes exposure of GpIIb-IIIa receptors on activated platelet membranes
- fibrinogen binds to these receptors and links platelets together in a rapidly snowballing sequence
- the enlarging platelet aggregate forms the **primary haemostatic plug**

- the primary haemostatic plug is reversible
- however, if the coagulation cascade is activated, **thrombin** is generated
- thrombin binds to a surface receptor on platelets and, together with ADP and thromboxane A_2, causes further platelet aggregation and catalyses the conversion of fibrinogen to fibrin

- the aggregated platelets then contract under the influence of **thrombospondin** (from platelet alpha granules) to form an irreversibly fused mass of platelets (**viscous metamorphosis**) which contributes with fibrin to the **secondary haemostatic plug**

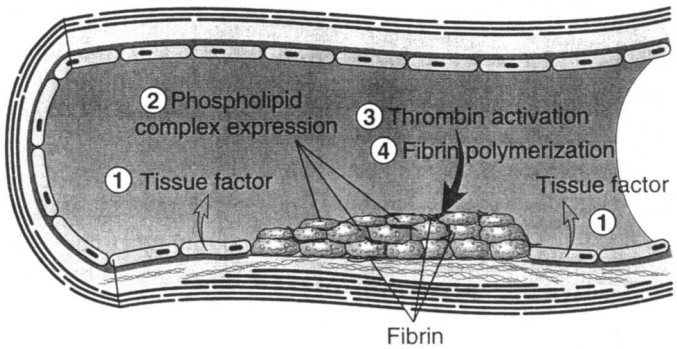
- **vasoconstriction and platelet plug formation provide an adequate, short-lived haemostatic seal following injury to capillaries, venules and small arterioles**
- **in larger vessels** (in which the hydrostatic pressure is greater), **a more secure and long-lasting fibrin plug is required to prevent haemorrhage**

PHASE 3 – SECONDARY HAEMOSTASIS

- following vascular injury, the **coagulation** (or **clotting**) **cascade** is activated almost simultaneously with platelet adhesion
- the coagulation cascade is a cascade of reactions in which circulating inactive proenzymes are converted to active protease enzymes
- by convention, the inactive proenzymes are designated by Roman numerals and the activated enzymes by the letter "a" after the Roman numeral
- all of the coagulation factors are present normally in circulation and most are produced in the liver

- the coagulation cascade culminates in the formation of **thrombin** which converts **fibrinogen** (a soluble plasma protein) into **fibrin** (an insoluble fibrillar protein)
- fibrin polymerises to form a meshwork that cements the fused mass of aggregated platelets of the primary haemostatic plug into a stable, lasting, **secondary haemostatic plug** at the site of vessel injury (Figure 5)

Figure 5



C SECONDARY HEMOSTASIS

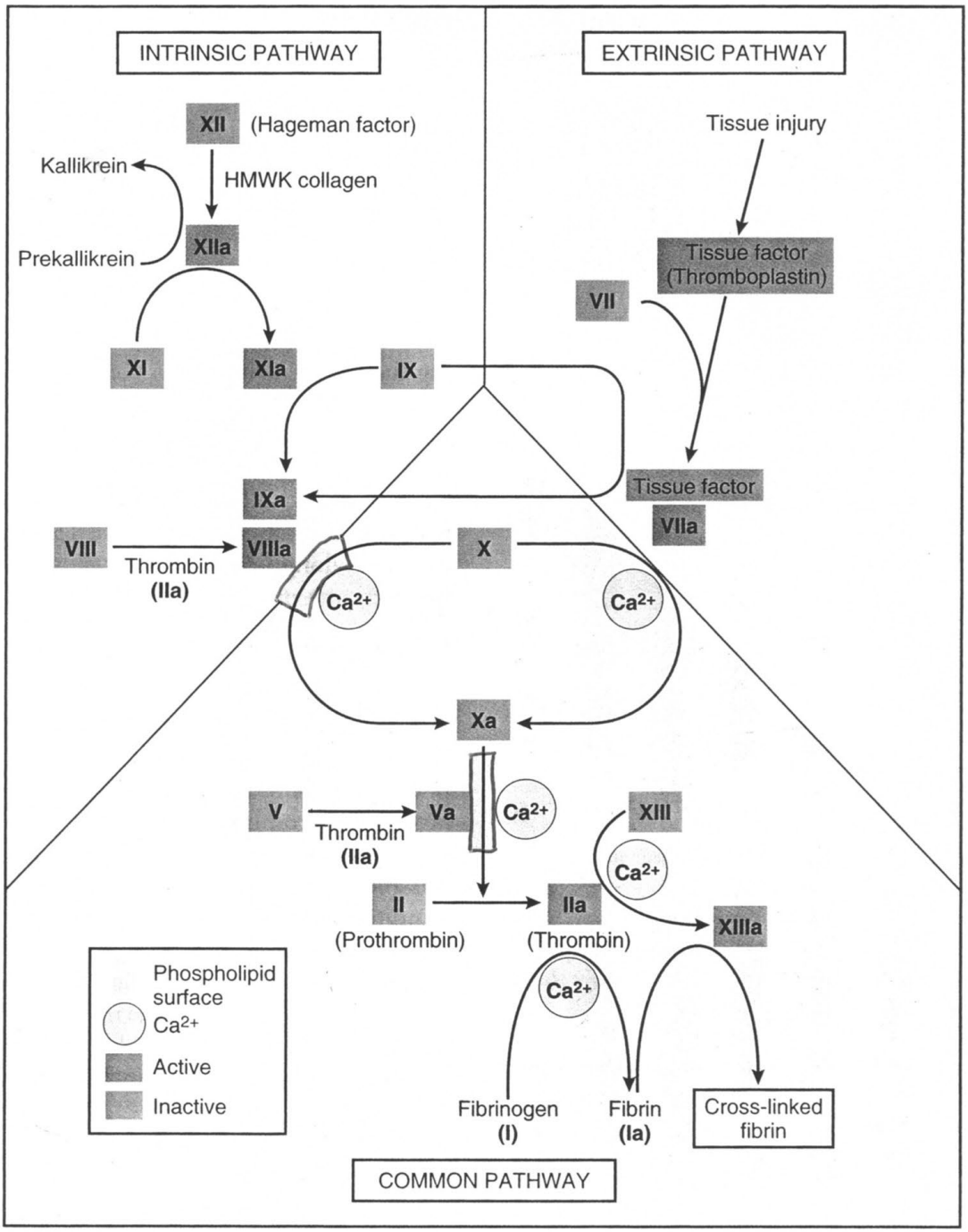
Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- the cascade involves amplification that enables a sufficient amount of fibrin to be produced from a small concentration of initiators

- traditionally, the coagulation cascade has been divided into two pathways, the **intrinsic system** and the **extrinsic system**, which converge where factor X is activated as the **common system**, a final common pathway leading to fibrin formation (Figure 6)

- each reaction in the coagulation cascade results from assembly of a complex composed of an enzyme (an activated coagulation factor), a substrate (a coagulation factor proenzyme) and a cofactor (a reaction accelerator)
- at multiple sites in the cascade, the complexes are assembled on a **phospholipid surface** and are held together by **calcium ions**; therefore, clotting tends to remain localised to sites where **such assembly can occur, e.g. the surface of activated platelets**

Figure 6



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

The Intrinsic System

- the intrinsic coagulation pathway (or **"contact" system**) involves components normally present in the circulation (i.e. intrinsic to blood)

- **Hageman factor (factor XII)** is activated by contact with a negatively charged surface (e.g. exposed subendothelial collagen or the primary haemostatic plug) (Figure 7)
- activated Hageman factor (factor XIIa), assisted by **high molecular weight kininogen** (HMWK), activates **prekallikrein** (to kallikrein) and factor XI (to factor XIa)
- factor XIa activates factor IX (to factor IXa) and so on down the cascade (Figure 6)

- there are **redundancies** in the coagulation cascade because of connections between the intrinsic and extrinsic systems
- for example, factor IX in the intrinsic system can be activated by a complex of factor VIIa and factor III from the extrinsic system
- platelets can also activate factor XII (in the presence of ADP) and factor XI (in the presence of collagen)
- because of these redundancies, deficiencies of factor XII, prekallikrein and high molecular weight kininogen do **not** cause clinical haemorrhage (although they may cause abnormal results in laboratory tests of haemostasis)

- it is now known that **the intrinsic pathway is not involved in the initiation of coagulation *in vivo***

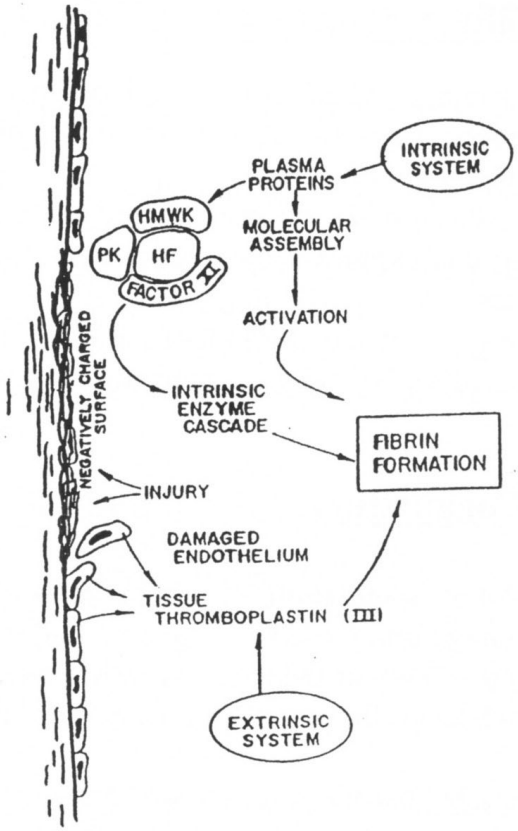
The Extrinsic System

- the extrinsic system is so named because it is initiated by **tissue factor (tissue thromboplastin**; **factor III**) derived from tissue rather than blood
- tissue factor is a membrane glycoprotein that is present in most tissues and exposed following cell injury
- small concentrations of tissue factor are also present in circulation (as a soluble form and as microparticles derived from membranes of haematopoietic cells)
- vascular injury causes expression of tissue factor by perivascular cells (e.g. fibroblasts)
- tissue factor forms a complex with circulating factor VII and calcium ions and activates factor VII to factor VIIa (Figures 6 and 7); this complex then activates factor IX (of the intrinsic pathway) and factor X (of the common pathway) (Figure 6)

- **activation of the extrinsic system by tissue factor is the major route of activation of coagulation *in vivo***

- however, Hageman factor (factor XII) of the intrinsic system is nevertheless of major importance because it can activate and link the kinin, complement, coagulation and fibrinolytic systems

Figure 7



Reference: "Mechanisms of Disease – A Textbook of Comparative General Pathology" – D.O. Slauson and B.J. Cooper, 2nd edition, Williams and Wilkins, Baltimore, 1990

The Common System

- see Figure 6

- factor Xa is capable of cleaving **prothrombin** (factor II) to **thrombin** on its own
- although only a small amount of thrombin is thereby generated, it is sufficient to activate platelets and factors V, VIII, XI and XIII

- the next phase of coagulation is the amplification phase
- factor IXa combines with factor VIIIa and Ca^{2+} on activated platelet membranes to form the **tenase complex**; this complex is the major activator of factor X

- factor Xa, factor Va and Ca^{2+} also form a complex (**prothrombinase**) on activated platelet membranes; this complex is the major activator of factor II
- **prothrombin** (factor II) is cleaved to **thrombin** (factor IIa)

- thrombin splits **fibrinogen** (factor I) bound to platelets to form a **fibrin monomer** (factor Ia)
- multiple fibrin monomers are then assembled into a still soluble **fibrin polymer** in a spontaneous, non-enzymatic self-assembly process
- stabilisation of fibrin occurs via **cross-linking**, in which covalent bonds are introduced into the fibrin polymer
- cross-linking is mediated by factor XIIIa (**fibrin stabilising factor**) in the presence of Ca^{2+}

- cross-linking renders the fibrin polymer insoluble, more elastic and less susceptible to lysis
- the cross-linked fibrin stabilises the primary haemostatic plug into a longer lasting **secondary haemostatic plug**

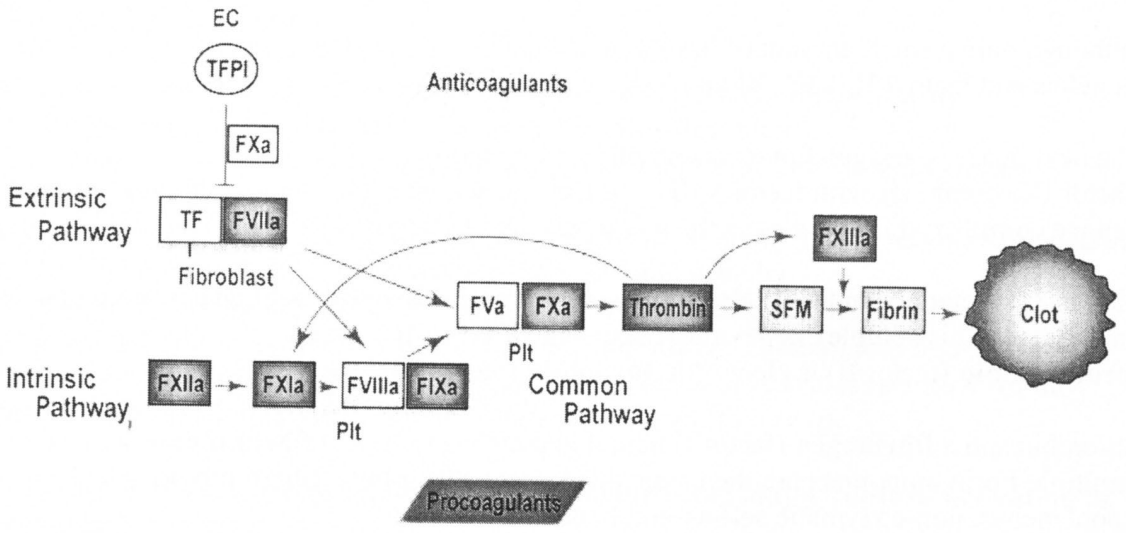
Other Actions of Thrombin

- thrombin is highly potent; thrombin present in 1 mL of blood can coagulate 3 L of blood
- in addition to cleaving fibrinogen, thrombin also has important effects on the local vasculature, platelets and leukocytes
- thrombin stimulates platelet adhesion, secretion and aggregation
- thrombin activates endothelium to express leukocyte adhesion molecules and various fibrinolytic molecules (e.g. tissue-type plasminogen activator), vasoactive molecules (e.g. nitric oxide and PGI_2 → vasodilation) and cytokines (e.g. PDGF)
- thrombin directly stimulates neutrophil and monocyte adhesion to endothelium

Tissue-based Model of Coagulation

- the current tissue-based model of coagulation (Figure 8) emphasises the essential role played by tissue factor in initiating coagulation *in vivo*
- it also emphasises the interrelatedness of the intrinsic and extrinsic pathways, the multiple positive and negative feedback loops that exist, and the amplification reactions that occur on activated cell membranes
- rather than being two independent parallel pathways converging on the common pathway, the intrinsic and extrinsic pathways are now believed to operate as an integrated web

Figure 8



Reference: N. Mackman. Tissue-specific hemostasis in mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **25**:2273-2281 (2005)

Although primary and secondary haemostasis have been presented above as separate processes, they are closely interlinked. For example, activated platelets accelerate several

steps in blood coagulation and thrombin (a product of blood coagulation) is a potent activator of platelets.

REGULATORY MECHANISMS IN HAEMOSTASIS

- once activated, the coagulation cascade must be restricted to the local site of vessel injury to prevent disseminated clotting
- the complicated system of normal coagulation has equally complicated regulatory controls to keep the system in check
- restriction of factor activation to sites of exposed phospholipids is one minor control mechanism
- other minor control mechanisms include local depletion of activated coagulation factors by blood flow and removal of activated factors by phagocytosis (e.g. by hepatic Kupffer cells), consumption, lysis or absorption

- the **four major regulatory mechanisms** are:

- the **antithrombin III system**
- the **protein C and protein S system**
- the **tissue factor pathway inhibitor**
- the **fibrinolytic system**

- each of these systems is essential for normal control of haemostasis and each controls different aspects of normal coagulation
- any imbalance in these regulatory mechanisms may cause a **hypercoagulable state** promoting **thrombosis**

The Antithrombin System

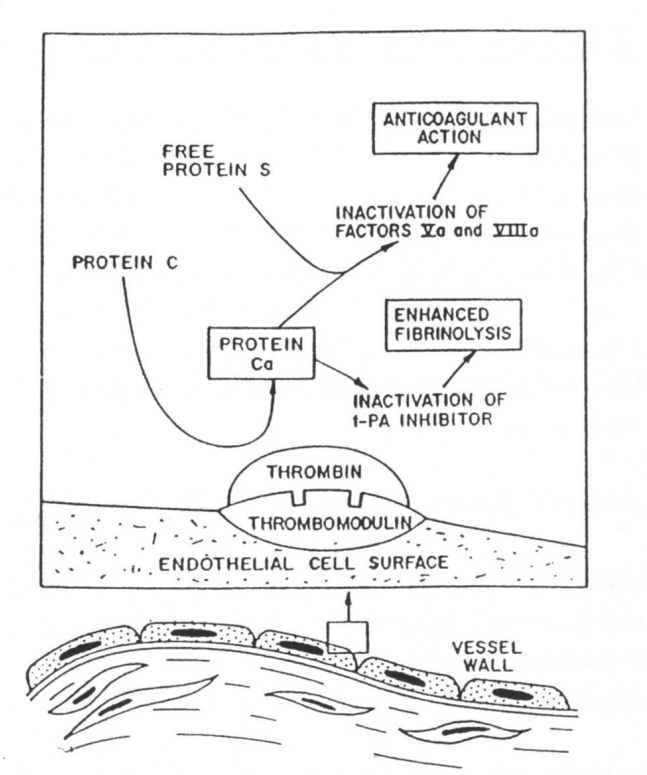
- **antithrombin** (**AT**) is the **most potent of the coagulation inhibitors**
- AT accounts for approximately 80% of naturally occurring coagulation inhibitory activity of plasma
- it is a glycoprotein protease synthesised by the liver and by endothelial cells
- AT is the **major inhibitor of activated thrombin (IIa)** and of **factor Xa**, two of the most important enzymes of the coagulation cascade, **as well as factors IXa, XIa and XIIa**
- AT also acts as a cofactor for the anticoagulant heparin
- AT is activated by binding to heparan sulphate molecules in the membranes of endothelial cells and platelets
- neutralisation of thrombin by AT also occurs on the surface of endothelial cells
- the AT-thrombin complex is then released back into the circulation and is eventually removed by specific receptors in the liver

The Protein C and Protein S System

- see Figure 9
- **protein C** is a vitamin K-dependent anticoagulant synthesised by the liver and is normally

present in plasma
- thrombin binds to a surface receptor on endothelial cells, **thrombomodulin**, which decreases the ability of thrombin to participate in platelet activation and fibrin generation
- the thrombin-thrombomodulin complex activates protein C to protein Ca

Figure 9



Reference: "Mechanisms of Disease – A Textbook of Comparative General Pathology" – D.O. Slauson and B.J. Cooper, 2nd edition, Williams and Wilkins, Baltimore, 1990

- protein Ca is released into the circulation where it **inactivates factors Va and VIIIa**
- protein Ca requires the presence of a cofactor, **protein S**, which is also a vitamin K-dependent anticoagulant synthesised by hepatocytes

- protein Ca also interacts with the fibrinolytic system by neutralising a circulating inhibitor of tissue-type plasminogen activator (t-PA), thereby accelerating conversion of plasminogen to plasmin to promote fibrinolysis (see below)

Tissue Factor Pathway Inhibitor

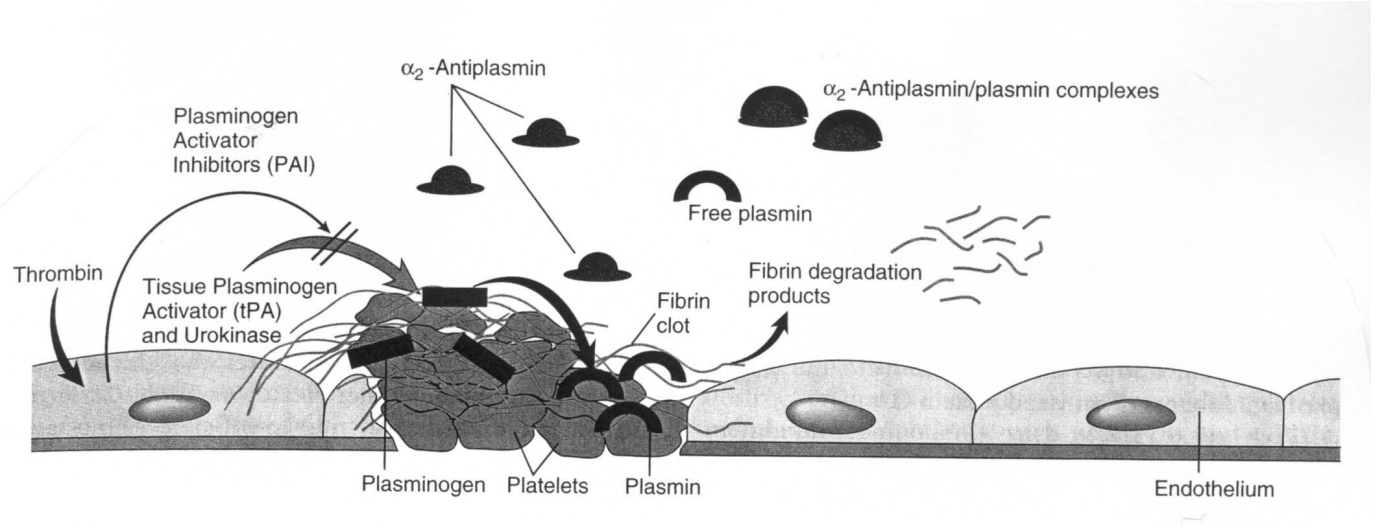
- tissue factor pathway inhibitor (TFPI) is a **significant inhibitor of the extrinsic pathway of coagulation** (Figure 8)
- it is present in plasma and is also expressed by endothelial cells and smooth muscle cells
- thrombin induces release of TFPI by endothelial cells and activated platelets
- TFPI forms a complex with factor Xa bound to the tissue factor-factor VIIa complex to inhibit subsequent factor X activation
- TFPI can interact with factor VIIa in the absence of factor Xa but only at a slow rate; TFPI

therefore does not substantially inhibit extrinsic coagulation until the concentration of factor Xa rises
- **following initiation of coagulation via the extrinsic pathway**, the tissue factor-factor VIIa complex is rapidly inhibited by TFPI, so that **components of the intrinsic pathway** (particularly factors VIIIa and IXa) **become the dominant regulators of thrombin generation**

The Fibrinolytic System

- see Figure 10
- **fibrinolysis = breakdown of fibrin**
- fibrinolysis commences as soon as the secondary haemostatic plug forms and is the major mechanism whereby blood clots are removed

Figure 10



Reference: "Robbins and Cotran Pathologic Basis of Disease" – V. Kumar, A.K. Abbas and N. Fausto. 7th edition, Saunders, Philadelphia, 2005

- **plasminogen** is produced by the liver and is a normal constituent protein of plasma, saliva, tears and milk

- plasminogen is activated to **plasmin** by the proteolytic action of **plasminogen activators**
- plasminogen activators may be **intrinsic** (e.g. kallikrein, factor XIIa or factor XIa) or **extrinsic**

- **extrinsic plasminogen activators** are of two types: **tissue-type plasminogen activator** (**t-PA**) and **urokinase-type plasminogen activator** (**u-PA**)
- **t-PA** is mainly synthesised by endothelial cells and functions largely in the circulation
- **u-PA** is synthesised by many cell types (including tissue macrophages) and functions largely within tissues but is also present in plasma
- plasminogen can also be activated by bacterial or fungal products (e.g. streptokinase)

- plasminogen activators proteolytically cleave plasminogen into plasmin

- **plasmin** cleaves factors V and VIII and splits fibrin into **fibrin degradation products** (**FDP**)
- FDP have weak anticoagulant activity by inhibiting platelet adhesion, secretion and aggregation and by inhibiting the effect of thrombin on fibrinogen
- some FDP can also cause smooth muscle contraction, increased capillary permeability and chemotaxis of neutrophils

- any free plasmin in the circulation is rapidly bound and neutralised by **α_2-antiplasmin**
- **t-PA** is most active when it is bound to fibrin; this helps to restrict activation of plasminogen to sites where fibrin is present (e.g. sites of haemorrhage or thrombosis)

- t-PA binding to fibrin is inhibited by **plasminogen activator inhibitors** (**PAI**) which are released by endothelial cells
- PAI are increased by thrombin and several inflammatory cytokines
- PAI probably play a role in thrombosis developing in sites of severe inflammation