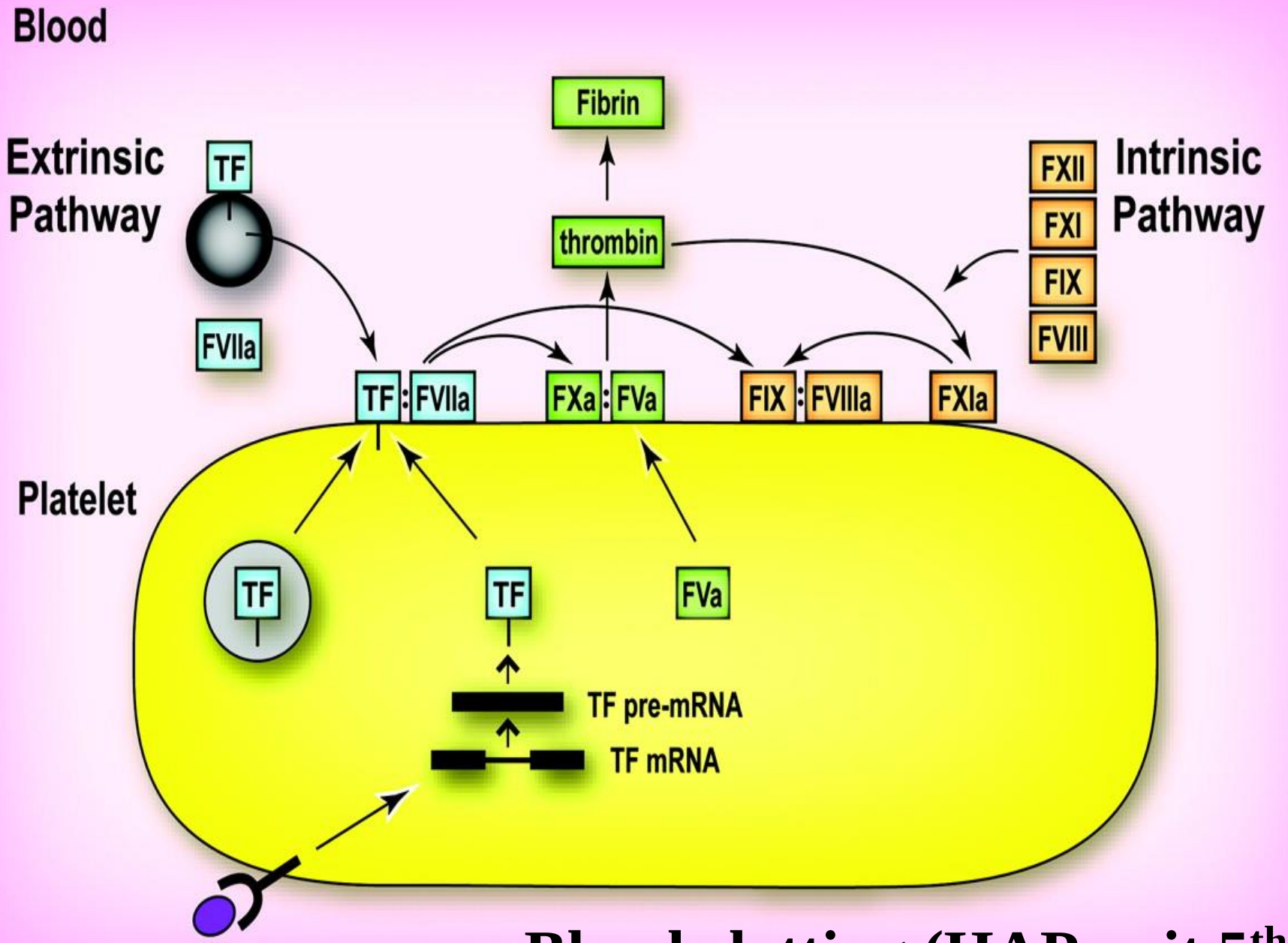
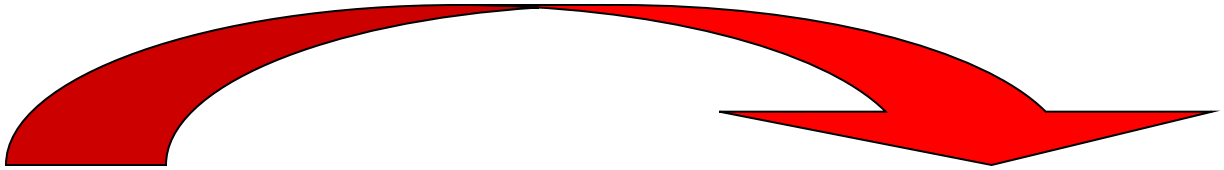


Blood clotting (HAP unit 5th)

Vessel injury



Antithrombogenic

(Favors fluid blood)

Thrombogenic

(Favors clotting)

3 Major systems involved

- **Vessel wall**
  - **Endothelium**
  - **ECM = BM (type 4 collagen + other proteins)**
- **Platelets**
- **Coagulation cascade**
  - **Coagulation factors (proteins)**
    - **Names and numbers**
    - **Active and inactive forms (zymogens)**

Coagulation Cascade

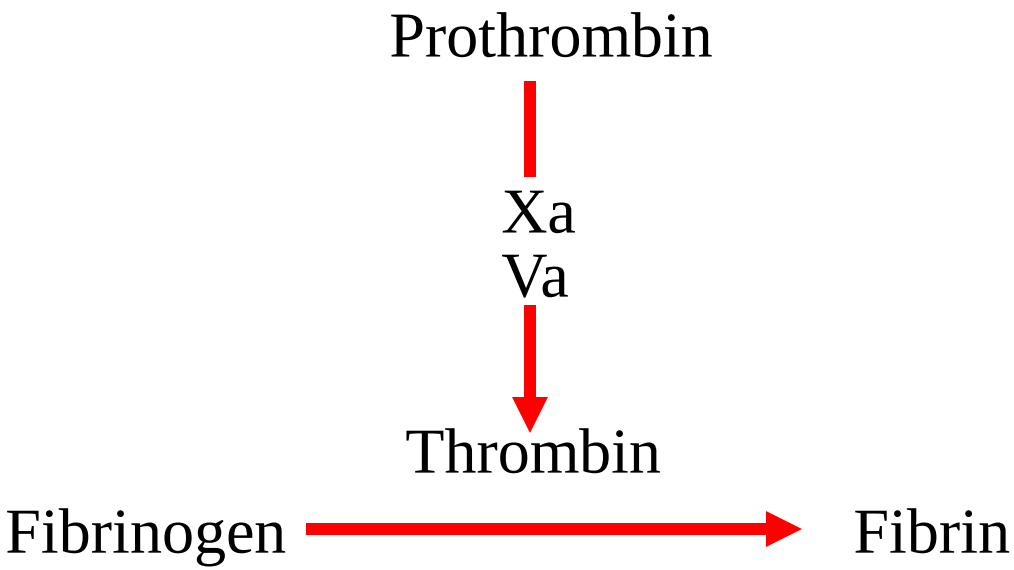
- **Enzymatic cascade** (amplification)
- **Several serine proteases**
  - Produced by liver (most)
  - Require Vit K (several)
- **3 protein cofactors (not enzymes)**
- **Requires Ca^{2+}**
- **Localized to site of injury**
- **Reversible (via production of plasmin)**

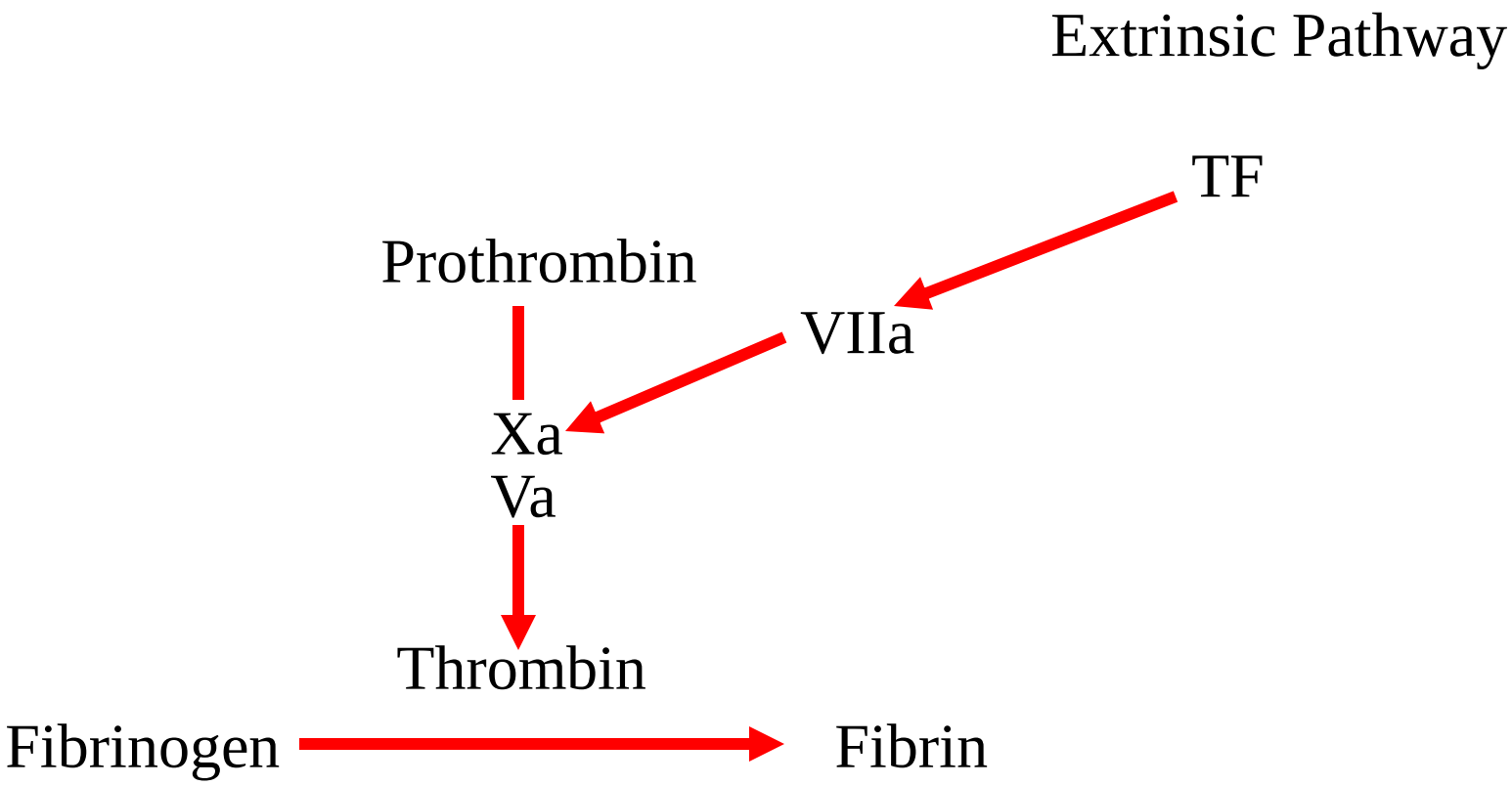
BLOOD COAGULATION

Coagulation factors and their properties

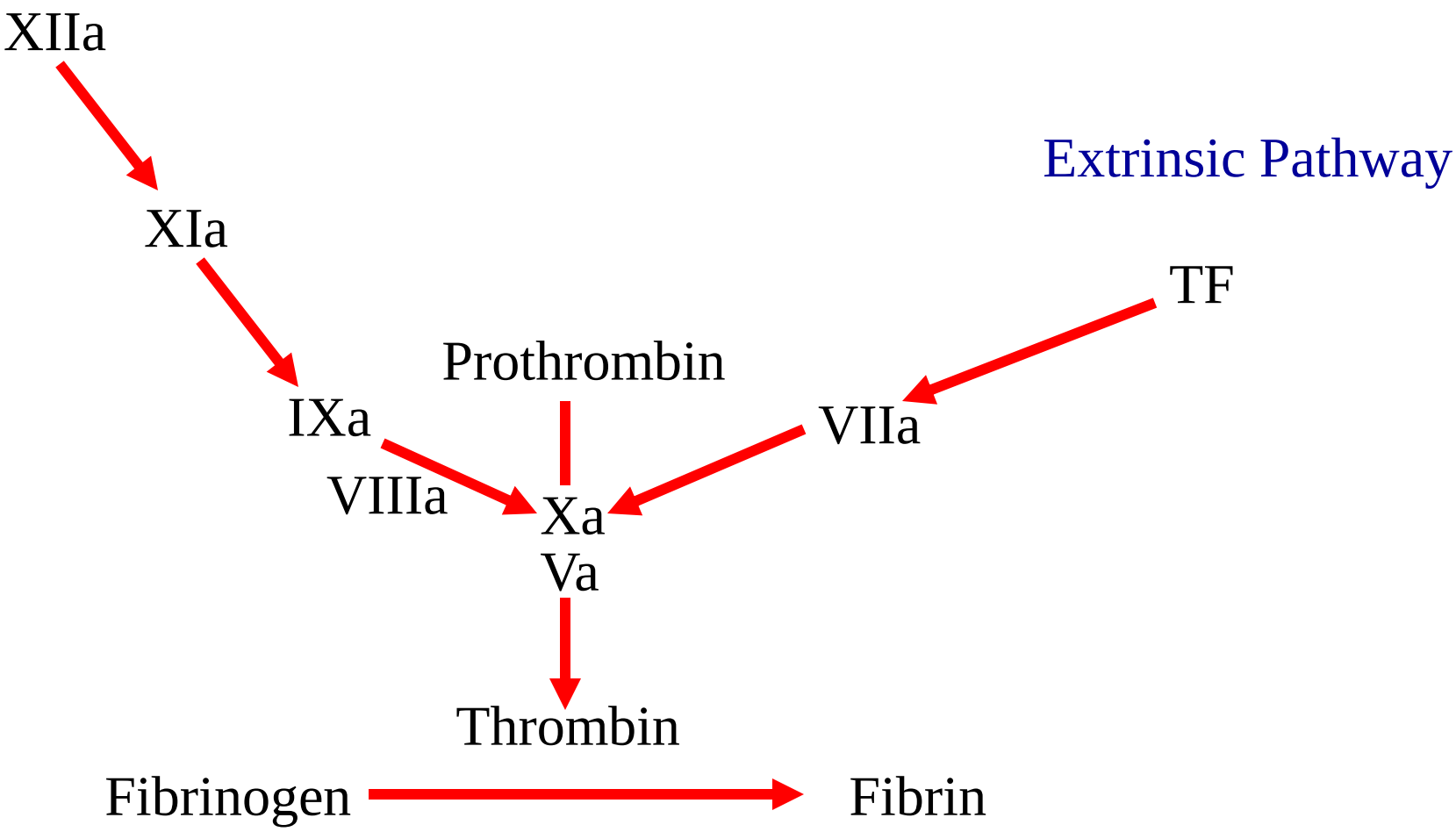
Factor	Synonyms	Molecular weight	Plasma concentration (mg/dL)
	Fibrinogen	340 000	200–400
	Prothrombin	70 000	10
	Tissue factor (thromboplastin)	44 000	0
	Calcium ion	40	9–10
V		330 000	1
VII		48 000	0.05
VIII		330 000	0.01
(vWF)		(250 000)n	1
IX		55 000	0.3
X		59 000	1
XI		160 000	0.5
XII		80 000	3
XIII		320 000	1–2
Prekallikrein		85 000	5
High-molecular-weight kininogen (HMWK)		120 000	6


Thrombin
Fibrinogen
Fibrin

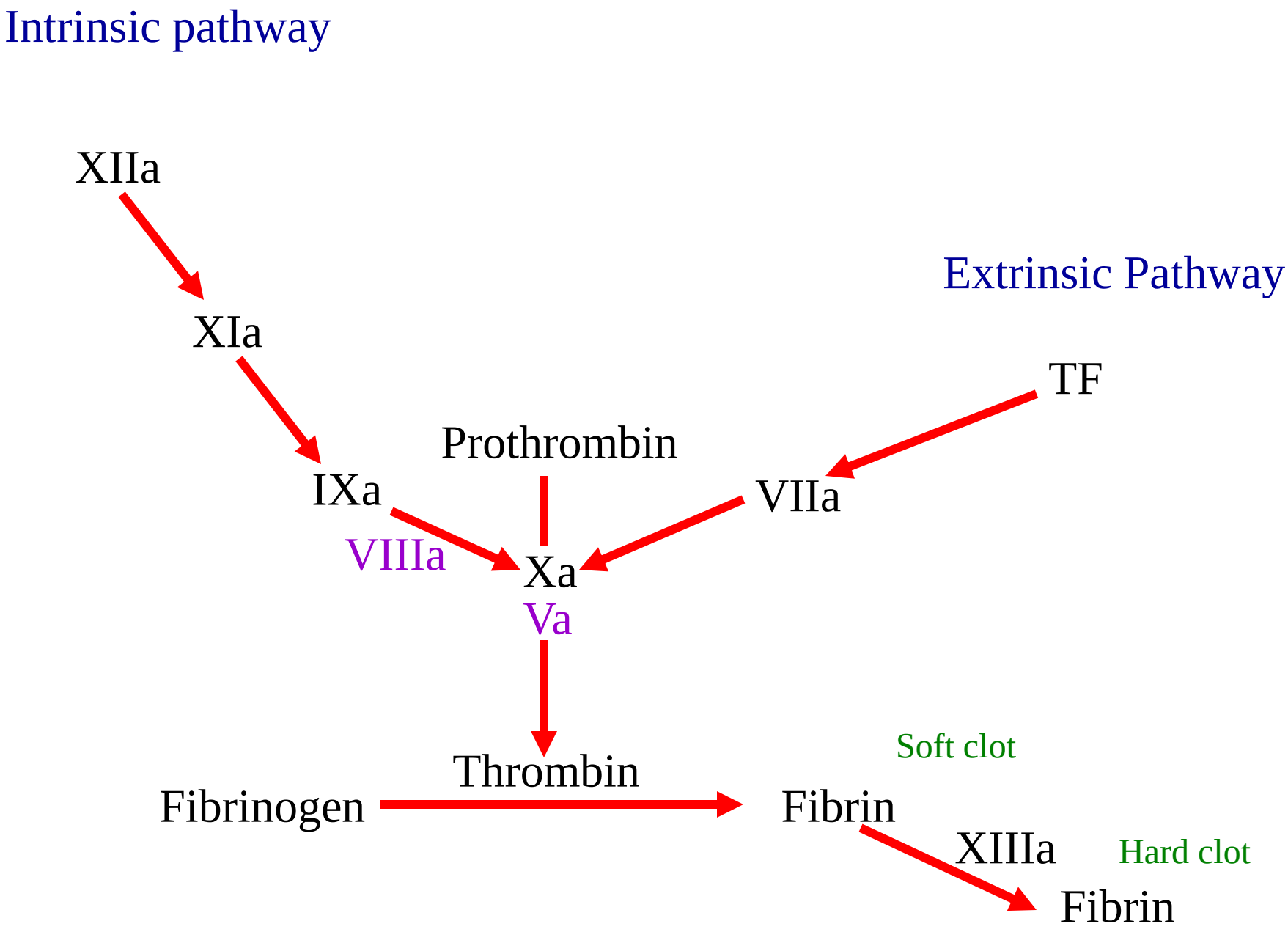

Prothrombin
Xa
Va
Thrombin
Fibrinogen
Fibrin


Extrinsic Pathway
TF
Prothrombin
VIIa
Xa
Va
Thrombin
Fibrinogen
Fibrin

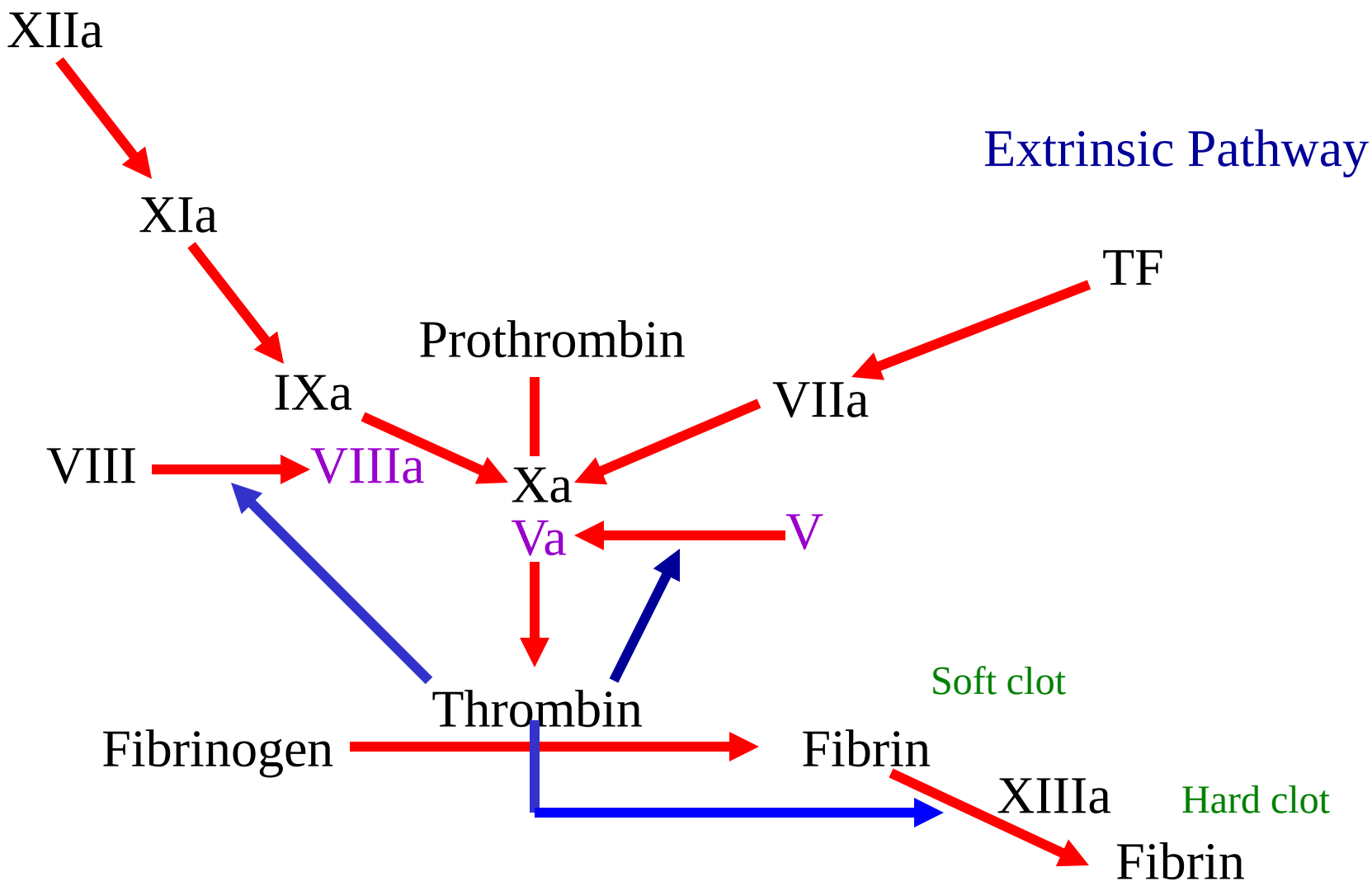
Intrinsic pathway



Extrinsic Pathway


Intrinsic pathway
XIIa
XIa
IXa
VIIIa
Prothrombin
Xa
Va
Extrinsic Pathway
TF
VIIa
Thrombin
Fibrinogen
Fibrin
Soft clot
XIIIa
Hard clot
Fibrin

Intrinsic pathway



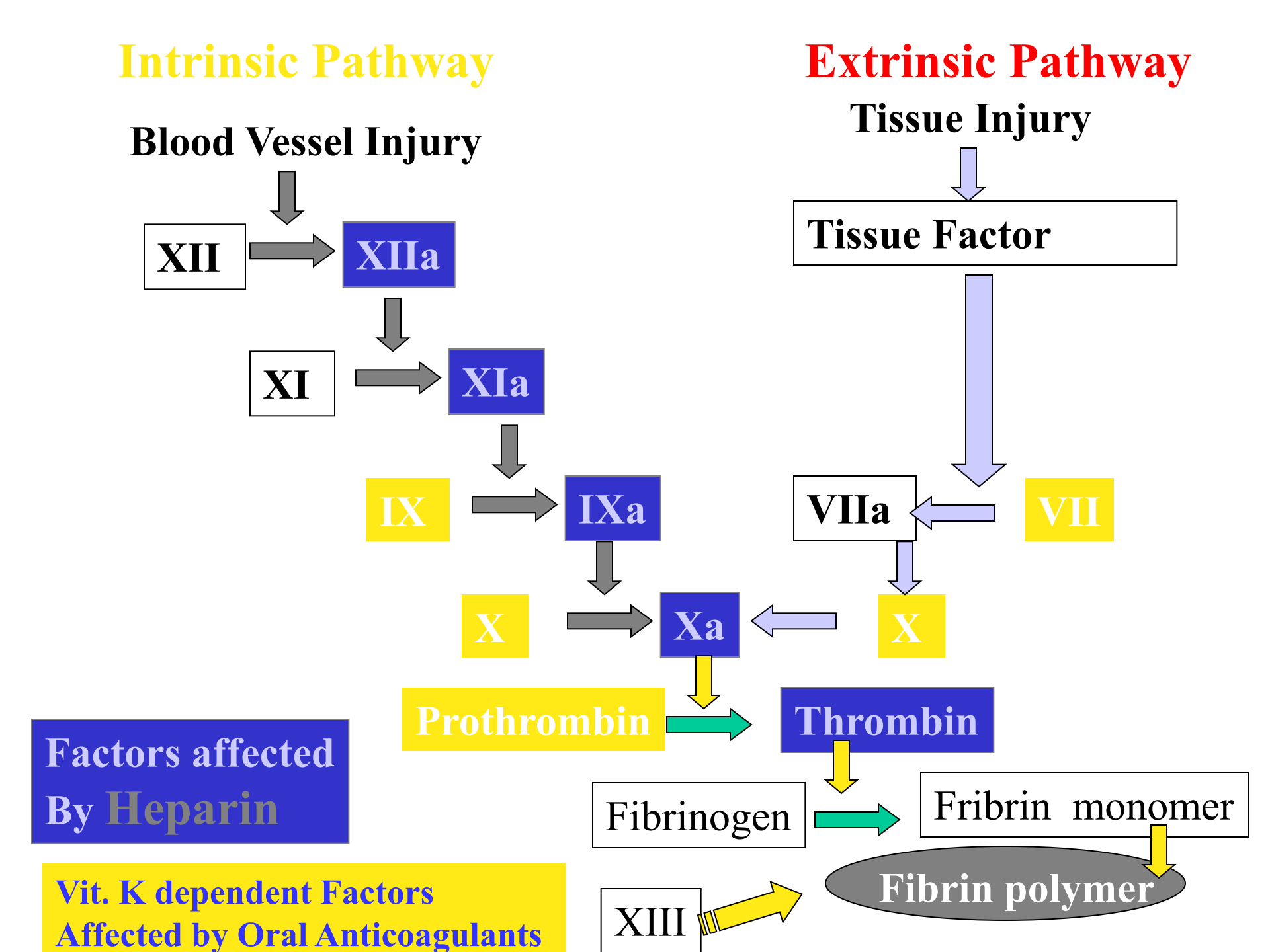
Extrinsic Pathway

Intrinsic Pathway

- All clotting factors are within the blood vessels
- Clotting slower
- Activated partial thromboplastin test (aPTT)

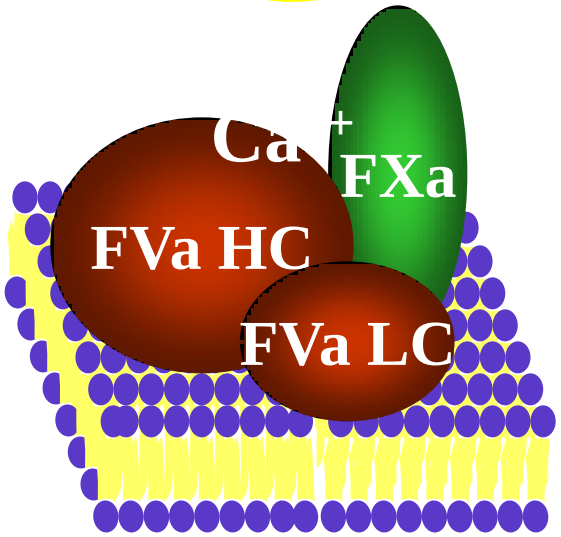
Extrinsic Pathway

- Initiating factor is outside the blood vessels - tissue factor
- Clotting - faster - in Seconds
- Prothrombin test (PT)

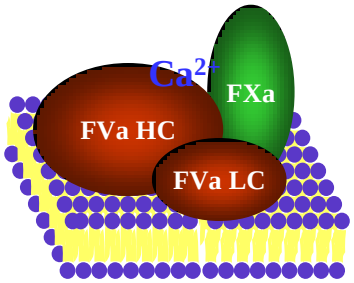
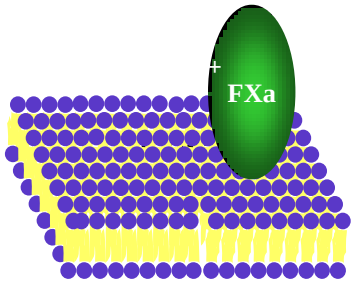
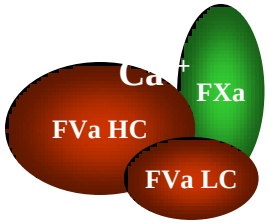

Intrinsic Pathway
Extrinsic Pathway
Blood Vessel Injury
Tissue Injury
Tissue Factor
XII
XIIa
XI
XIa
IX
IXa
VIIa
VII
X
Xa
X
Prothrombin
Thrombin
Fibrinogen
Fribrin monomer
Fibrin polymer
XIII
Factors affected By Heparin
Vit. K dependent Factors Affected by Oral Anticoagulants

Prothrombin

α-Thrombin



Prothrombinase

Prothrombinase Components	Relative Rate of Prothrombin Activation
	1
	300,000
	30
	300

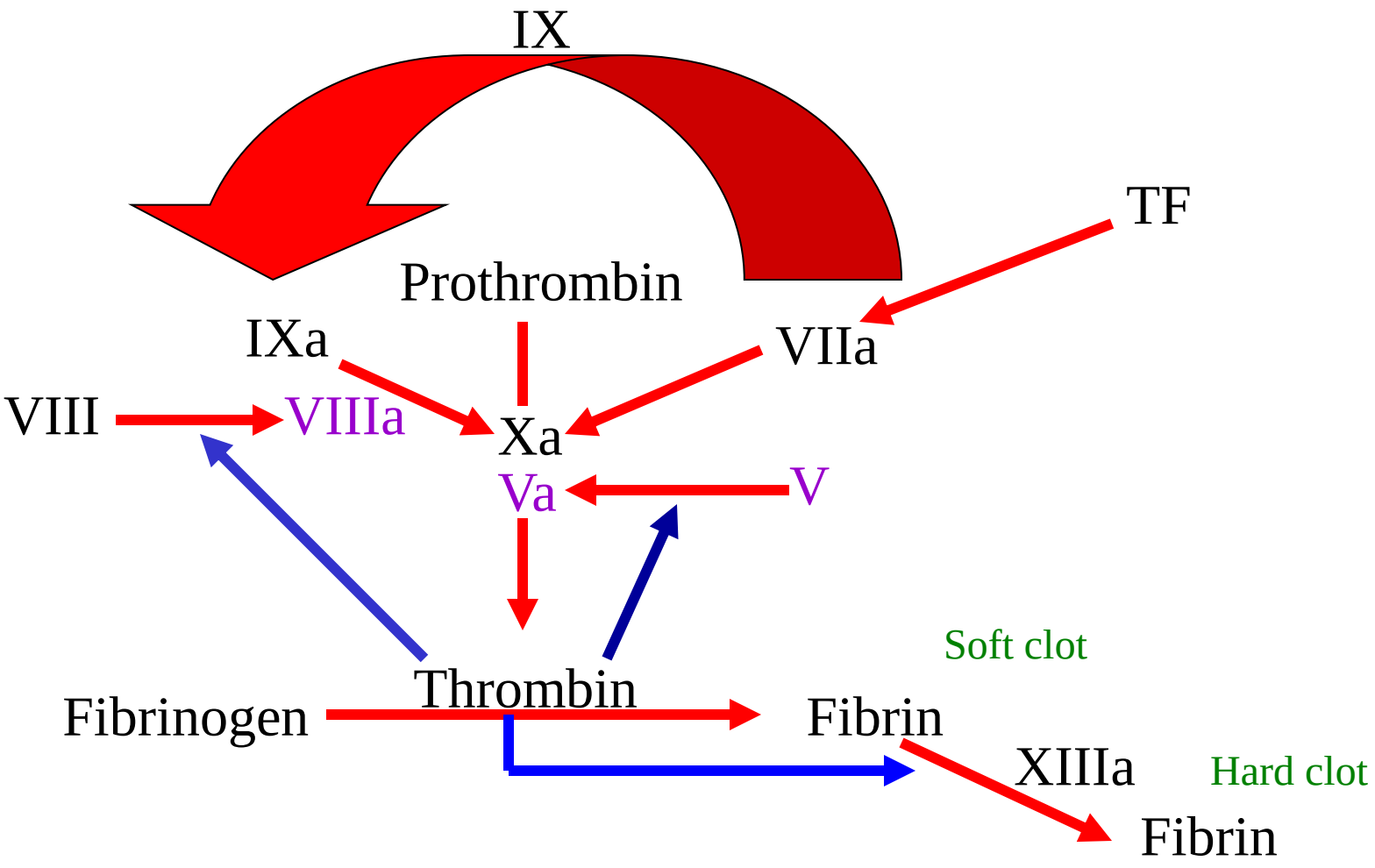
Relevance of complex formation and its constituents

Tissue Factor Pathway

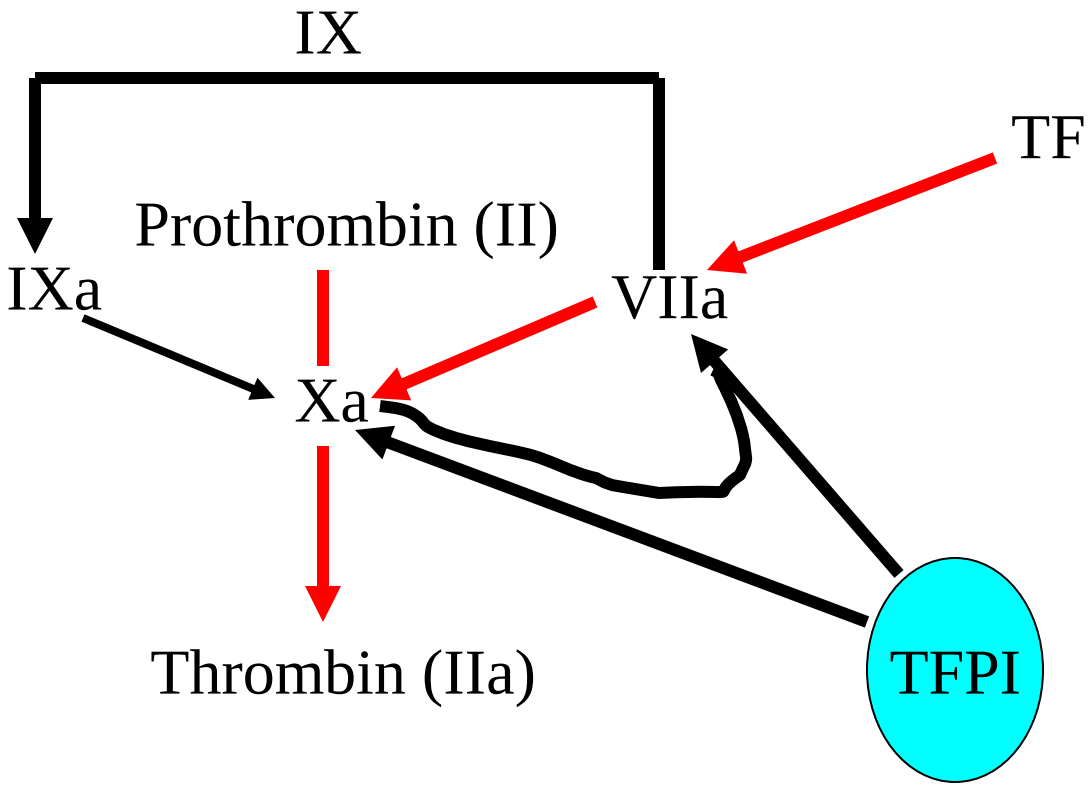
- **VIIa forms as usual via binding of VII to TF**
- **VIIa activates some X→Xa**
- **Xa converts a small amt of prothrombin to thrombin; this thrombin is used to produce small amts of VIIIa and Va**
- **As the conc of TF-VIIa-Xa-thrombin increases, Tissue Factor Pathway Inhibitor inactivates this complex stopping further production of thrombin.**
- **New: VIIa also activates IX→IXa (Key to new scheme)**
- **IXa, with VIIIa (produced as above), produces Xa; this Xa with Va (produced as above) produces new thrombin; this thrombin produces more VIIIa and Va and now we get lots of thrombin and fibrin!**

Tissue Factor Pathway

NB: activation of IX by VIIa



Tissue Factor Pathway Inhibitor



NB: Inhibition of Xa and VIIa

Tissue Factor Pathway Inhibitor

- Kunitz-type protease inhibitor (kringles)
- 34 and 41 kD forms in plasma (C-term truncation)
- **Directly inhibits Xa**
- **Inhibits VIIa-TF complex in a [Xa]-dependent manner**
- Bound to LDL, HDL and Lp (a)
- ~10% present in platelets (endothelium also)

Hemophilia A

Deficiency of/nonfunctional VIII

Hemophilia B

Deficiency of /nonfunctional IX

Why do they bleed?

Physiologic Inhibitors of coagulation

- **Antithrombin III** (serpin)
- **Activated Protein C + protein S**
  - Inactivates Va and VIIIa (via proteolysis)
  - NB: Factor V Leiden
- **Thrombomodulin (EC glycoprotein)**
  - Binds to thrombin
  - Decreases ability to produce fibrin
  - Increases ability to activate Protein C

Prothrombin time (PT)

Tissue Thromboplastin factor III

Mix with phospholipid extract

Add calcium and blood sample

Determine clotting time

Generally 12 - 14 seconds
Used to detect defects in extrinsic pathway

Activated partial thromboplastin time (APTT)

Blood sample + EDTA or Citrate

No clot (recalcification will result in clot in about 2 - 4 min)

Add calcium
Mix with negatively charged phospholipid
Kaoline (aluminum silicate)

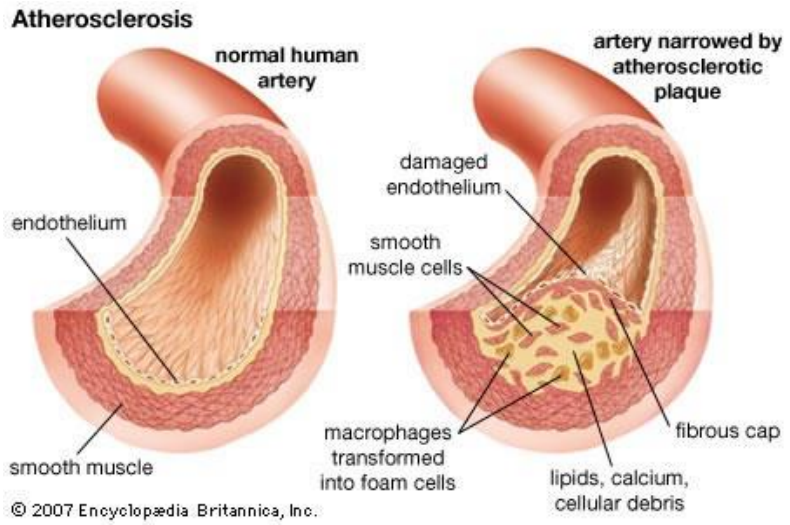
Determine clotting time

Generally clotting occurs in 26 to 33 seconds

Used to detect defects in the intrinsic pathway

Anticoagulant Properties of the Endothelium

- Anti-platelet properties
  - Covers highly thrombogenic basement membrane
  - Uninjured endothelium does not bind platelets
  - PGI2 (prostacyclin) and NO from uninjured endothelium inhibit platelet binding
  - ADPase counters the platelet aggregating effects of ADP



Anticoagulant properties of the endothelium

* **HEPARIN-LIKE MOLECULES:** activate anti-thrombin III (inactivates active proteases)

* **THROMBOMODULIN**: changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa

* Endothelial cells produce **t- PA** which activates fibrinolysis via plasminogen to plasmin

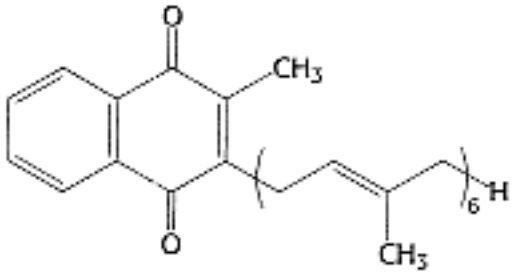
Prothrombotic Properties of the Endothelium

- Synthesis of von Willebrand factor
- Release of tissue factor, TF
- Production of plasminogen activator inhibitors (PAI)
- Membrane phospholipids bind and facilitate activation of clotting factors via Ca bridges

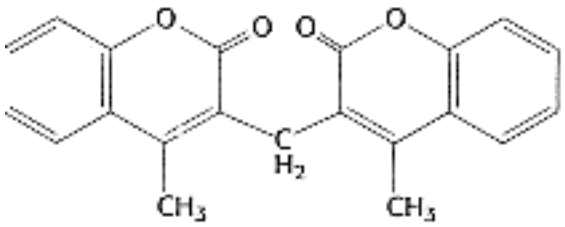
VITAMIN K

- Group of related, fat soluble compounds, which differ in the number of side-chain isoprenoid units
- Plant derived (vitamin K_1) and synthesized by intestinal bacteria (vitamin K_2)
- The reduced form of vitamin K_2 (vitamin KH_2) is required for the post-translational, gamma-carboxylation of several proteins involved in blood clotting

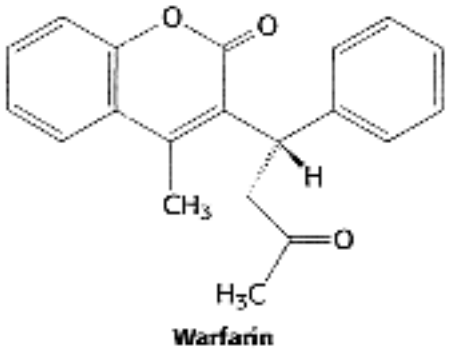
Role of vitamin K



Vitamin K



Dicoumarol



Warfarin

Role of vitamin K

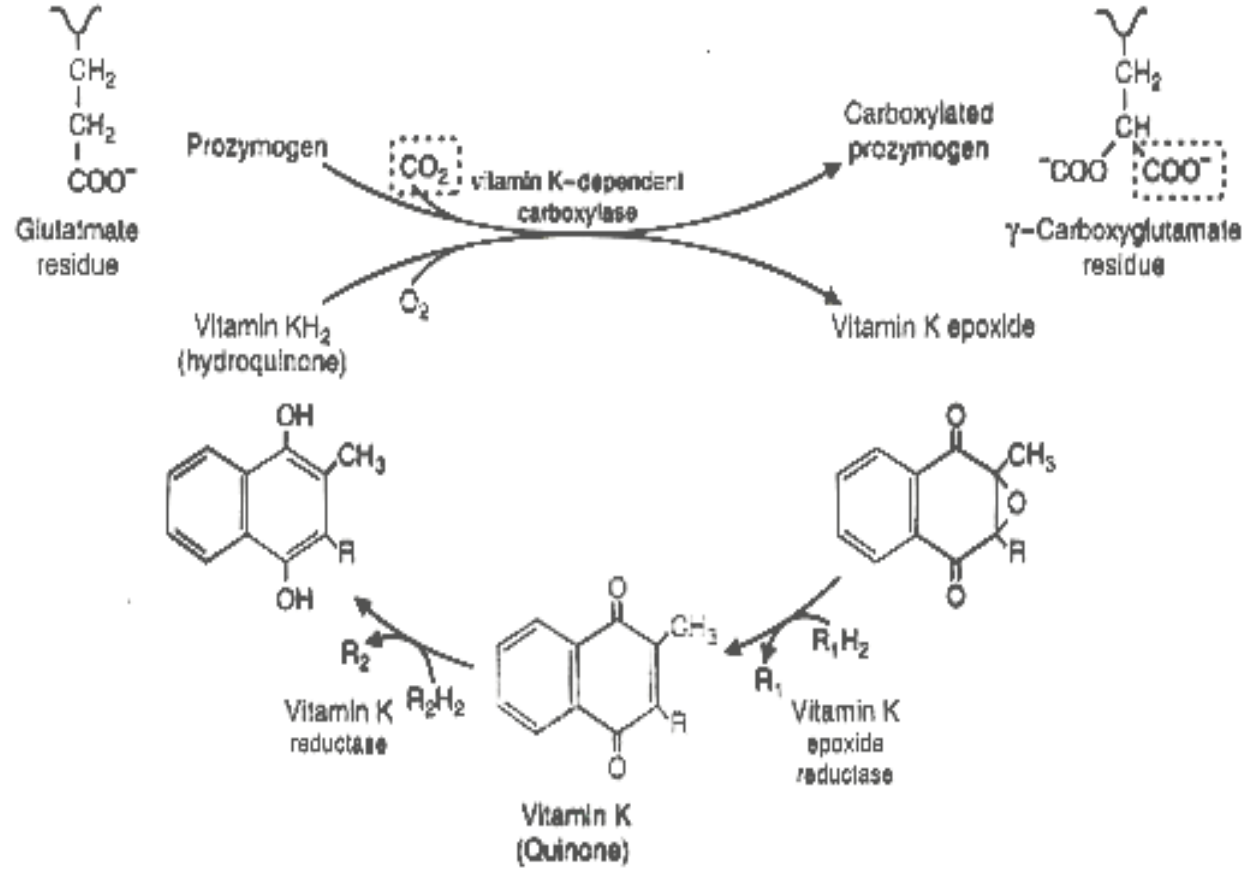
Some clotting factors require a post-translational modification before they are active in clotting

These factors are II, VII, IX, X, proteins C and S

This PTM involves the addition of a COO- to certain Glu residues in the clotting factors

This PTM results in the formation of several γ-carboxy glutamates = Gla

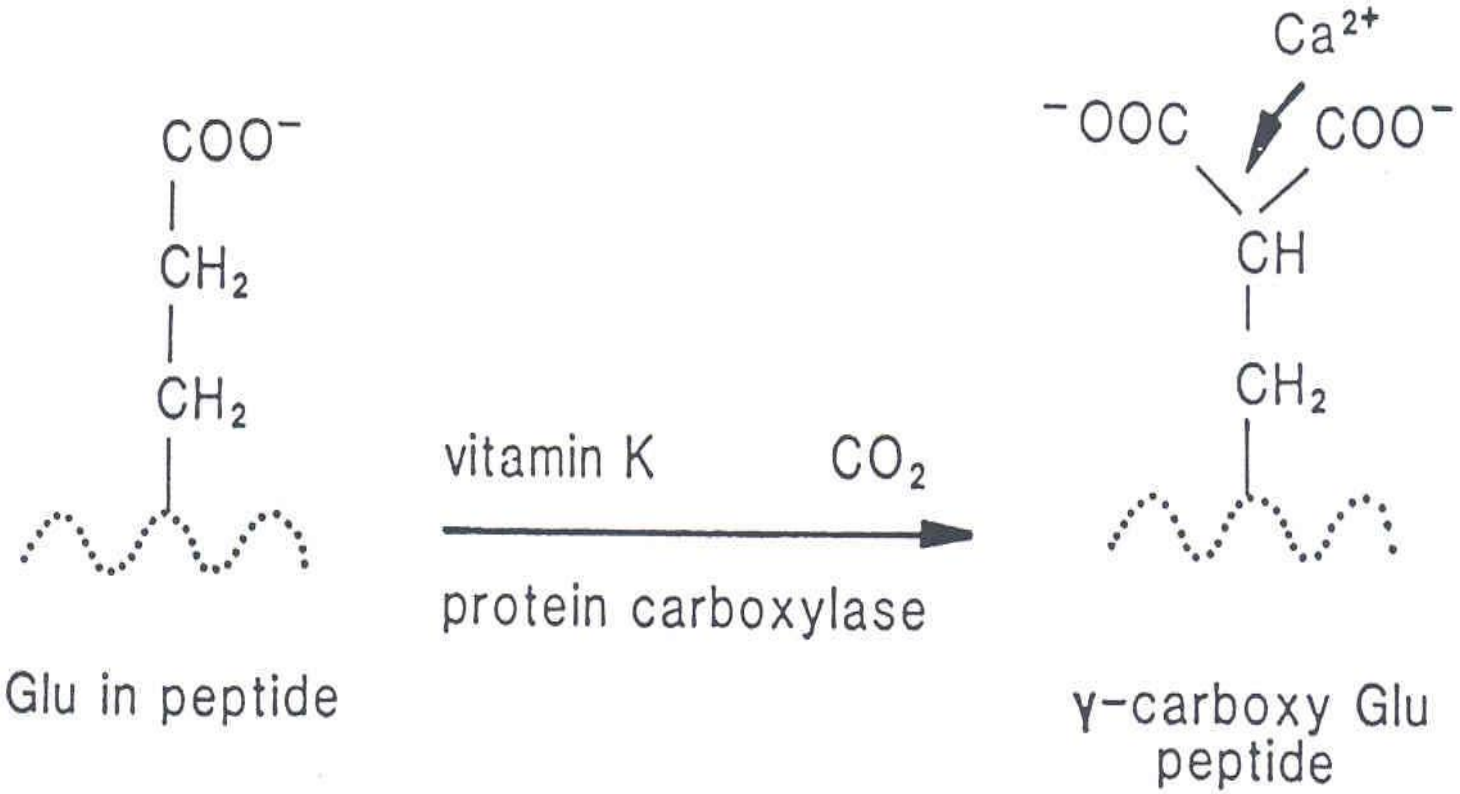
This PTM requires vitamin K



36. Vitamin K-dependent formation of γ-carboxyglutamate
s. Thrombin (II), Factor VII, Factor IX, and Factor X are bound

acid residues that have a high affinity for calcium. The vitamin K-dependent carboxylase, which adds the extra carboxyl group, uses a

Formation of Gla residues subsequent to protein synthesis (post-translational)



Role of vitamin K

- **Vit K is altered in carboxylation Rx and must be regenerated before reuse**
- **Regeneration involves 2 reductases which convert vit K epoxide to vit K**
- **Vit K antagonists inhibit these reductases**
- **Vit K-dependent PTM provides sites on modified coag factors for Ca bridgeing**

Non-physiologic inhibitors of coagulation

- **Vitamin K antagonists (in vivo)**
- **Ca chelators (in vitro only)**
  - **EDTA**
  - **Citrate**
  - **Oxalate**

*** Heparin (in vivo and in vitro)**

Vitamin K deficiency

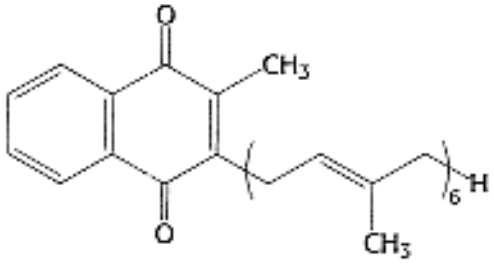
- Deficiency of vitamin K is rare because of its wide distribution in nature, and its production by intestinal bacteria

- Found in individuals with liver disease and fat malabsorption – it is associated with bleeding disorders

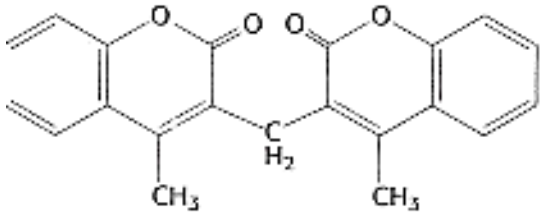
- Newborn infants (especially preemies) are also at risk
  - Placenta is insufficient in the transfer of maternal vitamin K
  - Concentration of circulating vitamin K drops immediately after birth, and it recovers upon absorption of food
  - Gut of the newborn is sterile

  Thus, newborns are given an injection of vitamin K following birth.

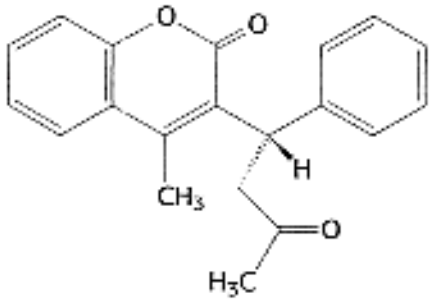
Vitamin K antagonists



Vitamin K



Dicoumarol



Warfarin

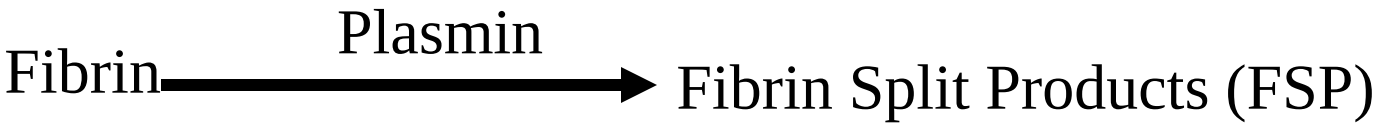
BLOOD COAGULATION (CONT.)

- Deficiencies in all of the factors, except factor XII, lead to a bleeding tendency in the affected individual
- Described as a 'waterfall' or 'cascade' sequence of zymogen (pro-enzyme) to enzyme conversions, with each enzyme activating the next zymogen in the seqeunce
- Activated factor enzymes are designated with an "a", e.g. factor Xa

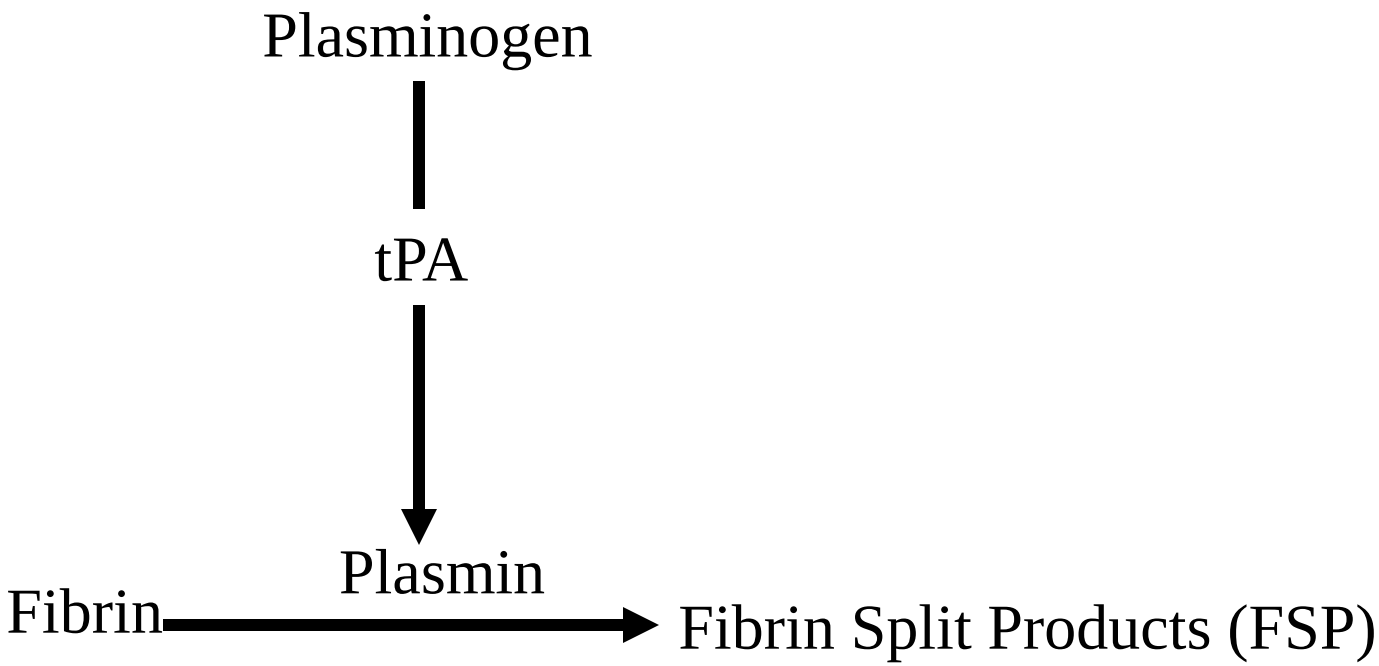
Clot removal

FIBRINOLYSIS

Fibrinolysis



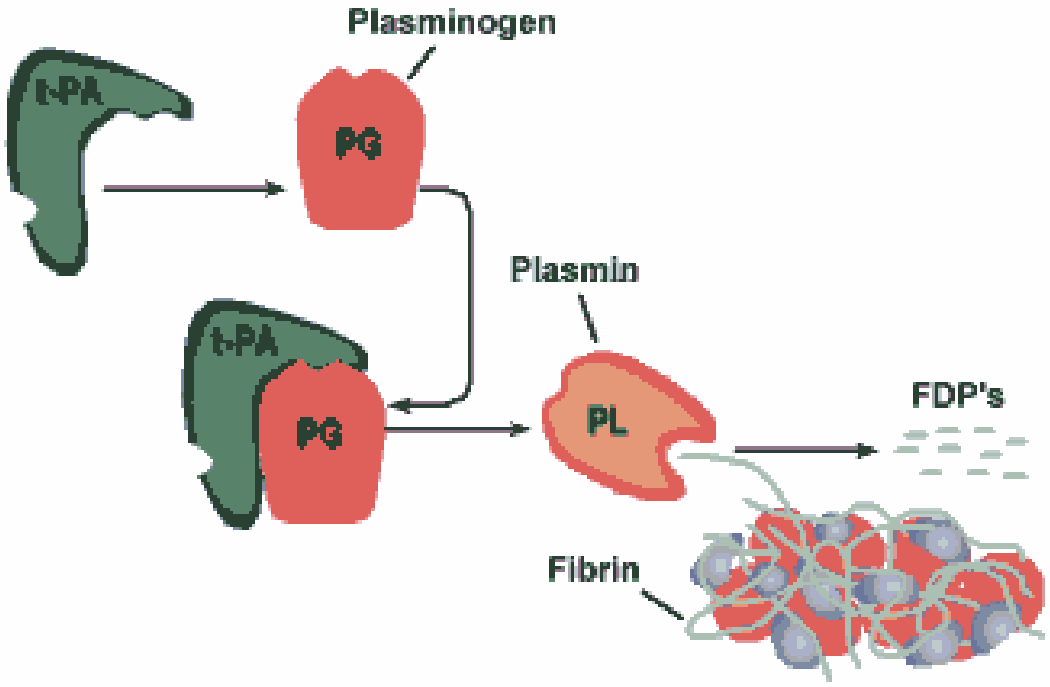
Fibrinolysis



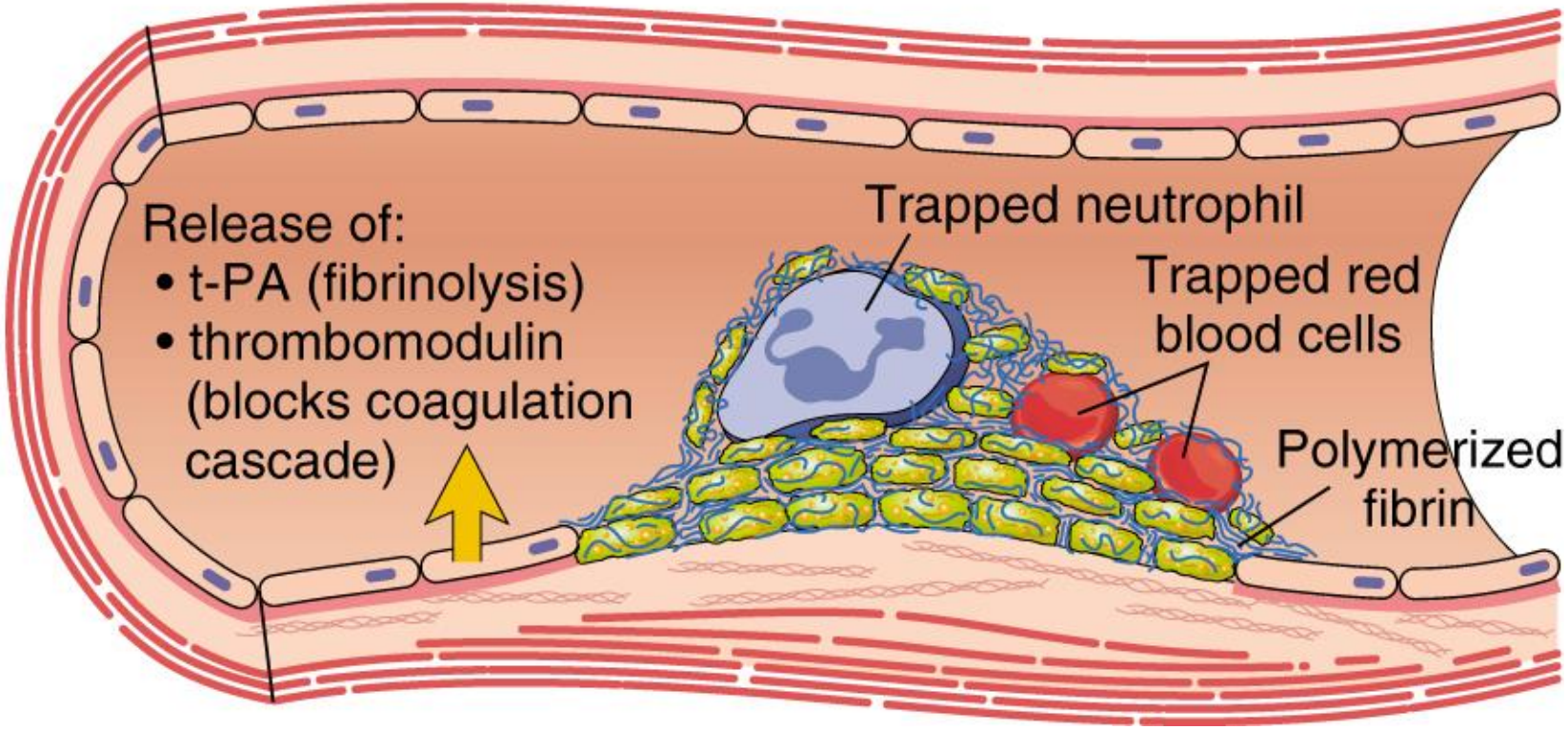
Fibrinolysis

- Enhance degradation of clots
- Activation of endogenous protease
- Plasminogen (inactive form) is converted to Plasmin (active form)
- Plasmin breaks down fibrin clots

Fibrinolysis



D. THROMBUS AND ANTITHROMBOTIC EVENTS



Inhibitors of fibrinolysis

- **Plasminogen activator inhibitors (PAIs)**

- **α_2-antiplasmin (serpin)**


Thank
you