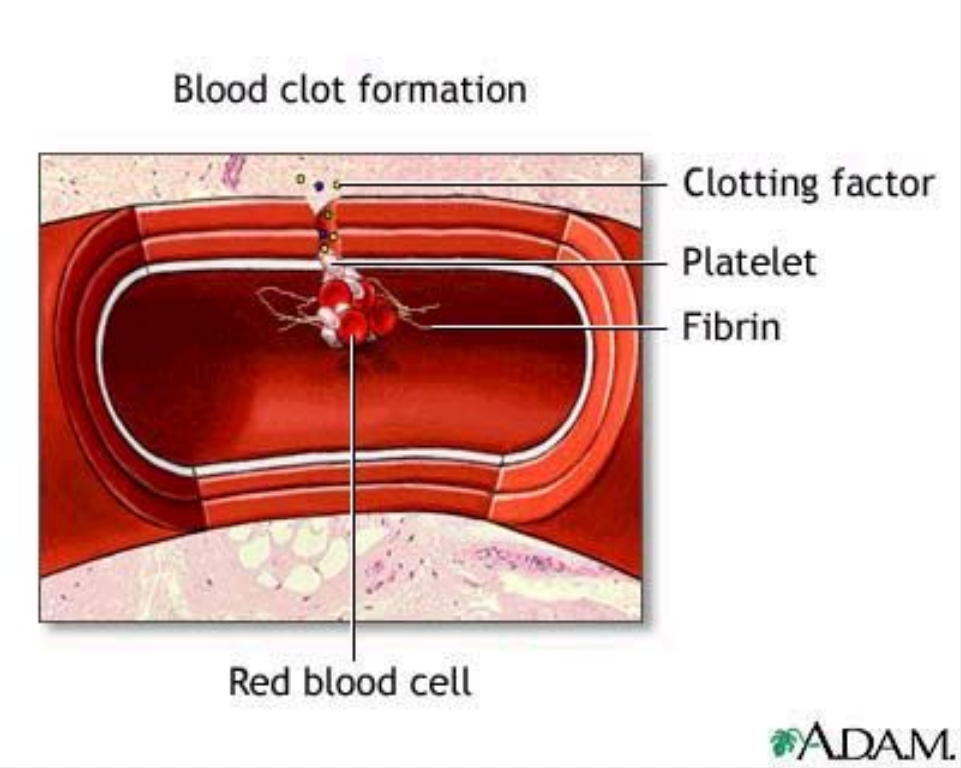
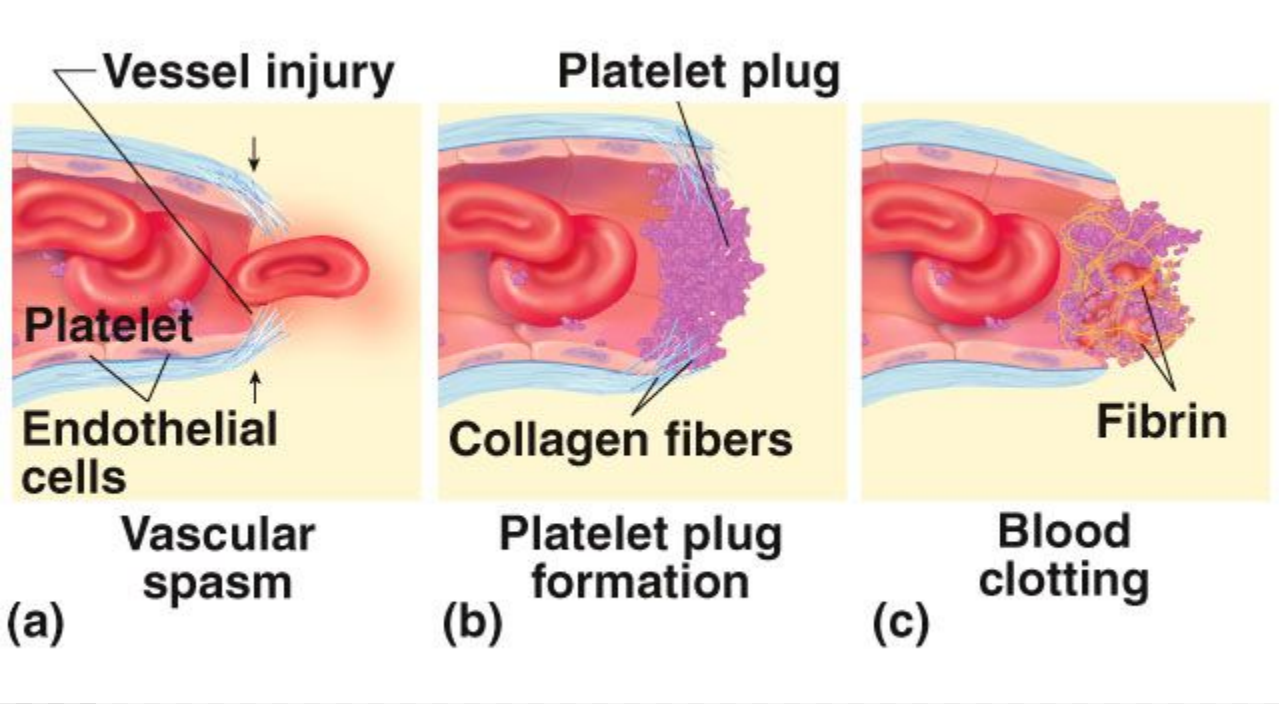
Hemostasis

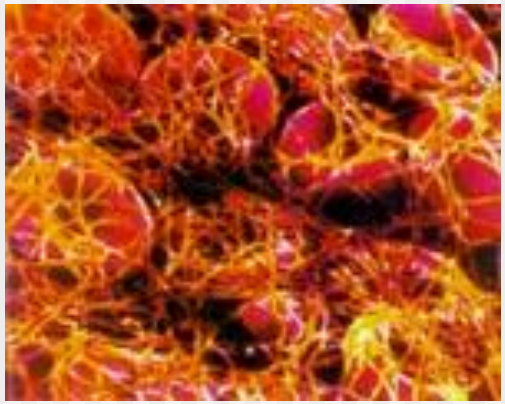

Blood clot formation
Clotting factor
Platelet
Fibrin
Red blood cell
A.D.A.M.

Hemostasis *(„hemo"=blood; sta=„remain")*

is the stoppage of bleeding, which is vitally important when blood vessels are damaged.

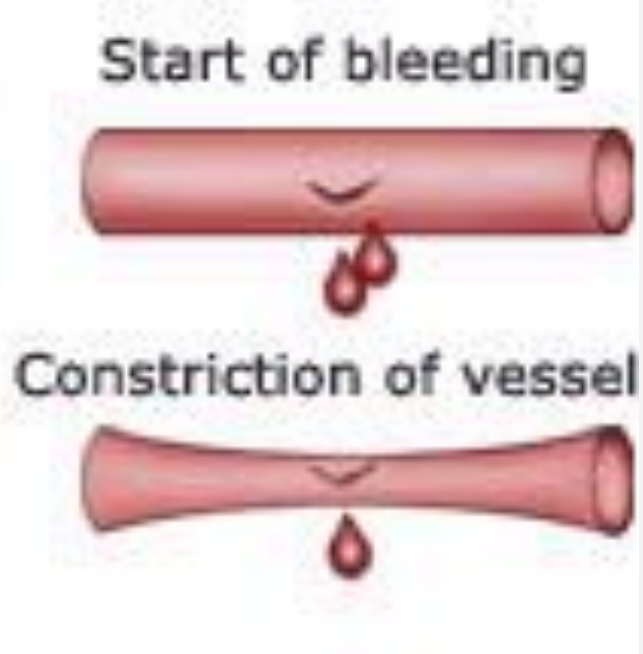
Following an injury to blood vessels several actions may help prevent blood loss, including:



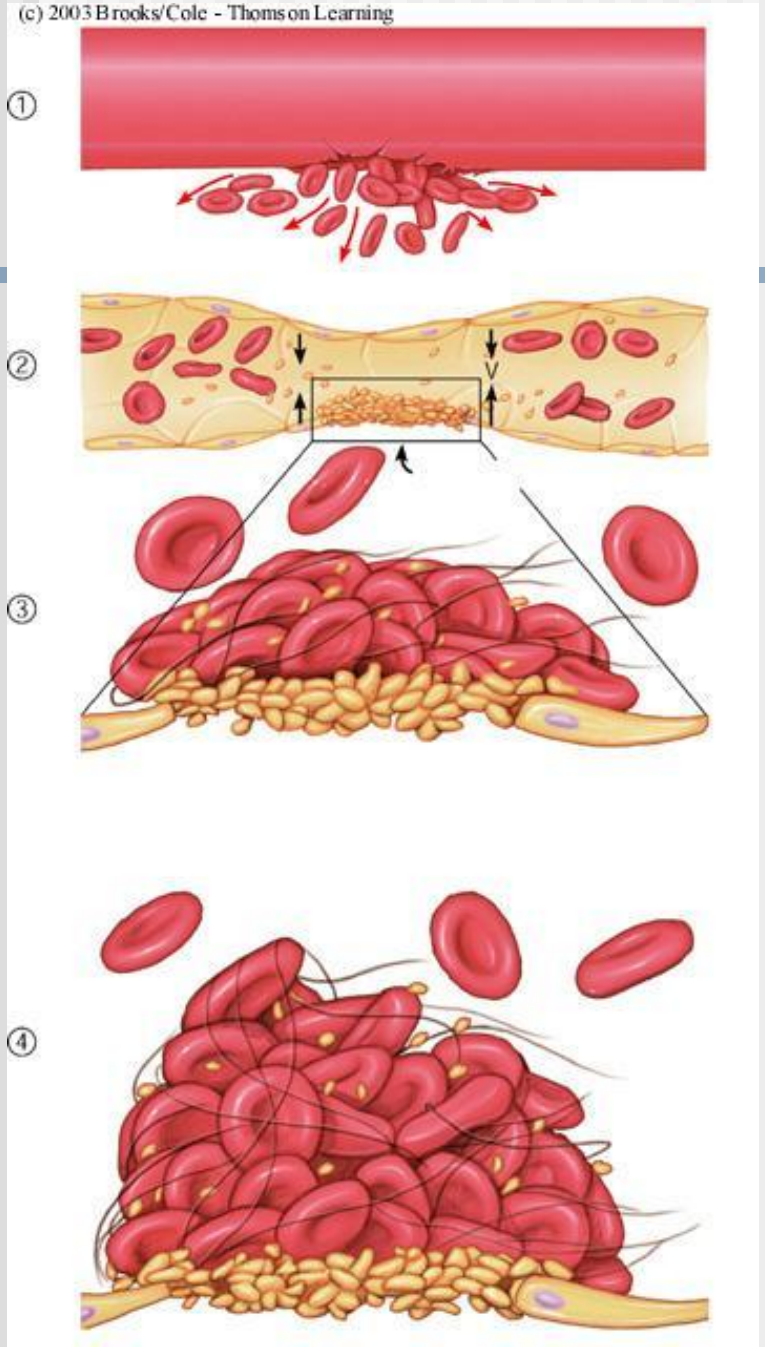


Formation of a clot

Local vasoconstriction



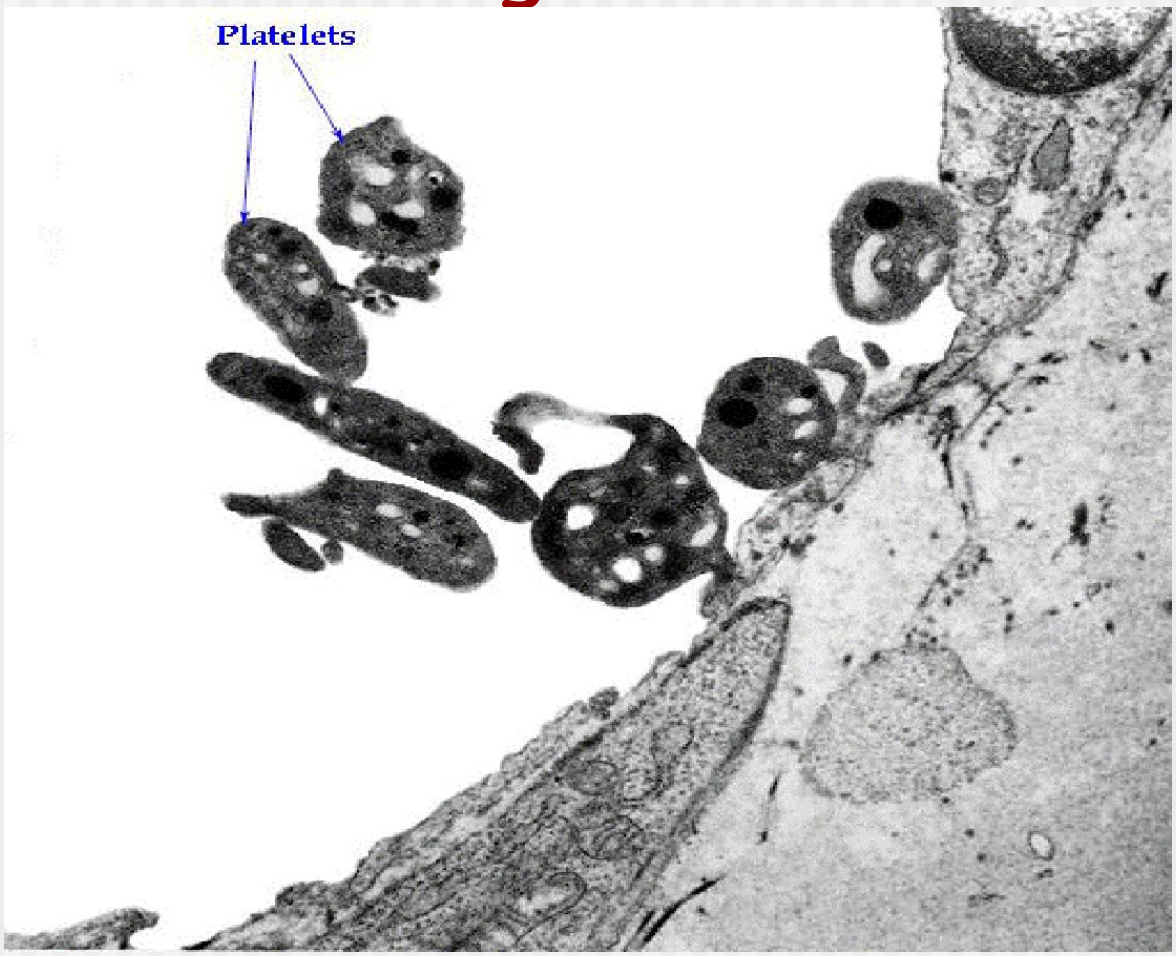
- is due to local spasm of the smooth muscle (symp. reflex)
- can be maintained by platelet vasoconstrictors



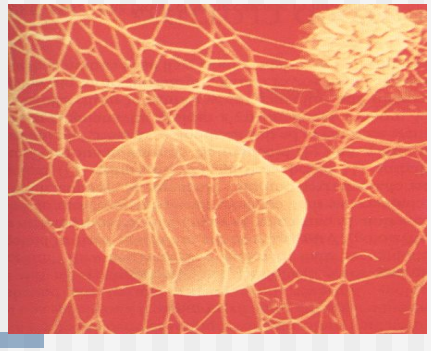
Formation of platelet aggregate

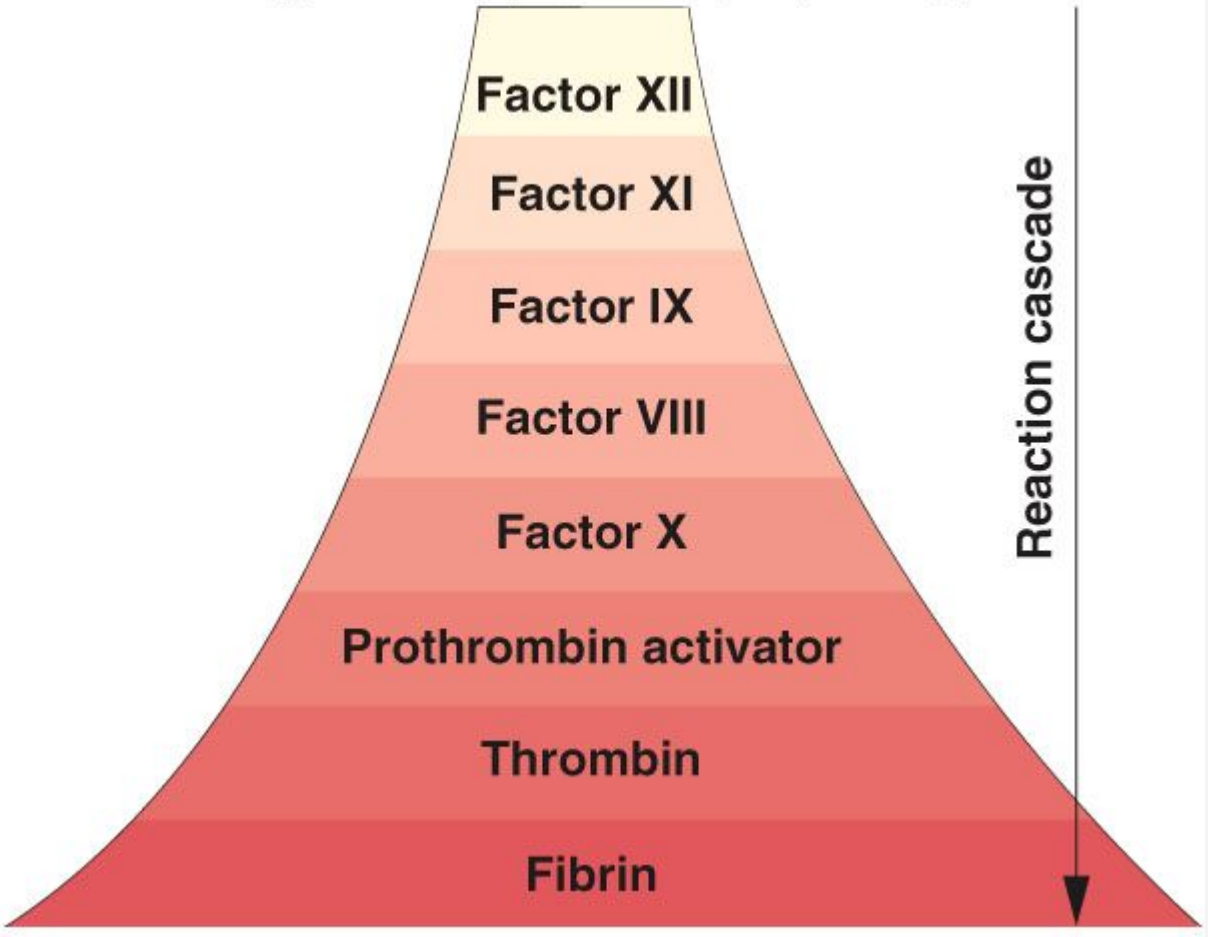
- Injured blood vessel releases ADP, which attracts platelets (PLT)
- PLT comming in contact with exposed collagen release: serotonin, ADP, TXA2, which accelerate vasoconstriction and causes PLT to swell and become more sticky

The micrograph shows activated platelets adhering to some damaged cells



Formation of blood clot

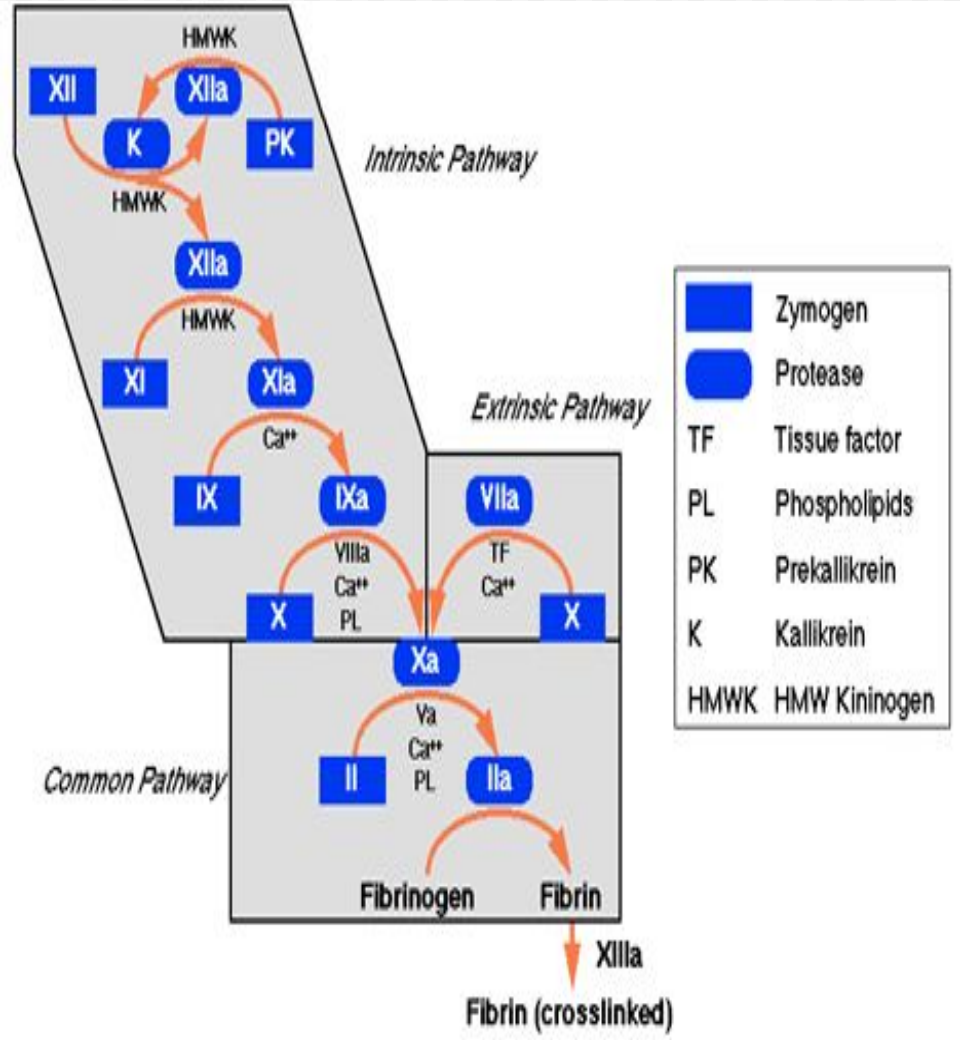


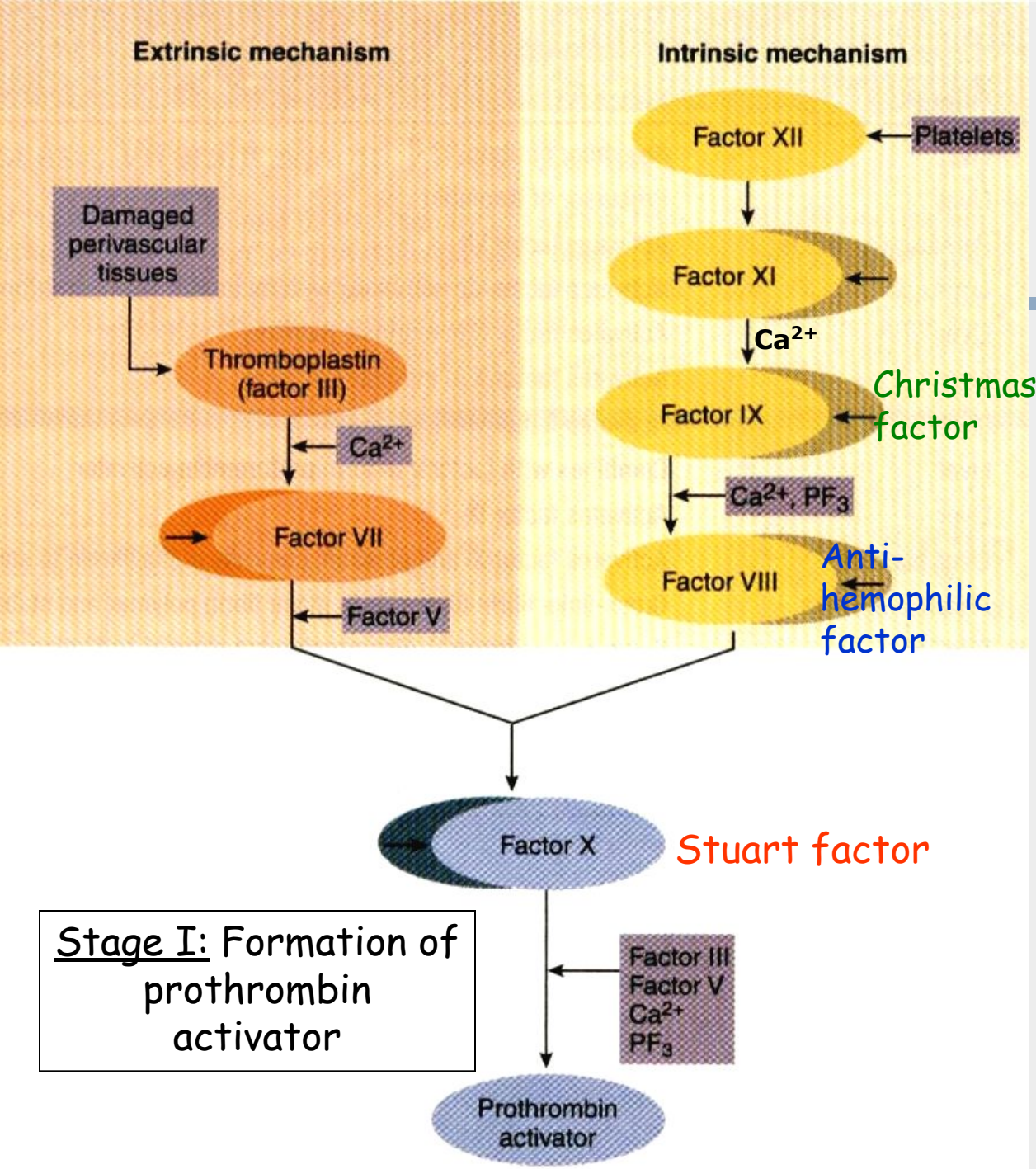


- In the formation of the clot, an enzyme called thrombin converts *fibrinogen* into insoluble protein, *fibrin*
- Fibrin aggregates to form a meshlike network at the site of vascular damage

Coagulation mechanism is composed of an extrinsic and intrinsic pathway, which eventually merge into one

- The intrinsic system is more complex and present only in „higher" life forms *(e.g. birds and reptiles possess only extrinsic system)*
- The complex sequence of events that produce fibrin are divided into three stages

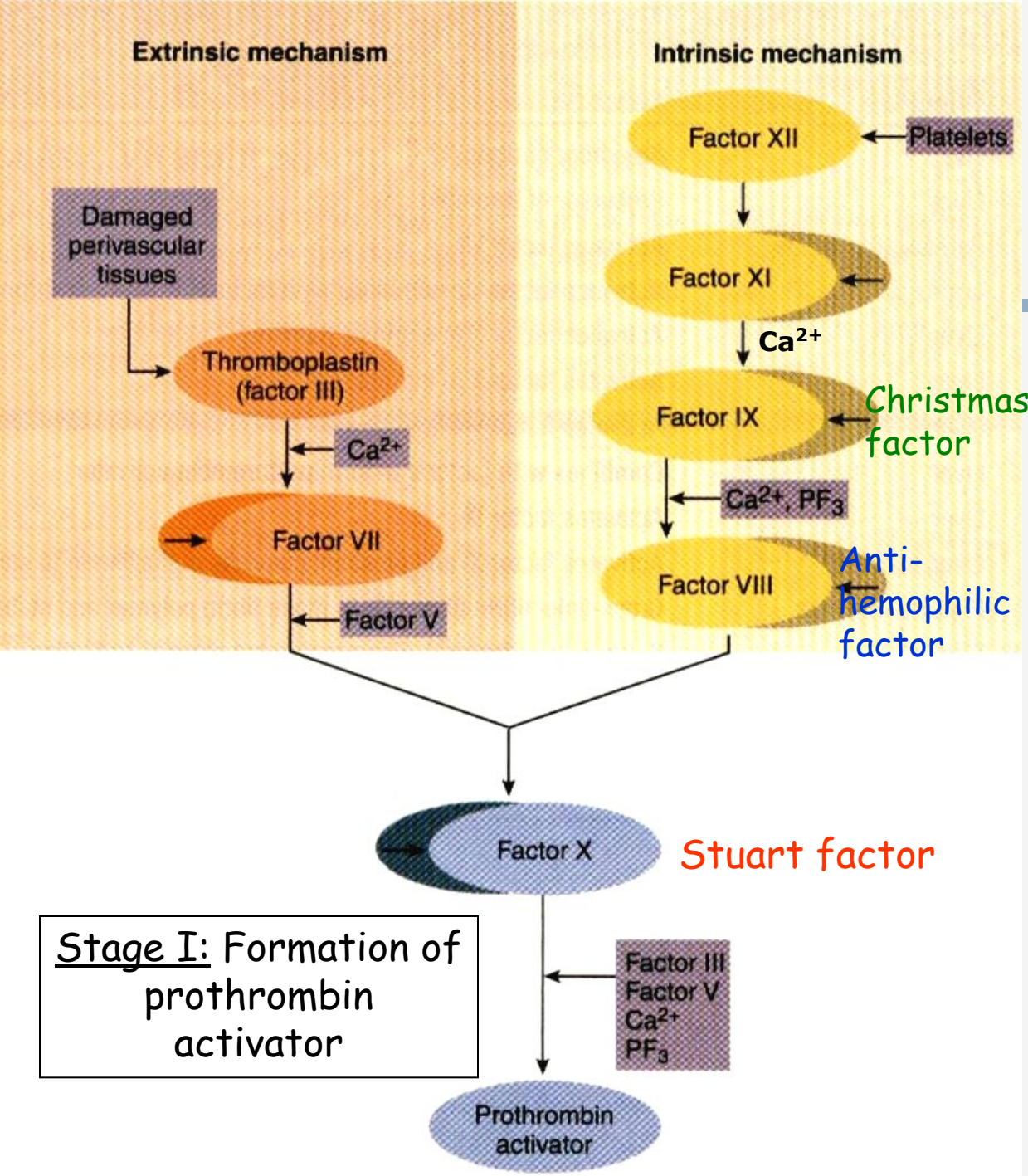




Stage I: Formation of prothrombin activator

Extrinsic pathway:

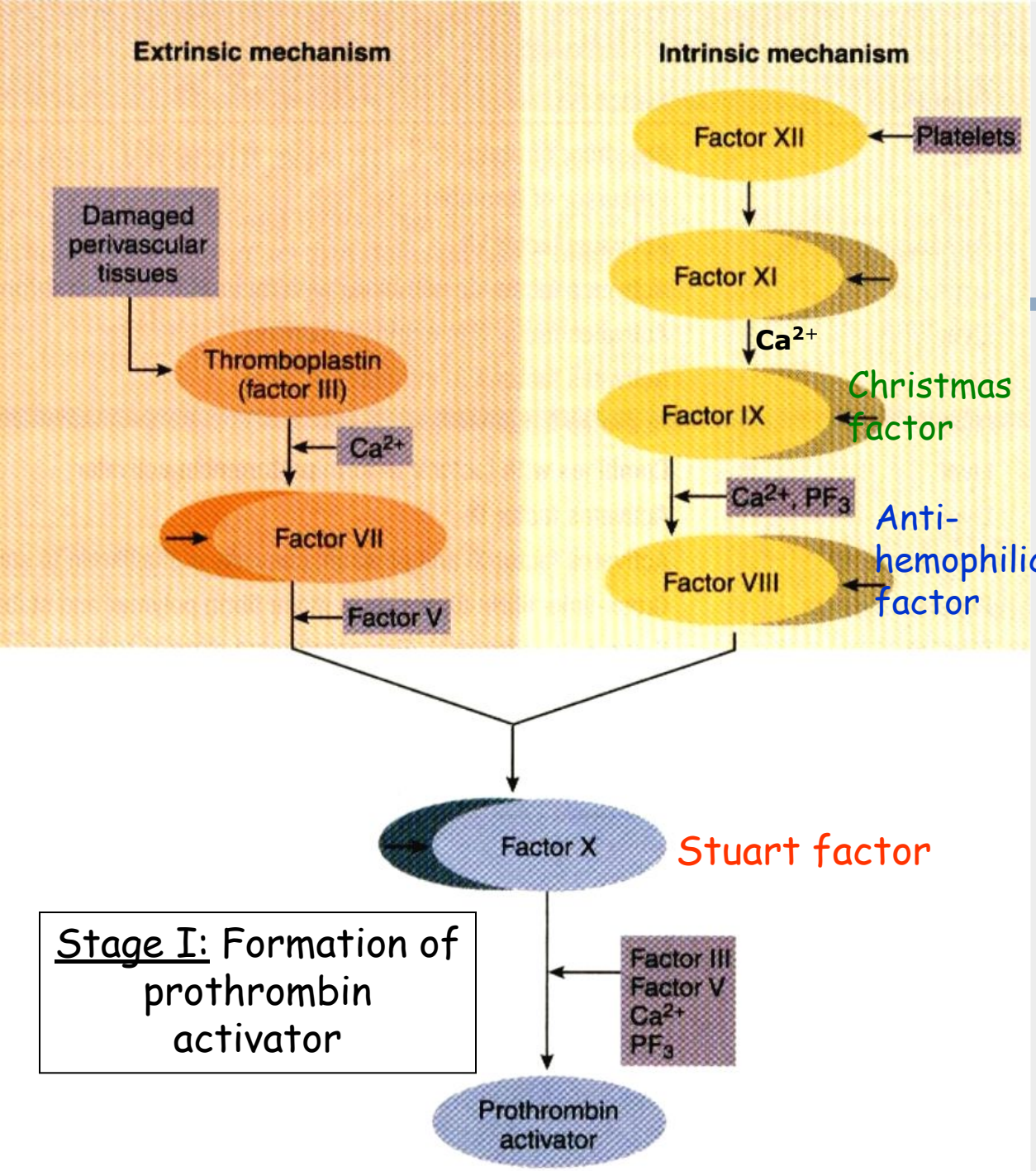
1. When blood comes in contact with injured tissue – tissue thromboplastin (F III) interacts with proconvertin (F VII), and Ca^{2+} activating Stuart factor (F X).



Stage I: Formation of prothrombin activator

Intrinsic pathway:

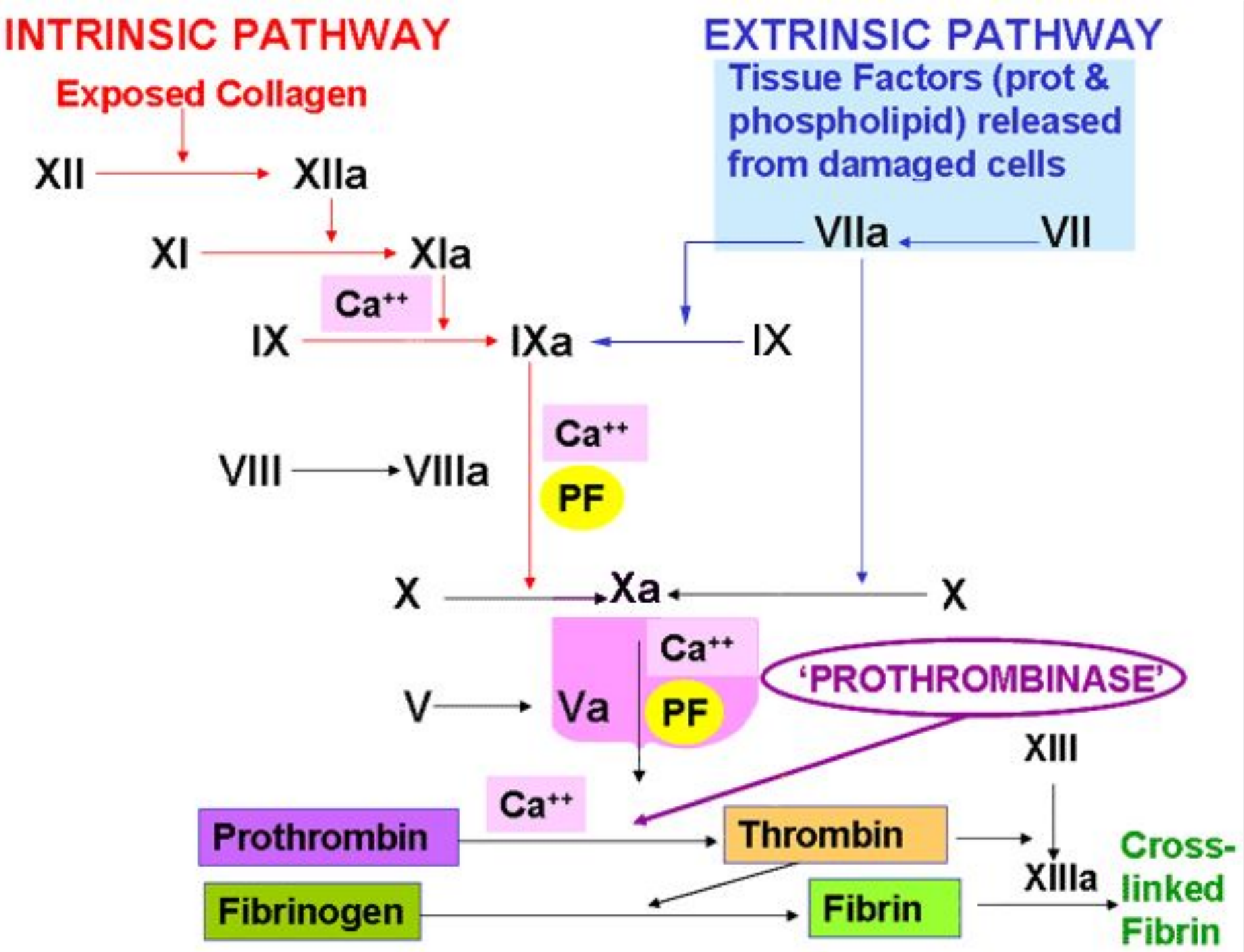
2. Exposed collagen activates Hageman factor (F XII). Activated F XII activates plasma enzyme – plasma thromboplastin antecedent (PTA; F XI, which in the presence of Ca^{2+} activates Christmas factor (F IX). F IX interacts with antihemophilic factor (F VIII), Ca^{2+} to form a complex that activates Stuart factor (F X).



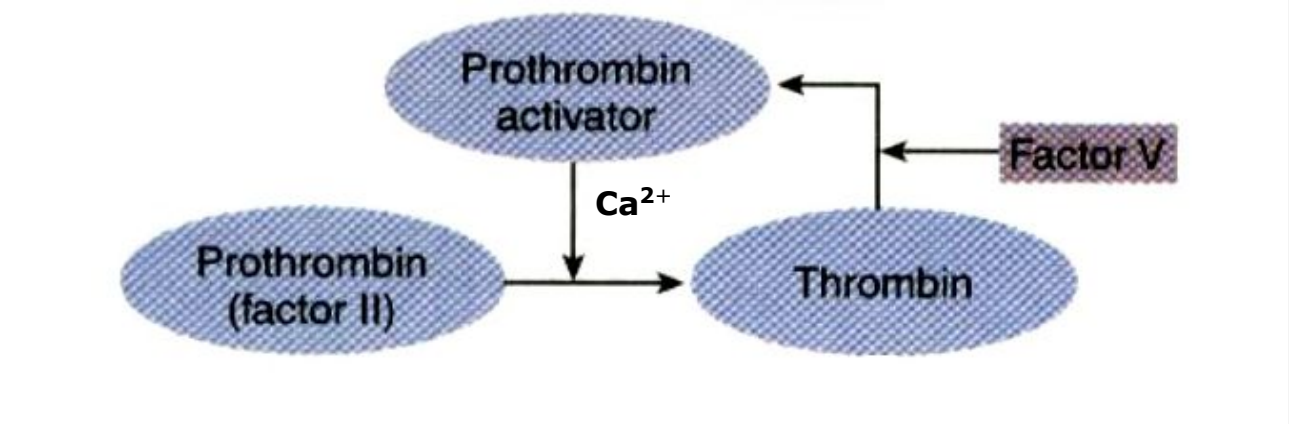
Stage I: Formation of prothrombin activator

3. Common pathway:

Activated F X in the presence of Ca^{2+} forms complexes with accelerin (F V) to form prothrombin activator

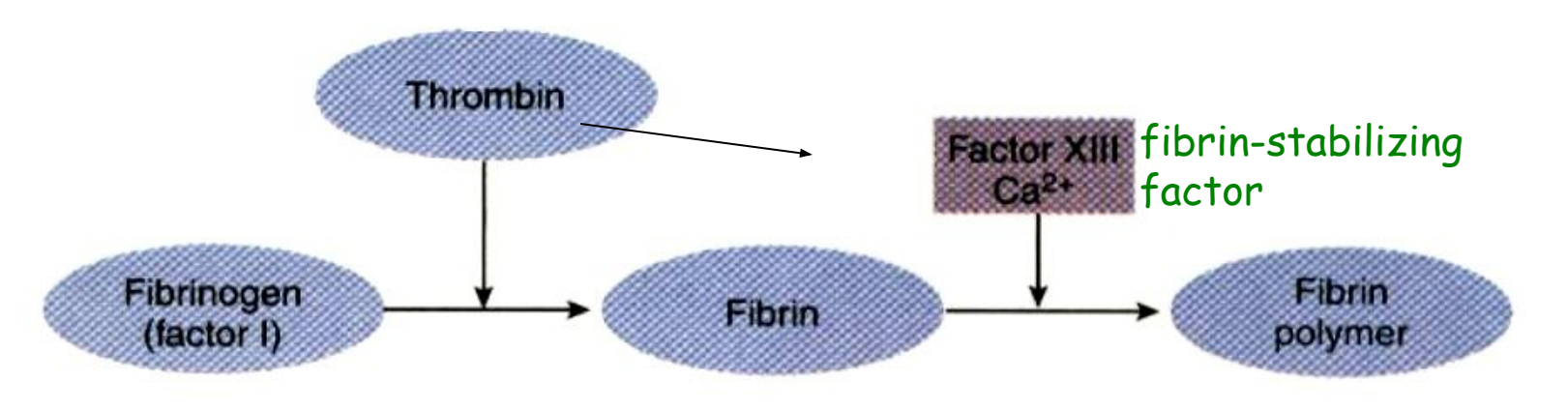

INTRINSIC PATHWAY
Exposed Collagen
XII
XIIa
XI
XIa
Ca++
IX
IXa
VIII
VIIIa
EXTRINSIC PATHWAY
Tissue Factors (prot & phospholipid) released from damaged cells
VIIa
VII
IX
Ca++
PF
X
Xa
X
Ca++
V
Va
PF
'PROTHROMBINASE'
XIII
Ca++
Prothrombin
Thrombin
XIIIa
Cross-linked Fibrin
Fibrinogen
Fibrin

Stage II: conversion of prothrombin to thrombin



- Prothrombin – inactive precursor of enzyme thrombin
- In the presence of prothrombin activator and Ca^{2+} prothrombin is converted to thrombin
- Thrombin itself increases its own rate of formation (positive feedback mechanism)

Stage III: conversion of fibrinogen to fibrin



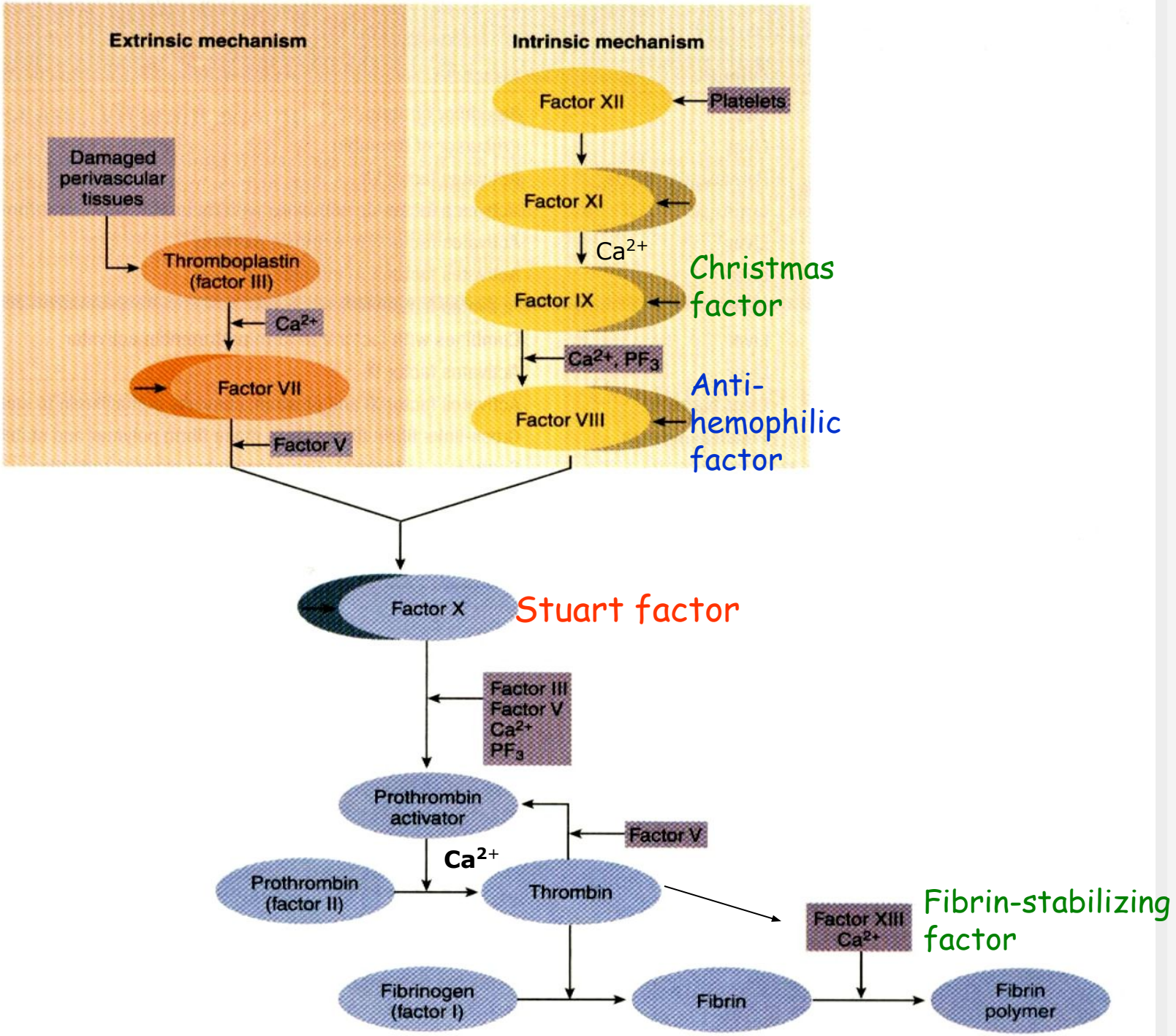
- Fibrinogen – plasma protein produced by the liver
- Thrombin converts fibrinogen to fibrin
- Thrombin also activates fibrin-stabilizing factor (F XIII), which in the presence of Ca^{2+}, stabilizes the fibrin polymer through covalent bonding of fibrin monomers

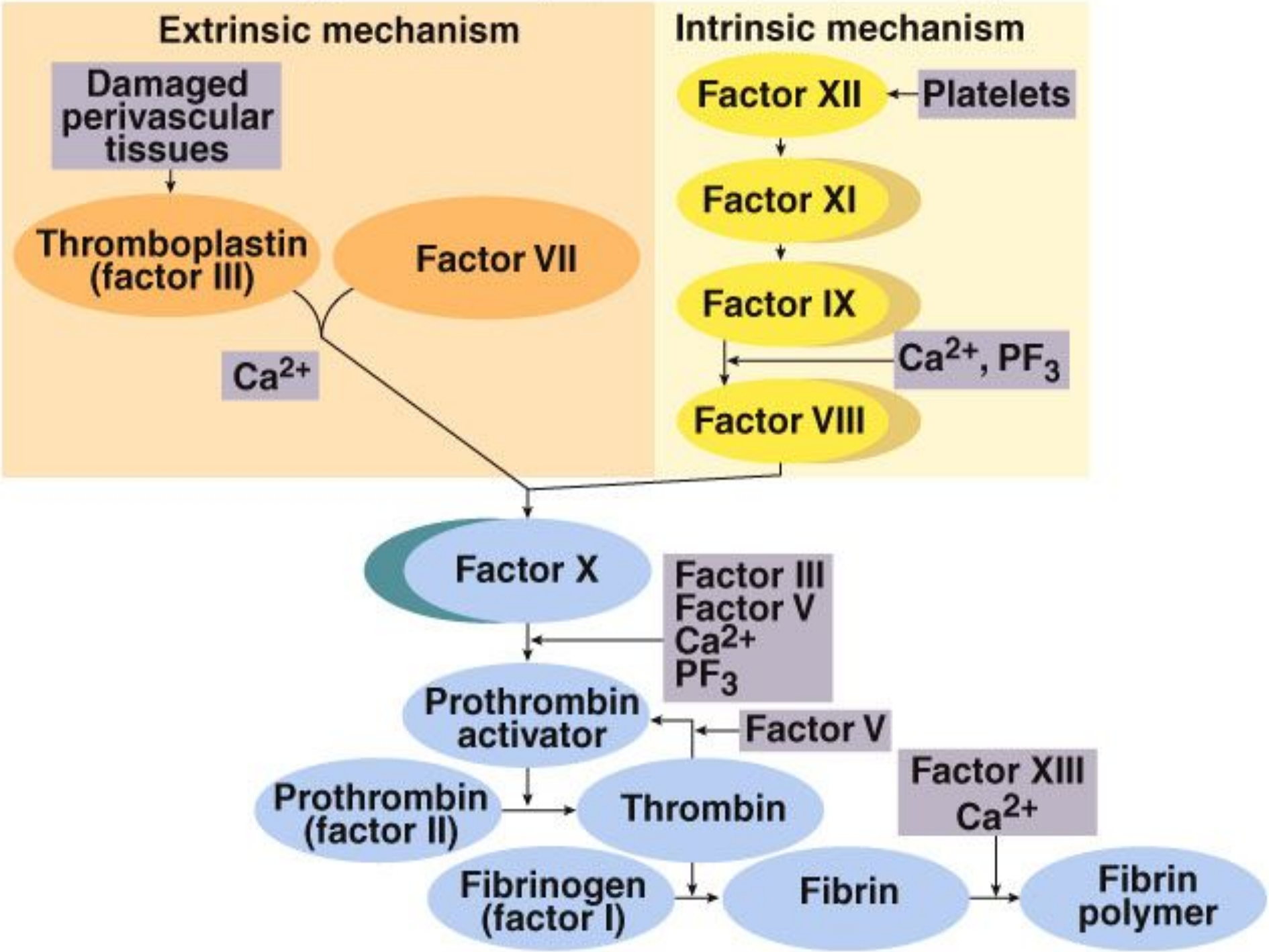
Calcium ions

- Are required for promotion and acceleration of almost all blood clotting reactions
- Except: activation of XII and XI (intrinsic mechanism)

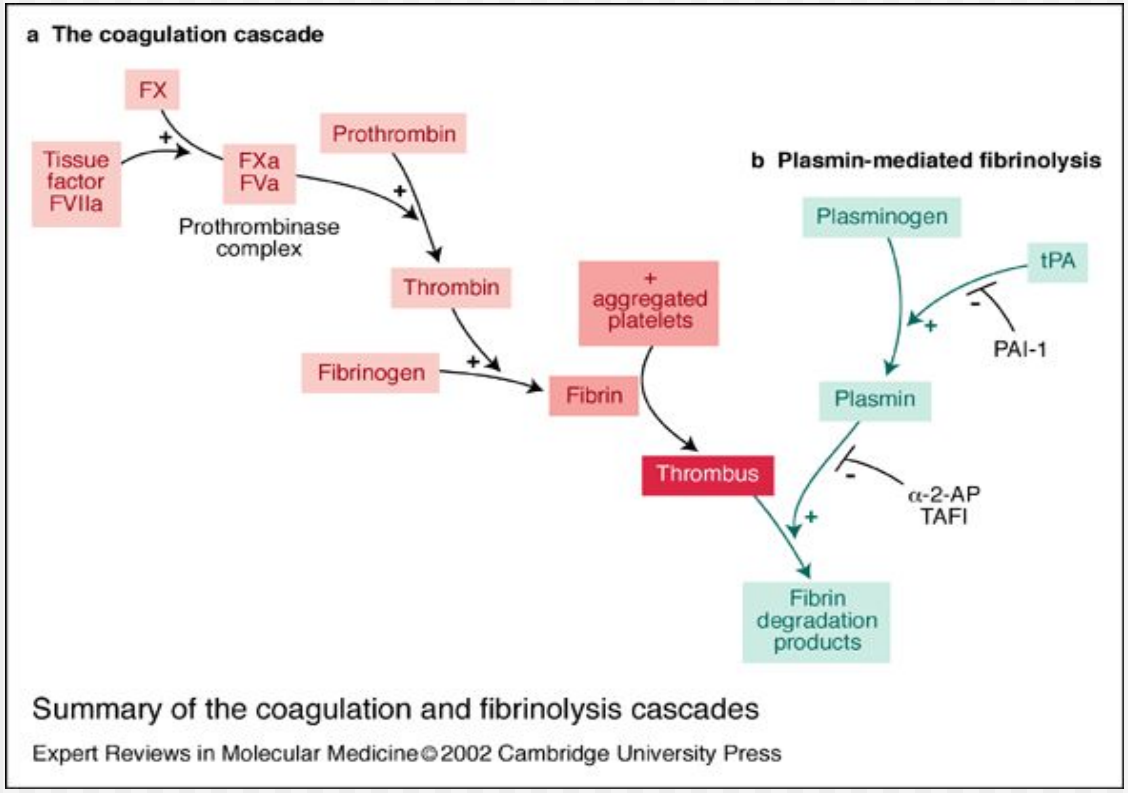
Ca^{2+}

http://www.mhhe.com/biosci/esp/2002_general/Esp/folder_structure/tr/m1/s7/trm1s7_3.htm


Extrinsic mechanism
Intrinsic mechanism
Damaged perivascular tissues
Thromboplastin (factor III)
Ca2+
Factor VII
Factor V
Factor XII
Platelets
Factor XI
Ca2+
Factor IX
Christmas factor
Ca2+, PF3
Factor VIII
Anti-hemophilic factor
Factor X
Stuart factor
Factor III
Factor V
Ca2+
PF3
Prothrombin activator
Factor V
Ca2+
Prothrombin (factor II)
Thrombin
Factor XIII
Ca2+
Fibrin-stabilizing factor
Fibrinogen (factor I)
Fibrin
Fibrin polymer


Extrinsic mechanism
Intrinsic mechanism
Damaged perivascular tissues
Thromboplastin (factor III)
Factor VII
Ca^{2+}
Factor XII
Platelets
Factor XI
Factor IX
Ca^{2+}, PF_3
Factor VIII
Factor X
Factor III
Factor V
Ca^{2+}
PF_3
Prothrombin activator
Factor V
Prothrombin (factor II)
Thrombin
Factor XIII
Ca^{2+}
Fibrinogen (factor I)
Fibrin
Fibrin polymer

Fibrinolysis



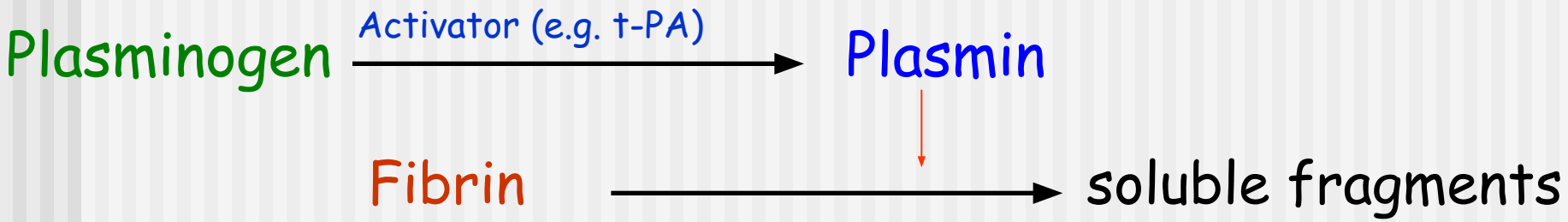
Summary of the coagulation and fibrinolysis cascades

Expert Reviews in Molecular Medicine©2002 Cambridge University Press

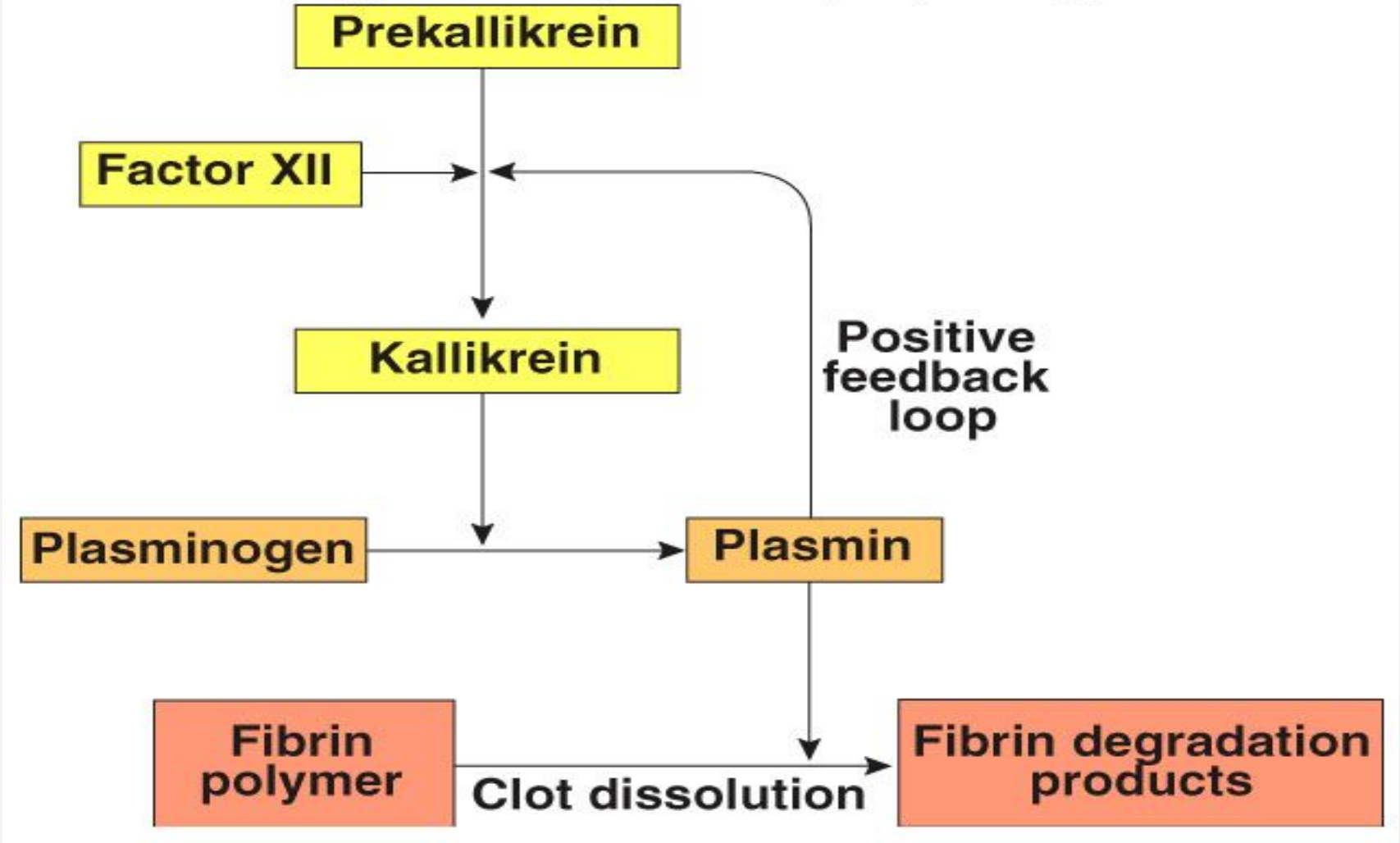
Clot Dissolution

1. Plasmin is formed from plasminogen - enzyme called activator (e.g. enzymes from urine, tears, saliva or bacterial enzyme streptokinase)

2. Plasmin as an enzyme is involved in breaking down fibrin into soluble fragments (fibrinolysis)



***Plasminogen** may be produced by eosinophils*


Prekallikrein
Factor XII
Kallikrein
Positive feedback loop
Plasminogen
Plasmin
Fibrin polymer
Clot dissolution
Fibrin degradation products

Anticoagulants

Hirudo medicinalis produce

Hirudin that inhibits Thrombin

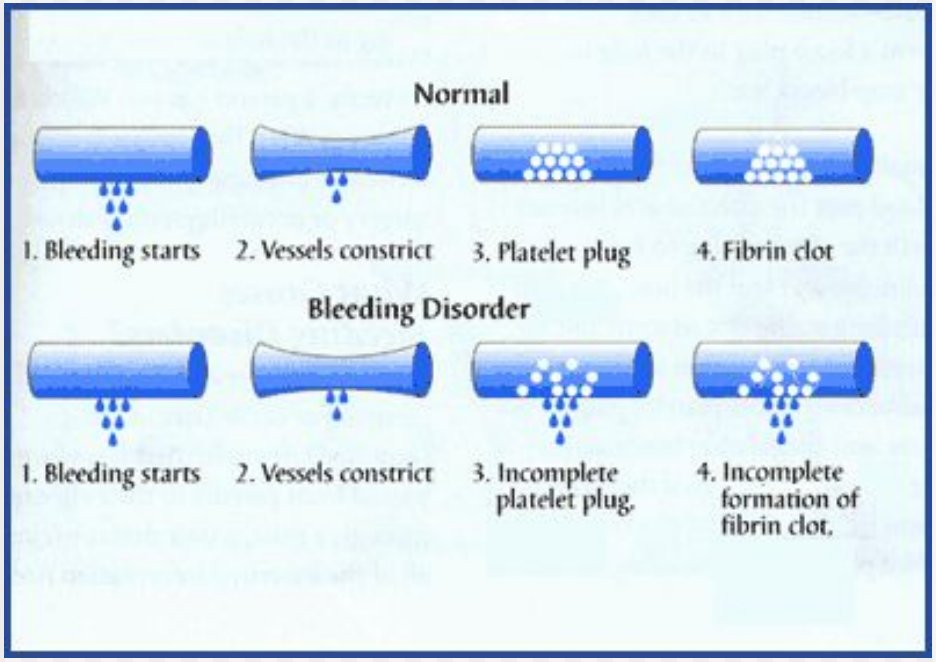
Anticoagulants

- Although tissue breakdown and platelets destruction are normal events in the absence of trauma, intravascular clotting does not usually occur because:
  - the amounts of procoagulants released are very small
  - natural anticoagulants are present *(Antithrombin III, Heparin, Antithromboplastin, Protein C and S, fibrin fibers)*

Natural anticoagulants

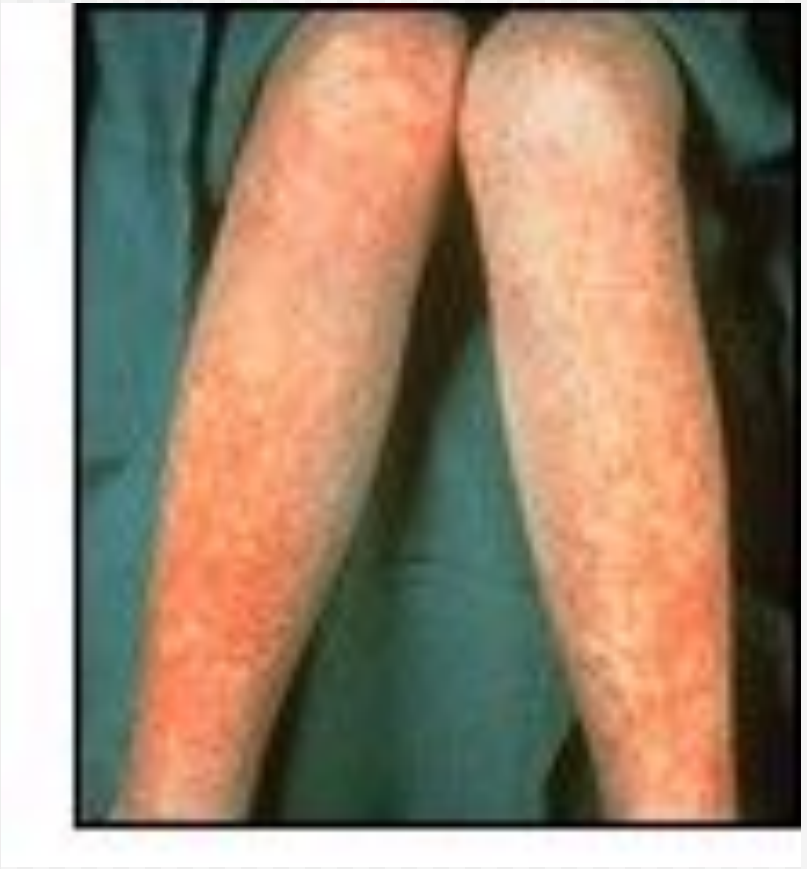
- Antithrombin III – inhibits factor X and thrombin
- Heparin from basophils and mast cells potentiates effects of antithrombin III (together they inhibit IX, X, XI, XII and thrombin)
- Antithromboplastin (inhibits „tissue factors" – tissue thromboplastins)
- Protein C and S – activated by thrombin; degrade factor Va and VIIIa

Abnormalities of hemostasis



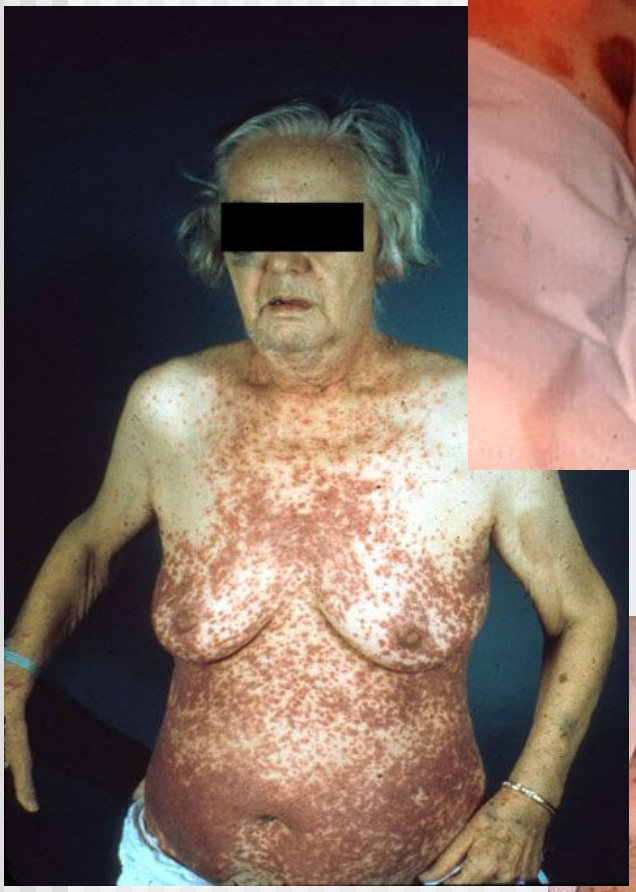
Thrombocytopenia

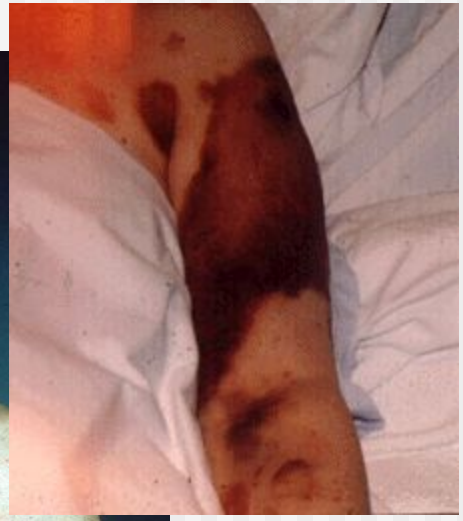
- Severe reduction in the number of PLTs - thrombocytopenia
- this causes spontaneous bleeding as a reaction to minor trauma
- in the skin - reddish-purple blotchy rash
- it may result from:
  - decreased production (toxins, radiation, infection, leukemias)
  - increased destruction (autoimmune processes)
  - increased PLTs consumption (DIC)

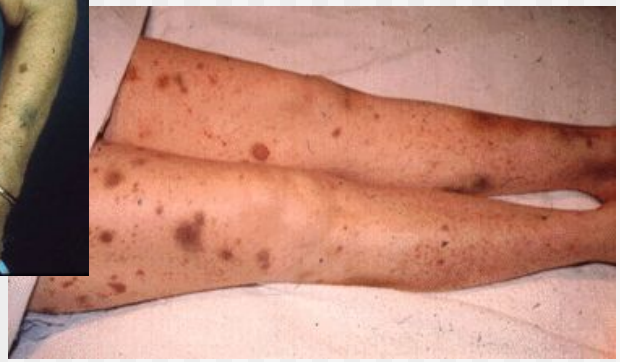


Hemorrhagic spots (petechiae)

Thrombocytopenia

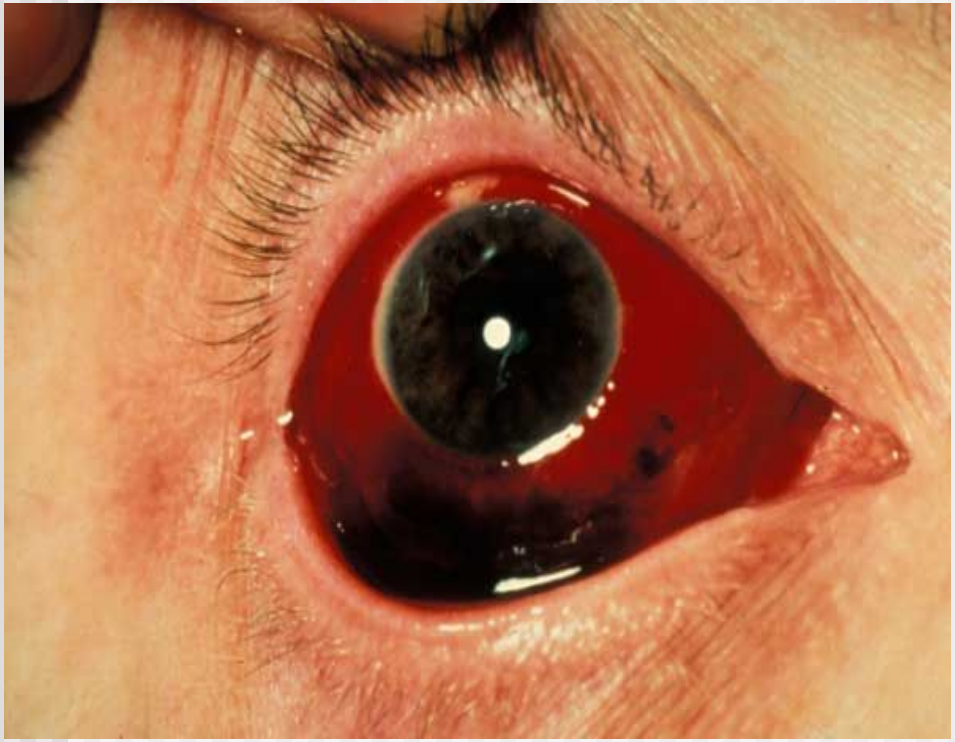






- Lethal when PLTs<10G/L
- Bleeding occurs when PLTs<50G/L
- Norm: 150-400G/L

Hepatic failure



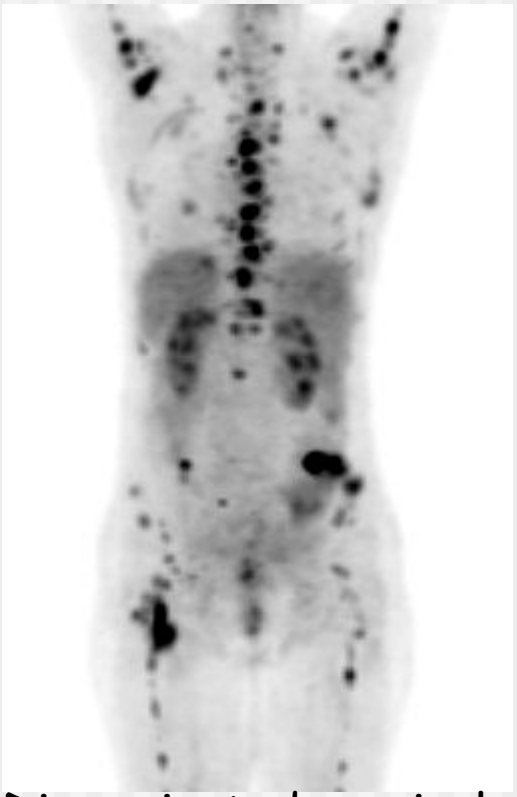
Subconjunctival hemorrhage

- Most of the clotting factors are formed in the liver

Disseminated intravascular coagulation (DIC)

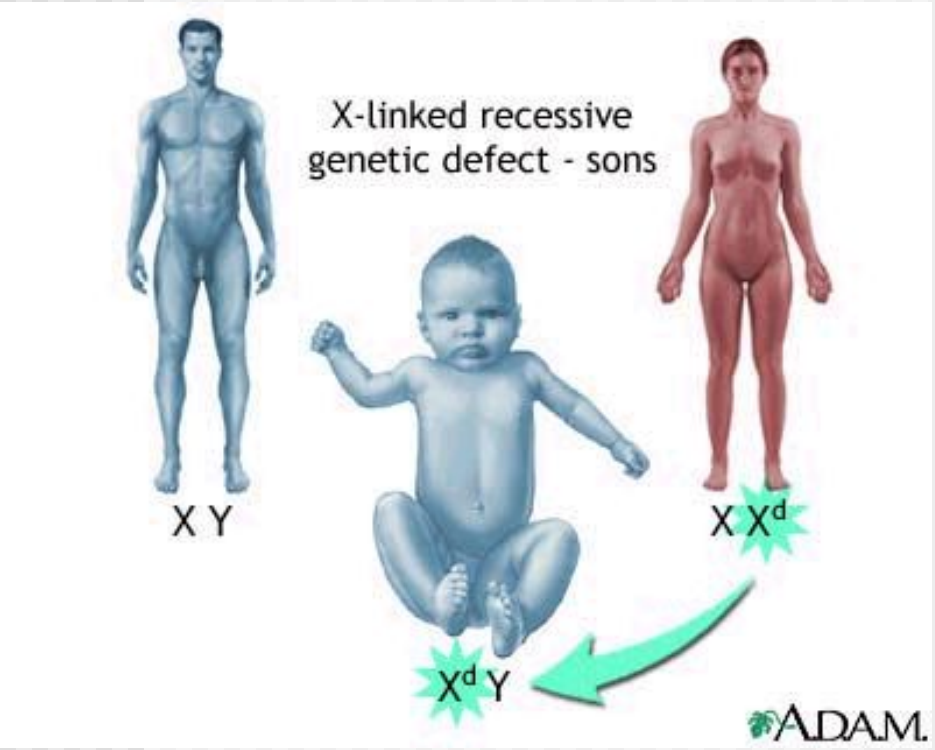
- Widespread coagulation → thrombosis in small blood vessels → increased fibrinolysis, and depletion of coagulating factors → generalized bleeding

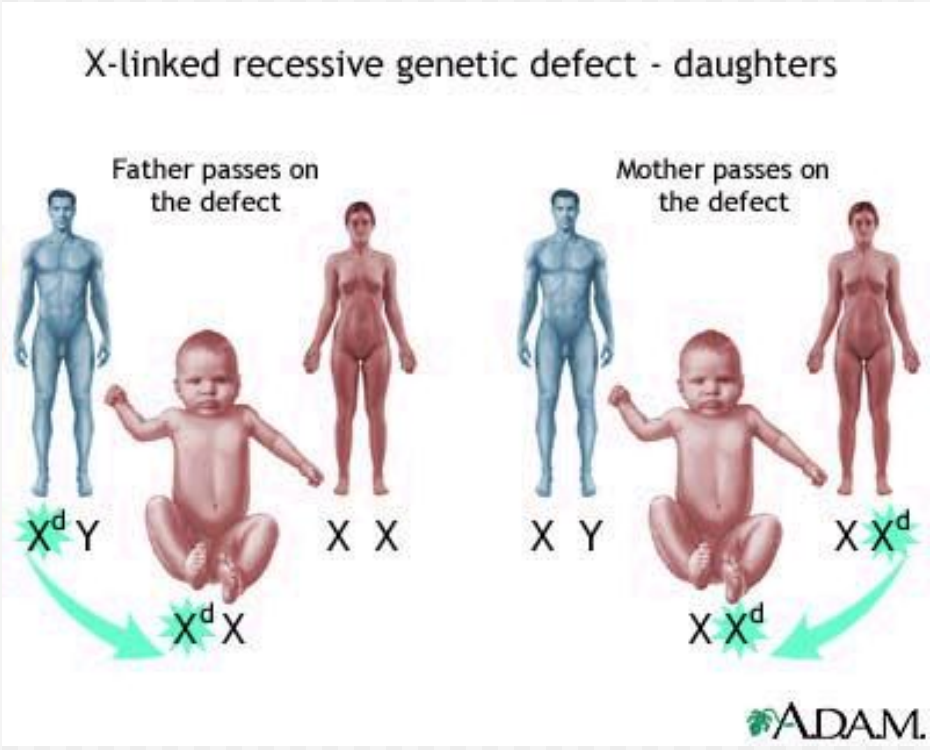
- It may result from:
  - bacterial infections (endothelial damage)
  - disseminated cancers (release of procoagulants)
  - complications of pregnancy
  - severe catabolic states



Disseminated cervical cancer metastases (PET imaging)

Hemophilia A (lack of F VIII) and B (lack of F IX) are transmitted genetically and affect only males. Females carry the gen but do not show symptoms.

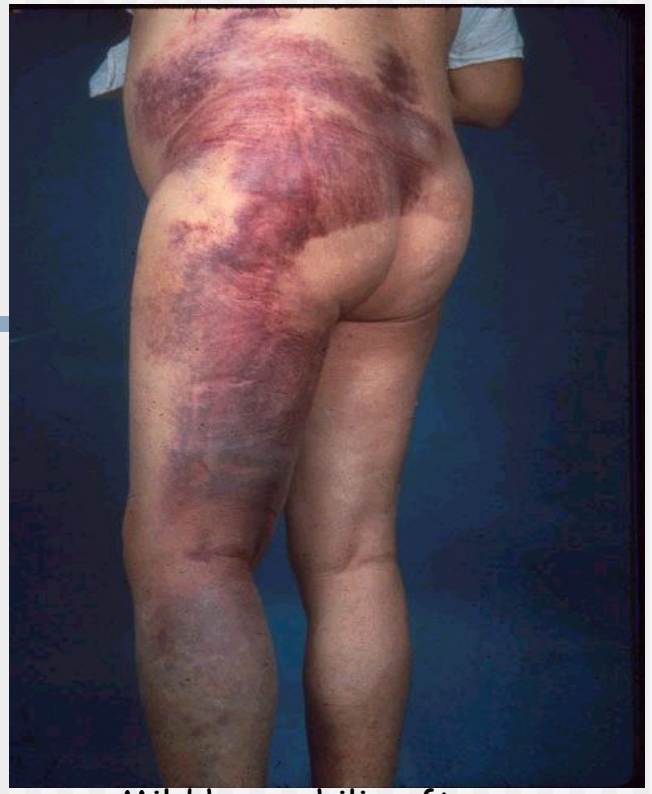




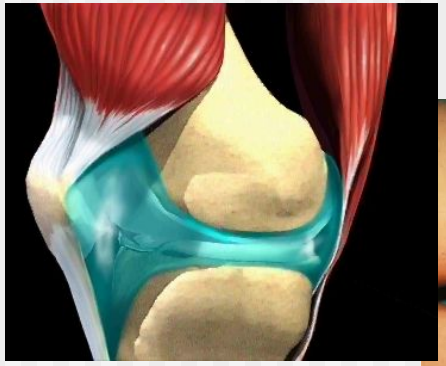
Von Willebrand's disease – loss of large component of fVIII

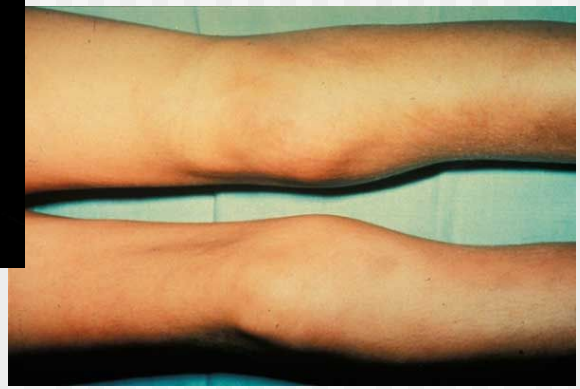
Hemophilia A (lack of F VIII; 85%)

- Spontaneous or traumatic subcutaneous bleeding
- Blood in the urine
- Bleeding in the mouth, lips, tongue
- Bleeding to the joints, CNS, gastrointestinal tract



Mild hemophilia after injection in buttock

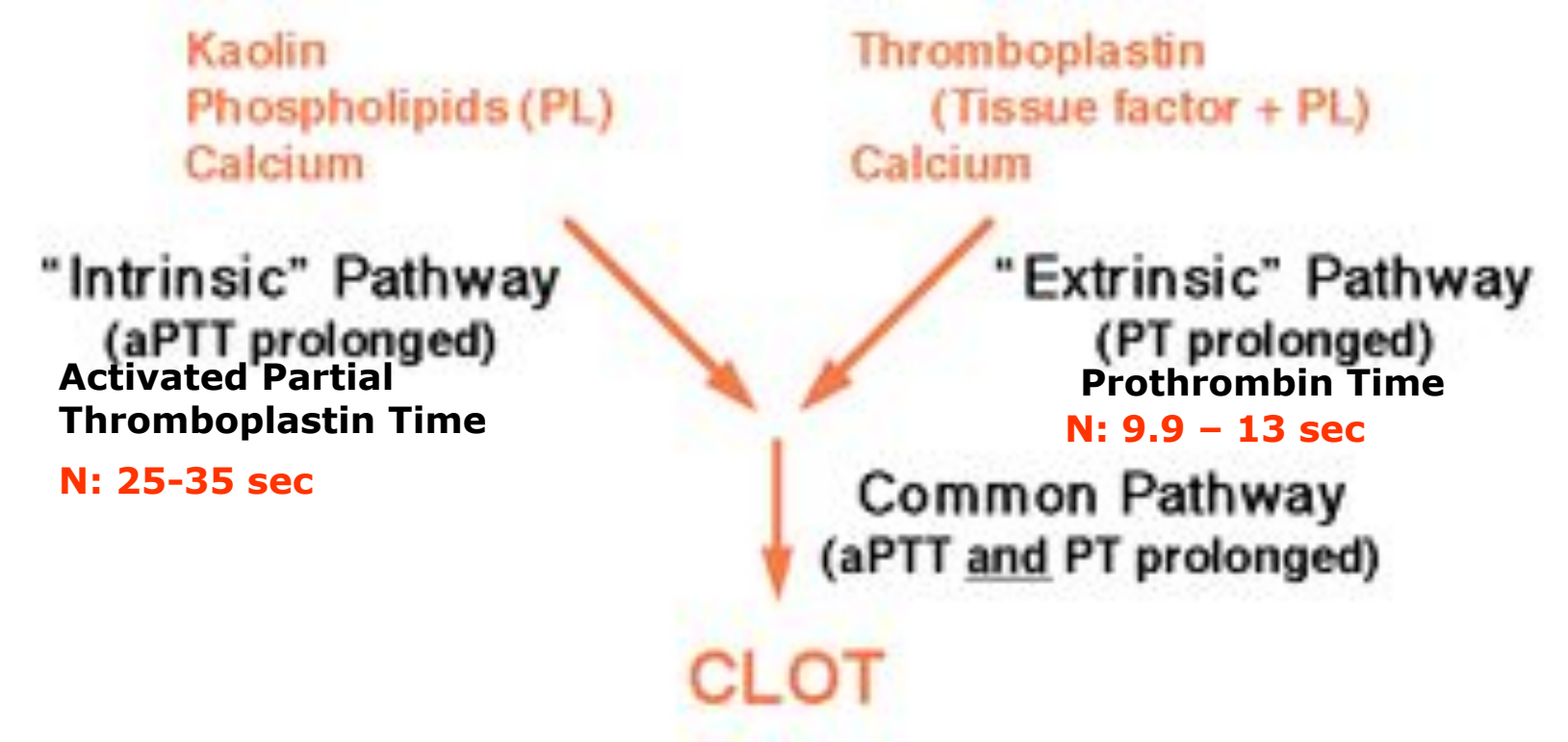




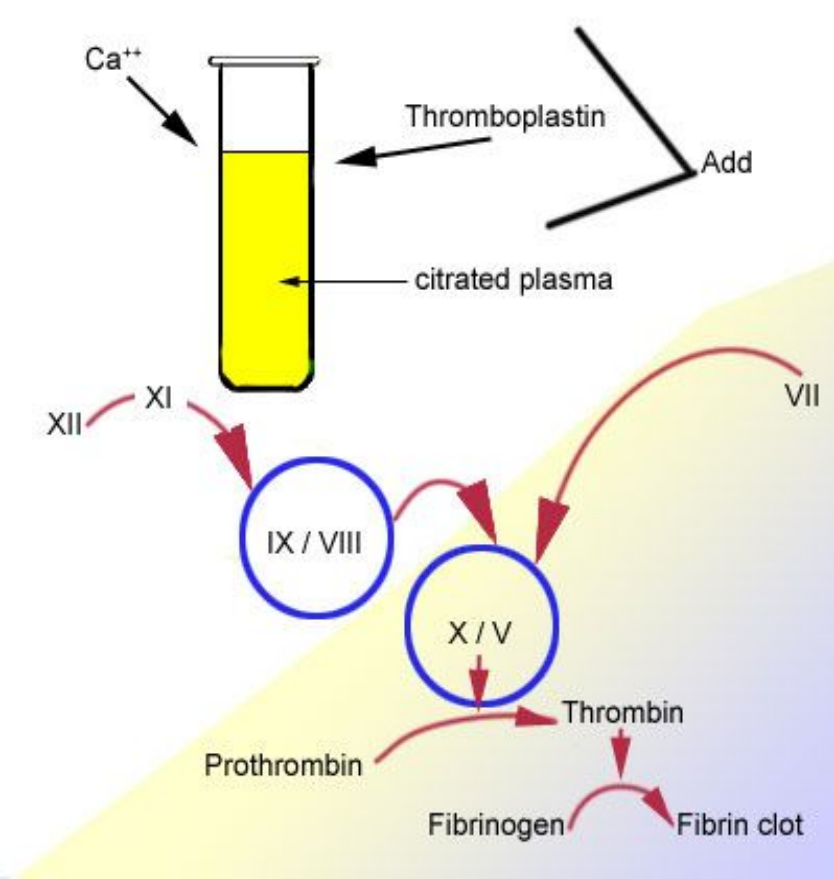
- Son of the last Tsar of Russia – Aleksy Romanow suffered from Hemophilia A

Tests of coagulation

"Intrinsic" and "extrinsic" coagulation pathways



Prothrombin time (PT) test – norm 11 -15 sec
evaluates extrinsic system (*VII, X, V, II, fibrinogen*)



- prolonged PT indicates a deficiency in any of factors VII, X, V, prothrombin (factor II), or fibrinogen (factor I).

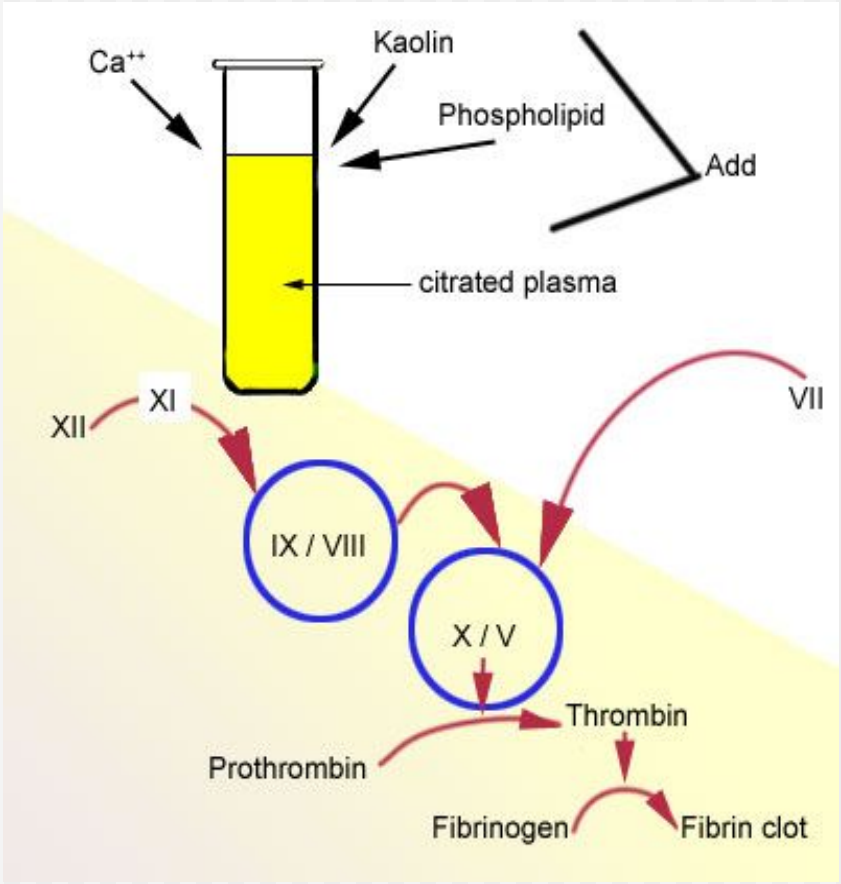
- Prolonged PT:
- *a vitamin K deficiency* (vitamin K is a co-factor in the synthesis of functional factors **II (prothrombin), VII, IX and X**)
- *liver disease*
- *Warfarin therapy*
- *DIC*
- *excesive heparin*

International Normalised Ratio (INR)

- The result for the **PT** is expressed as a ratio (prothrombin clotting time for patient plasma divided by time for control plasma);
- Correction factor (International Sensitivity Index) is applied to the prothrombin ratio and the result issued as INR.
- **Therapeutic interval**: Therapeutic interval for oral anticoagulant therapy: 2.0-4.5.
- **Application**: Monitoring oral anticoagulant therapy (eg. Warfarin);
- note that heparin will not prolong INR *(heparinase is included within the INR reagent)!!!!!!!!!!!!*
  For heparin therapy we monitor aPTT and/or aPTT ratio

Activated Partial Thromboplastin Time test (aPTT) – norm: 25-35 s; evaluates intrinsic system

(VIII, IX, XI, XII, X, V, II, fibrinogen)



- an isolated prolongation of the aPTT (PT normal) suggests deficiency of factor VIII, IX, XI or XII
- prolongation of both the APTT and PT suggests factor X, V, II or I (fibrinogen) deficiency, all of which are rare
- aPTT is normal in factor VII deficiency (PT prolonged) and factor XIII deficiency

Most common case of prolonged aPTT – heparin!!!

Thrombin time (TT) – norm: 14-15 sec

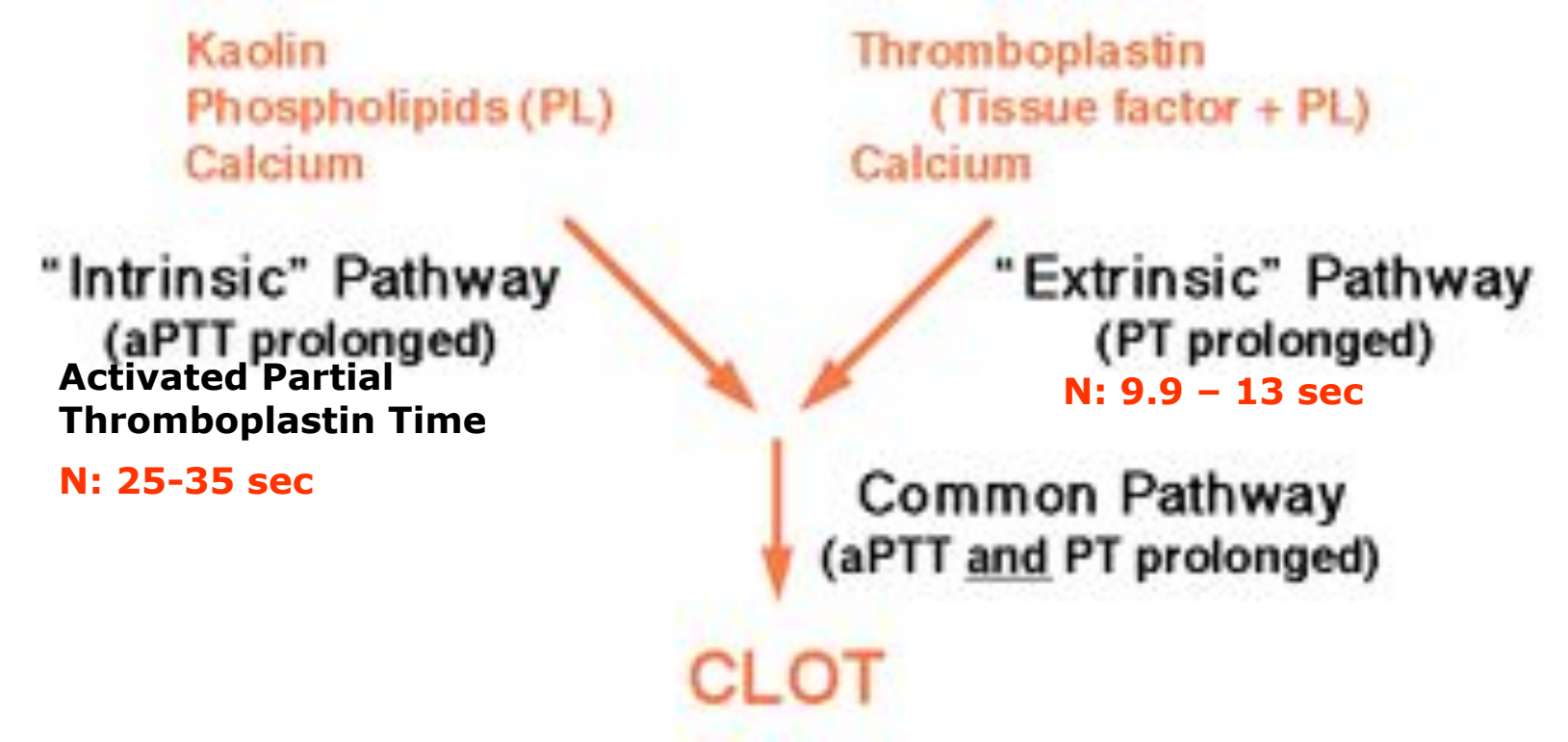
Prolonged TT:

- Heparin (much more sensitive to heparin than aPTT)
- Hypofibrinogenemia

Selected causes of abnormal coagulation tests

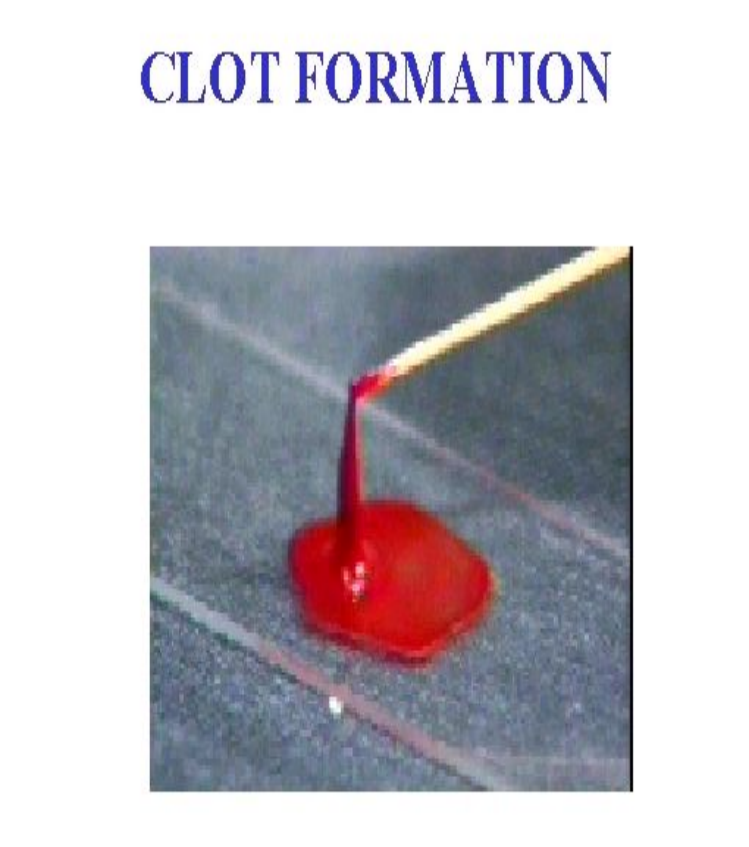
Partial Thromboplastin Time (aPTT)	Prothrombin Time (PT)	Thrombin Time (TT)	Bleeding Time (BT)
Factor deficiency (except VII)	VII, X, V, II, fibrinogen deficiency	Low or absent fibrinogen	Thrombocytopenia
Antibodies to clotting factors	Antibodies	Dysfibrinogenemia, hypofibrinogenemia	Von Willebrand's disease
Heparin	Warfarin; Vit K defficiency (mild to severe)	Heparin	Drugs (Aspirin, NSAIDs, high dose penicillins, etc.)
Excessive Warfarin	Excessive Heparin		Cirrhosis, Uremia, PLTs dysfunction

"Intrinsic" and "extrinsic" coagulation pathways



Whole blood clotting time

- The time taken for blood to clot mainly reflects the time required for the generation of thrombin
- The surface of the glass tube initiates the clotting process. This test is sensitive to the factors involved in the intrinsic pathway
- The expected range for clotting time is 4-10 mins.



Whole blood clotting time – *procedure:*

- Clean the tip of the finger with an alcohol
- Prick the finger tip with an automatic lancet
- Note the time when blood first appears on the skin
- Touch the tube to the drop of blood
- Break gently 1cm of the tube at the end of 2 min, and every 30 sec these after
- When fibrin is formed between the two broken pieces of tube the coagulation or clotting time is noted

Bleeding time

- This is a test that measures the speed in which small blood vessels close off (the condition of the blood vessels and platelet function)
- This test is useful for detecting bleeding tendencies
- The bleeding stops within 1 to 9 minutes. This may vary from lab to lab, depending on how the test is measured
- Using the ear lobe method, a normal bleeding time is between 1 and 4 minutes.

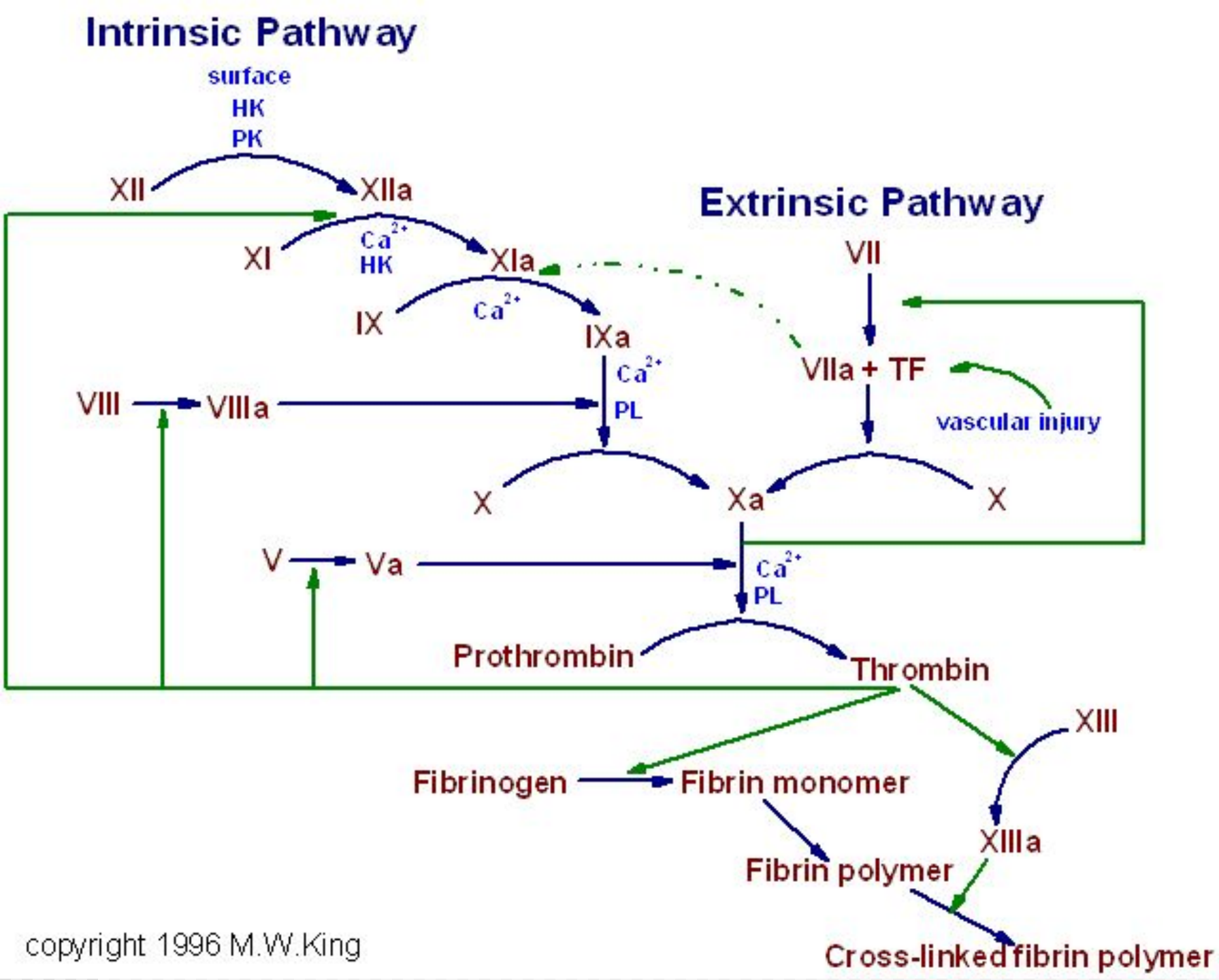
Bleeding time – procedure:

- Clean the earlobe with an alcohol
- Prick the earlobe with an automatic lancet
- Note the time when blood first appears on the skin
- After half a minute (30sec) place the edge of the filter paper on the top of the drop of blood.
- Perform the operation at half minute (30 sec) interval
- The end point or bleeding time is the first half minute when no blood is seen on the filter paper.

Abnormal Bleeding Time

- Prolonged bleeding time may indicate:
  - A vascular (blood vessel) defect
  - A platelet function defect (see platelet aggregation)
  - platelets count defect (low platelets)
- Drugs that may increase times include dextran, indomethacin, and salicylates (including aspirin).

http://www.medicine.mcgill.ca/physio/vlab212D/bloodlab/images/clottime5.mpg



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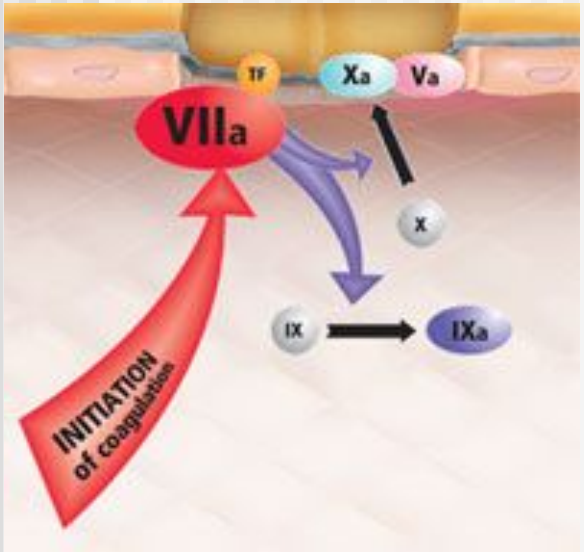
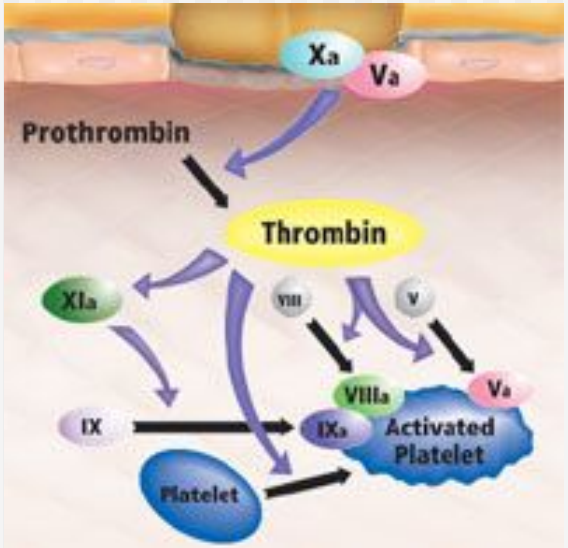
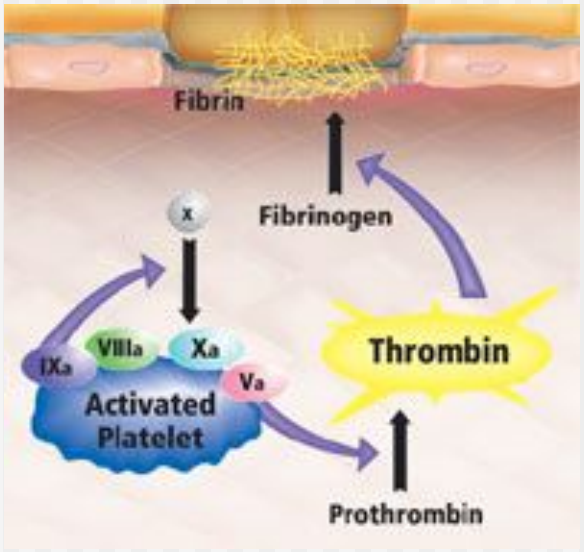
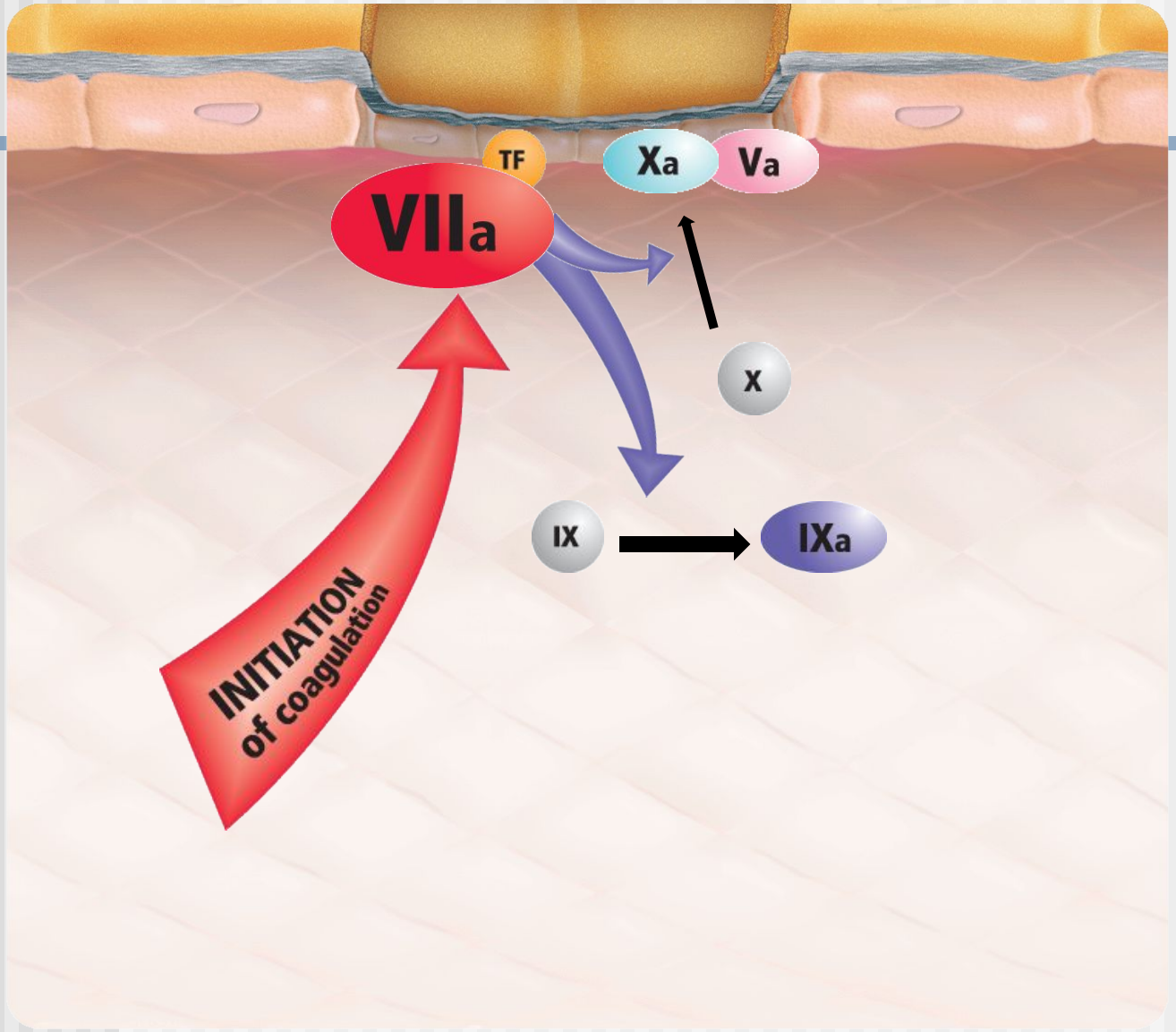
The new model of haemostasis







1. Initiation phase



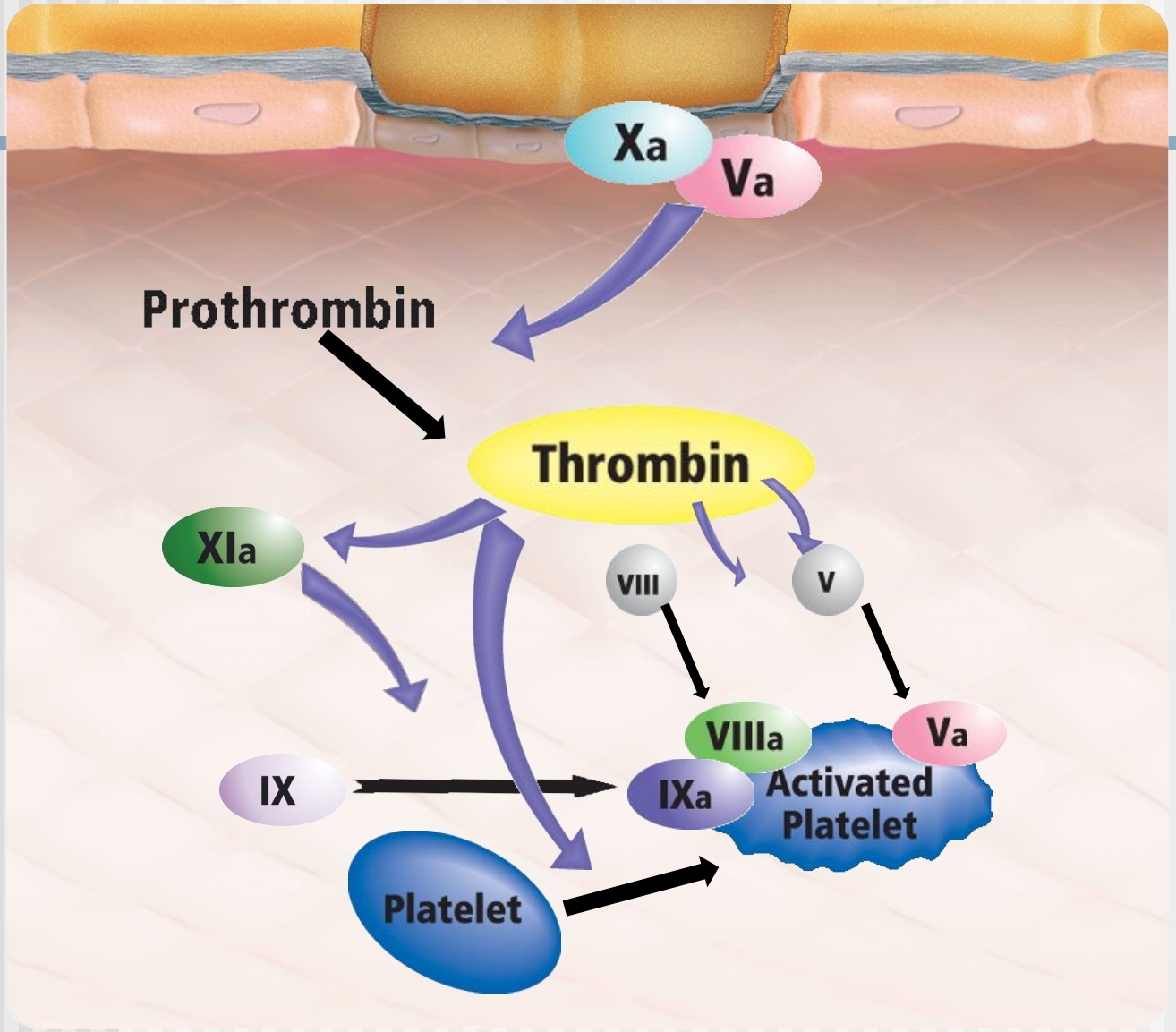
Injury of vessels wall leads to contact between blood and subendothelial cells

Tissue factor (TF) is exposed and binds to FVIIa or FVII which is subsequently converted to FVIIa

The complex between TF and FVIIa activates FIX and FX

FXa binds to FVa on the cell surface

2. Amplification phase

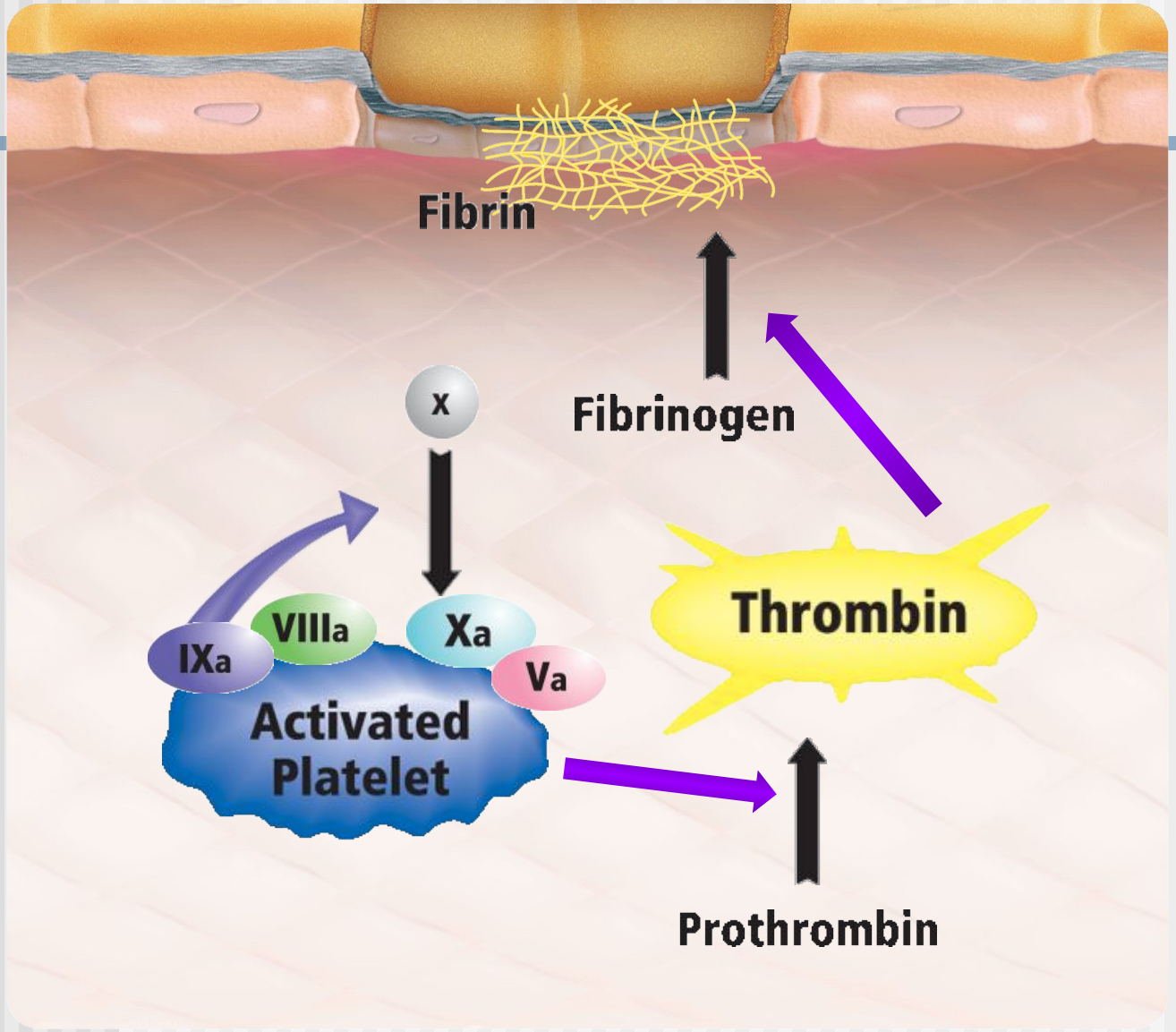


The FXa/FVa complex converts small amounts of prothrombin into thrombin

The small amount of thrombin generated activates FVIII, FV, FXI and platelets locally. FXIa converts FIX to FIXa

Activated platelets bind FVa, FVIIIa and FIXa

3. Propagation phase



The FVIIIa/FIXa complex activates FX on the surfaces of activated platelets

FXa in association with FVa converts large amounts of prothrombin into thrombin creating "thrombin burst".

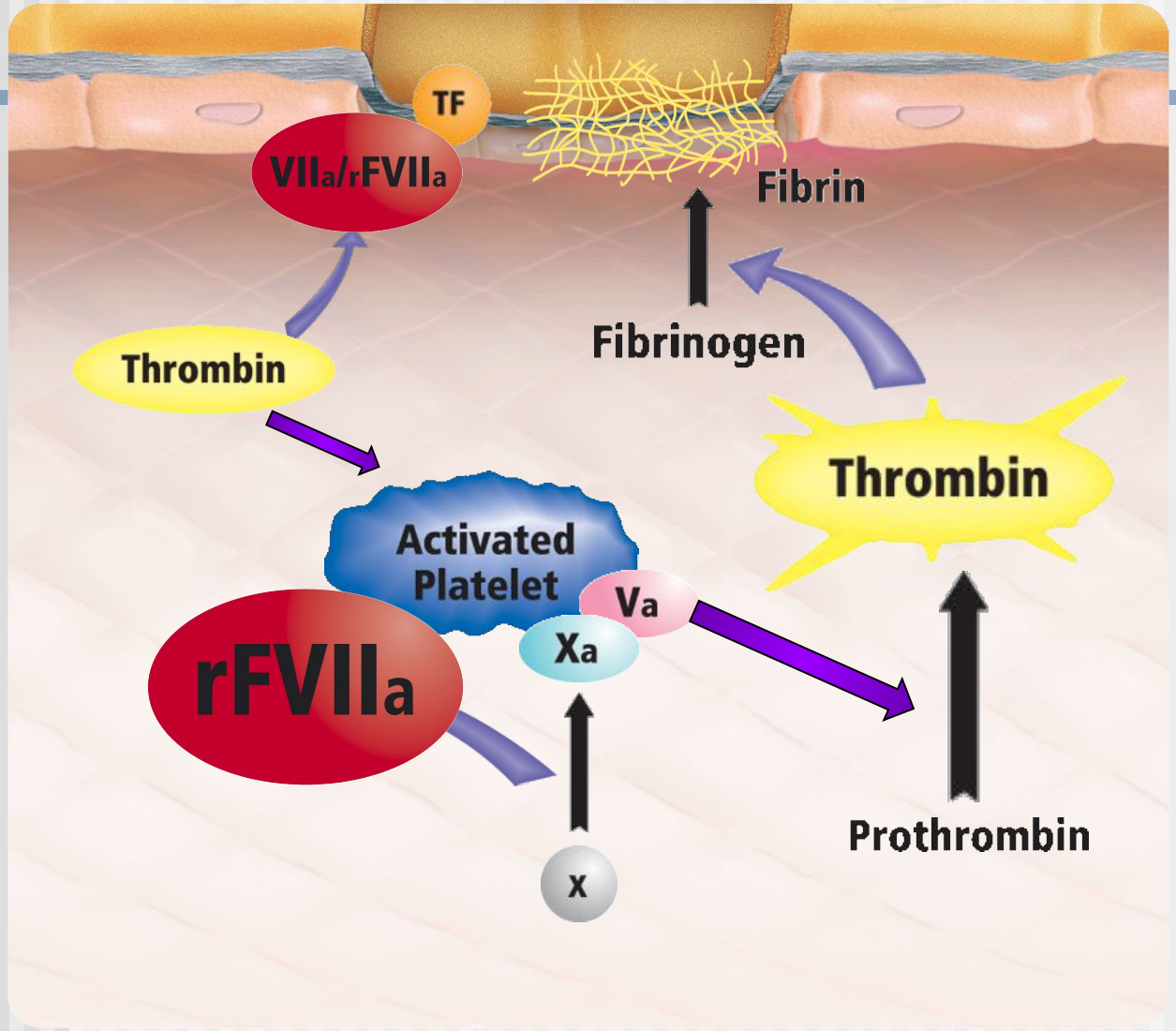
The "thrombin burst" leads to the formation of a stable fibrin clot.

Summary:

- Haemostasis starts with the interaction between TF and FVIIa on the surface of subendothelial cells.
- The small amount of thrombin generated during the amplification phase activates platelets locally on whose surface the subsequent reactions take place.
- The resulting thrombin burst results in the formation of a stable clot.

NovoSeven® Mode of Action

Eptacog alfa (activated)



Tissue factor (TF)/FVIIa, or TF/rFVIIa interaction, is necessary to initiatiate haemostasis

At pharmacological concentrations rFVIIa directly activates FX on the surface of locally activated platelets.
This activation will initiate the ”thrombin burst” independently of FVIII and FIX.
This step is independent of TF.

The thrombin burst leads to the formation of a stable clot

Conclusion:

- In high doses rFVIIa binds to the surface of the locally activated platelets where it leads to the formation of a ”thrombin burst”

Prescribing Information

NovoSeven® Eptacog alfa (activated) Abbreviated Prescribing Information: NovoSeven [Recombinant Coagulation Factor VIIa (rFVIIa)] **Presentation:** Powder for injection with accompanying solvent for reconstitution (Water for Injections). Available in packs containing 1.2, 2.4 or 4.8 mg rFVIIa. **Uses**: Treatment of bleeding episodes and prevention of bleeding during surgery or invasive procedures in patients with: - congenital haemophilia with inhibitors to coagulation factors VIII or IX > 5 BU or who are expected to have a high anamnestic response to FVIII or FIX. - acquired haemophilia - congenital FVII deficiency - Glanzmann's thrombasthenia with antibodies to GP IIb-IIIa and/or HLA, and with past or present refractoriness to platelet transfusion. **Dosage**: The rFVIIa is dissolved in the accompanying solvent before use. After reconstitution the solution contains 0.6 mg rFVIIa/ml. Administer by intravenous bolus injection over 2-5 minutes; must not be mixed with infusion solutions or given in a drip. *Haemophilia A or B with inhibitors or acquired haemophilia* Initial dose of 90µg per kg body weight. Duration of, and interval between, repeat injections dependent on severity of haemorrhage or procedure/surgery performed. For mild to moderate bleeding episodes (including ambulatory treatment): 1-3 doses at 3 hour intervals (90µg per kg b.w.) to achieve haemostasis, with additional dose to maintain haemostasis. Duration of ambulatory treatment should not exceed 24 hours. For serious bleeding episodes, initial dose 90µg per kg. b.w.; dose every two hours until clinical improvement. If continued therapy indicated, dosage interval can be increased successively. Major bleeding episode may be treated for 2-3 weeks or longer if clinically warranted. For invasive procedures/surgery administer initial dose of 90µg per kg. b.w. immediately before the procedure. Repeat dose at 2-3 hour intervals for first 24-48 hours. In major surgery continue dosing at 2-4 hour intervals for 6-7 days. Dosage interval may then be increased to 6-8 hours for further 2 weeks. Treatment may be up to 2-3 weeks until healing has occurred. *Factor VII deficiency* For bleeding episodes and for invasive procedures/surgery administer 15-30µg per kg b.w. every 4-6 hours until haemostasis achieved. Adapt dose and frequency to individual. *Glanzmann's thrombasthenia* For bleeding episodes and for invasive procedures/surgery administer 90µg (range 80-120µg) per kg b.w. every 2 hours (1.5-2.5 hours). At least three doses should be administered to secure effective haemostasis. For patients who are not refractory platelets are first line treatment. **Contra-indications**: Known hypersensitivity to active substance, excipients, or to mouse, hamster or bovine protein. **Precautions**: For severe bleeds NovoSeven should only be administered in hospitals specialised in the treatment of patients with coagulation factor VIII or IX inhibitors or in close collaboration with a physician specialised in treatment of haemophilia. Ambulatory treatment should not exceed 24 hours. Possibility of thrombogenesis or induction of DIC in conditions in which tissue factor could be expected in circulating blood, e.g. advanced atherosclerotic disease, crush injury, septicaemia, or DIC. Since NovoSeven may contain trace amounts of mouse, bovine and hamster proteins there is a remote possibility of the development of hypersensitivity. Monitor FVII deficient patients for prothrombin time and FVII coagulant activity; suspect antibody formation if FVIIa activity fails to reach expected level or bleeding not controlled with recommended doses. Avoid simultaneous use of prothrombin complex concentrates, activated or not. **Use in pregnancy**: Only administer to pregnant women if clearly needed. Not known if excreted in human milk; exercise caution when administering NovoSeven to nursing women. **Side Effects**: Adverse reactions (serious and non-serious) reported during post-marketing period: Rare (>1/10,000, <1/1,000): Lack of efficacy. Very rare <1/10,000): Coagulopathic disorders such as increased D-dimers and consumptive coagulopathy; myocardial infarction; nausea; fever; pain, especially at injection site; increase of ALT, ALP, LDH and prothrombin levels; cerebrovascular disorders including cerebral infarction and cerebral ischaemia; skin rashes; venous thrombotic events; haemorrhage.
Serious adverse reactions include: Arterial thrombotic events (such as myocardial infarction or ischaemia, cerebrovascular disorders and bowel infarction); venous thrombotic events (such as thrombophlebitis, deep vein thrombosis and pulmonary embolism). In the vast majority of cases patients were predisposed to such events. No spontaneous reports of anaphylactic reactions, but patients with a history of allergic reaction should be carefully monitored. No reports of antibodies against FVII in haemophilia A or B patients. Isolated cases of FVII-deficient patients developing antibodies against FVII reported after treatment with NovoSeven. These patients previously treated with human plasma and/or plasma derived FVII. Monitor FVII deficient patients for FVII antibodies. One case angioneurotic oedema reported in patient with Glanzmann's thrombasthenia after administration of NovoSeven. **Marketing Authorisation numbers**: NovoSeven 60 KIU EU/1/96/006/001 NovoSeven 120 KIU EU/1/96/006/002 NovoSeven 240 KIU EU/1/96/006/003 **Legal Category**: POM **Basic NHS Price**: NovoSeven 1.2 mg £664.72 NovoSeven 2.4 mg £1329.44 NovoSeven 4.8 mg £2658.88 Further information: Full prescribing information can be obtained from: Novo Nordisk Limited Broadfield Park Brighton Road Crawley West Sussex RH11 9RT Tel: 01293 613555 Fax: 01293 613535 Date of preparation: May 2004 Ref N7/03/039a

A 35-year-old man complains of chronic physical fatigue, which began 3-4 weeks ago. He said he felt tired all of the time even through his occupation as a software developer was mentally but not physically demanding. He breathed comfortably at rest but, when he exerted himself, he experienced difficulty in breathing and had hard time catching his breath. He also complained of „more than usual" mental fatigue, confessing an increasing inability to concentrate and focus his attention on tasks at hands. Colleagues noticed his pallor and his inattentiveness at brainstorming sessions and suggested he reschedule his annual physical examination for an earlier date. He complained of vague abdominal pain and sense of abdominal fullness. His appetite was depressed, and he thought perhaps his physical and mental symptoms were caused by poor diet. However, attempts to increase eating resulted in nausea. His stools, he said, were sometimes loose and tarry. Eventually, increased heart palpitations and chest pain made him seek medical advice

Laboratory findings revealed the following:

Laboratory test	Patient	Normal
RBC (red blood cell count)	3.5 T/L	4.5-6.0 T/L
HCT (hematocrit ratio)	28%	40-52%
Hb (hemoglobin)	8.0g/dL	13-17g/dL
MCV (mean corpuscular volume)	70fL	78-95fL
MCH (mean corpuscular hemoglobin)	22.8pg	29pg
MCHC (mean corpuscular hemoglobin concentration)	28%	34%

Case history questions:

1. What general medical condition is suggested by the person's symptoms?
2. What fundamental change in function of blood related to the red blood cells could simultaneously affect the function of several systems (cardiovascular, respiratory, gastrointestinal, and others)?
3. What specific diagnosis is supported by the laboratory findings?
4. How could the stool be related to the laboratory findings?

Answers:

1. Anemia
2. A reduction in oxygen-carrying capacity of the blood and thus a reduction in the delivery of oxygen to various body tissues
3. An iron defficiency anemia
4. Most cases of iron-defficiency anemia result from internal blood loss.
   Dark, tarry loose stools suggest bleeding from the gastrointestinal tract and warrant further tests to determine the exact cause