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BCSLS Telehealth Video Broadcast

Blood Coagulation: From Clotting Proteins to INR

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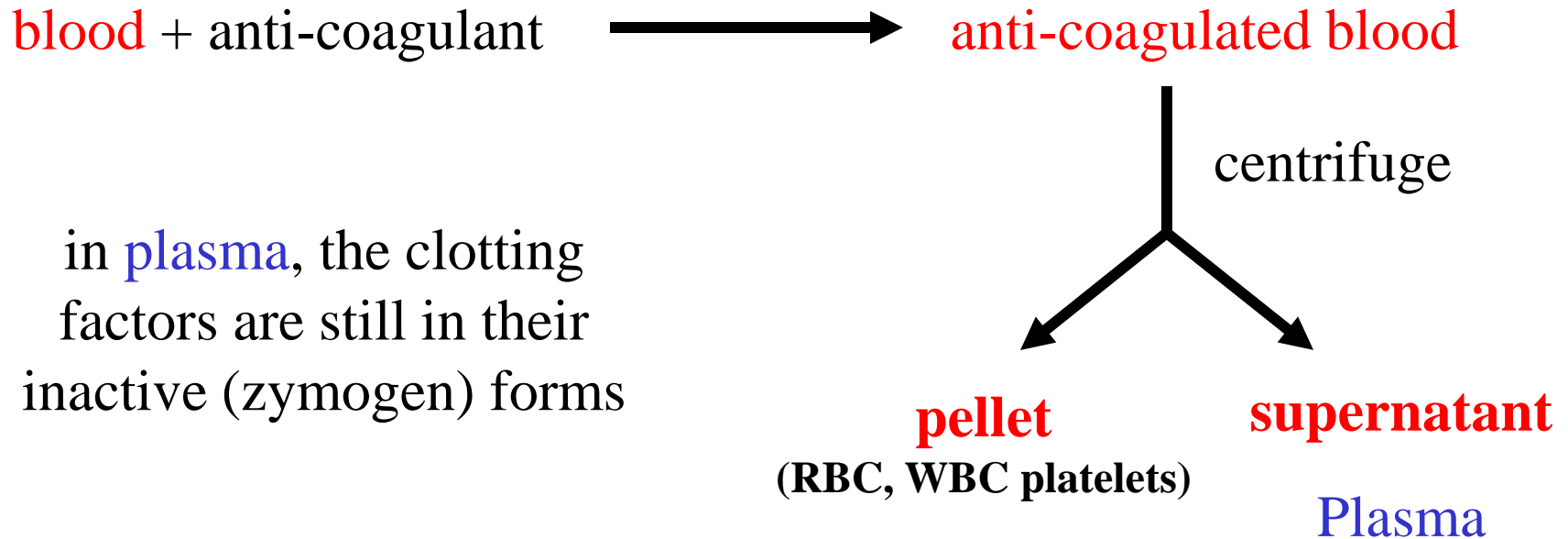


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October 20th, 2011

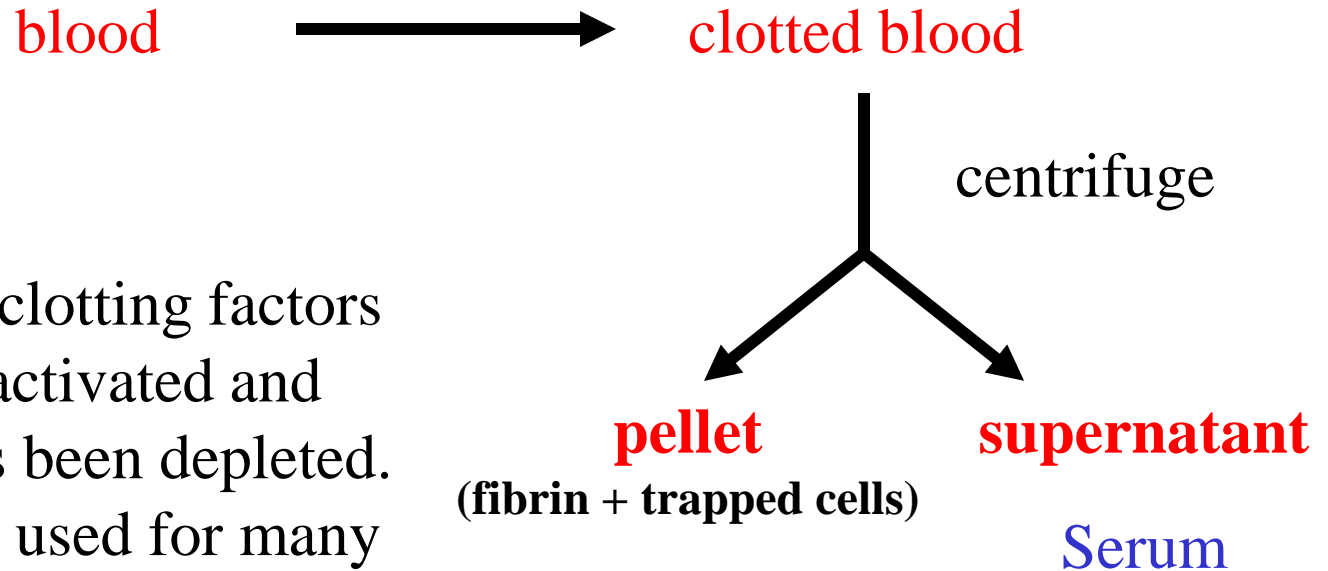
Plasma

Plasma is obtained when blood is collected into an anticoagulant and the cells (red blood cells, white blood cells and platelets) are removed:



Serum

Serum is obtained when blood is allowed to clot. The clot can then be separated into a fibrin clot (fibrin plus trapped cells) and serum:



in **serum**, the clotting factors have been activated and fibrinogen has been depleted. **Serum** can be used for many blood chemistry tests

Hemostasis

mechanism that ensures retention of the blood within the vascular system

involving:

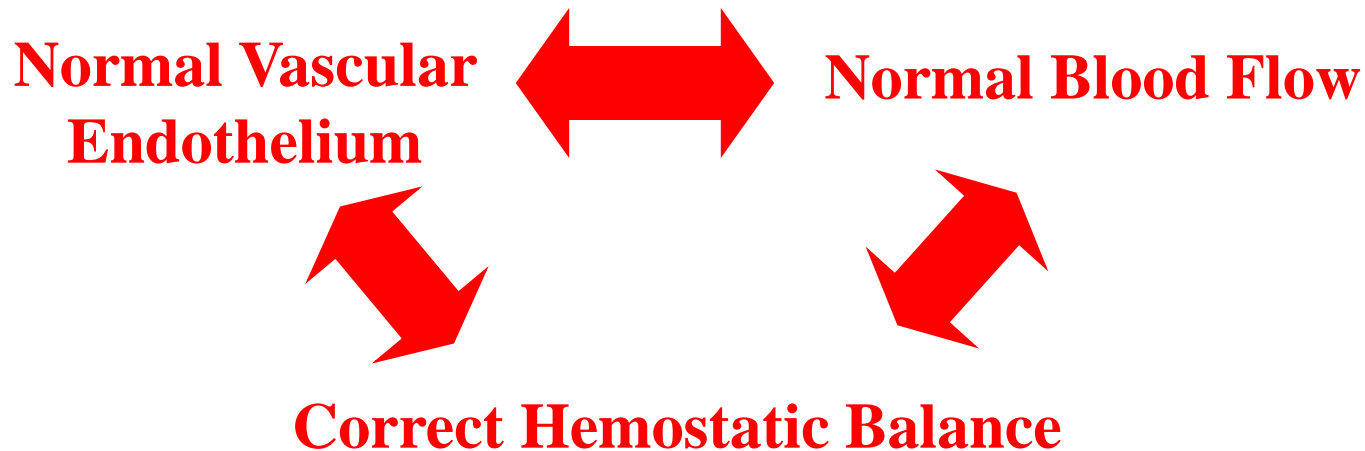
endothelial cells lining the blood vessels

platelets – non-nucleated cells in blood

a group of blood proteins called the clotting factors

Virchow's Triad of Normal Hemostasis

Normal Hemostasis



(pro-coagulant, anti-coagulant, fibrinolysis)

Overview of Clotting & Thrombolysis

CLOTTING

tissue damage leading to
fibrin clot formation



THROMBOLYSIS

tissue repair and fibrin
clot dissolution

Platelets – the first defense

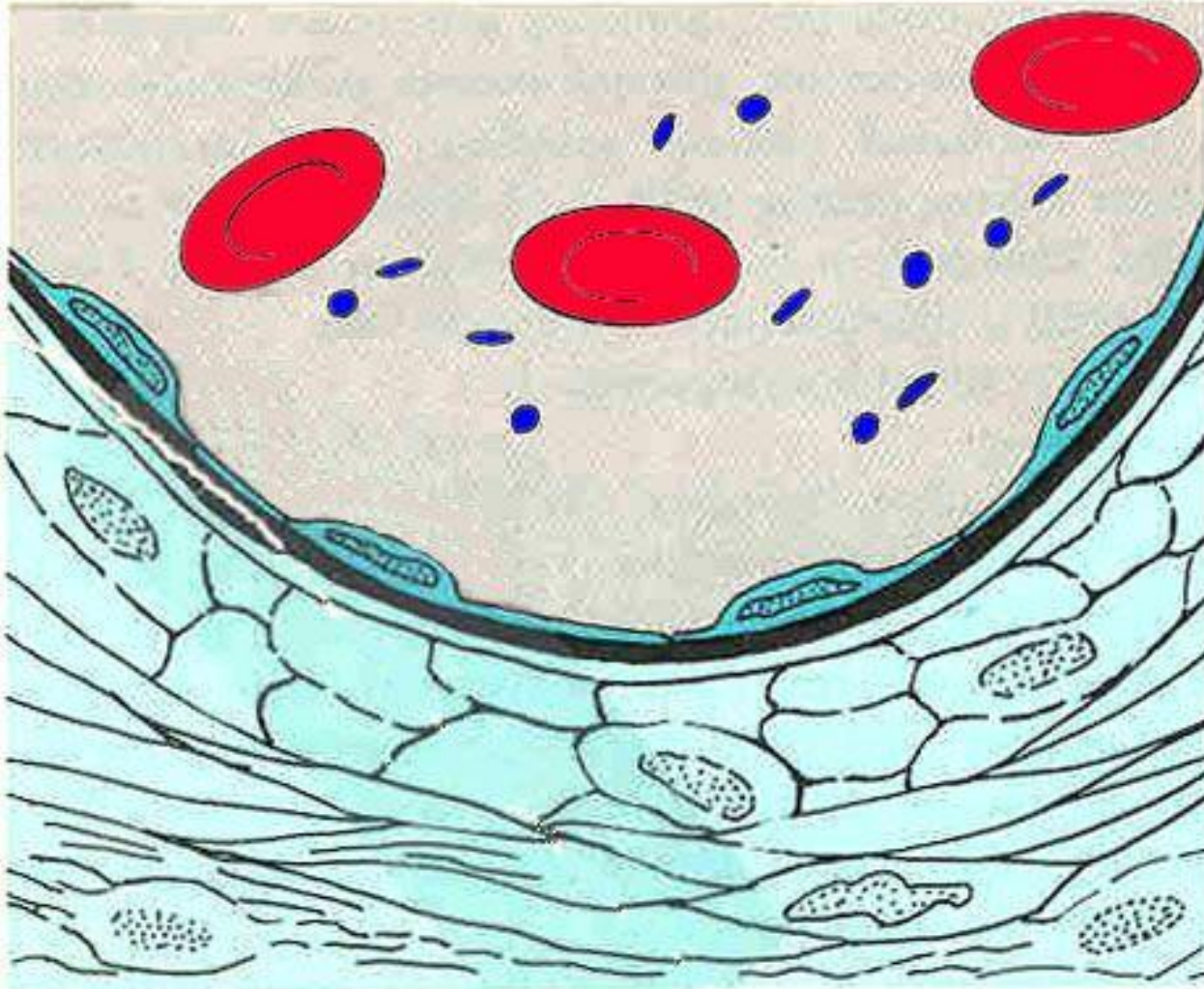


Resting platelets



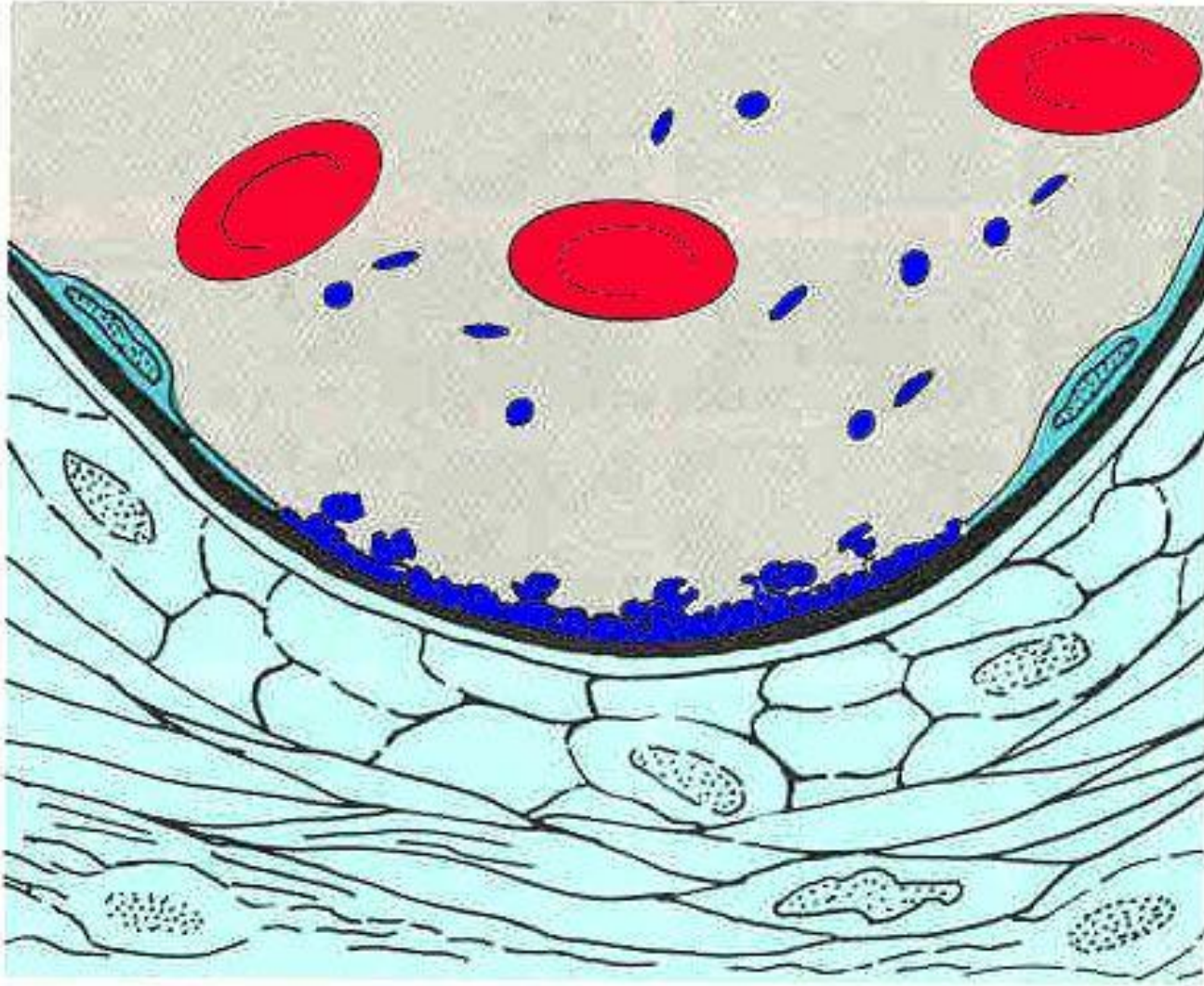
Activated platelets

Physiological Events Leading to Clot Formation (Thrombosis)



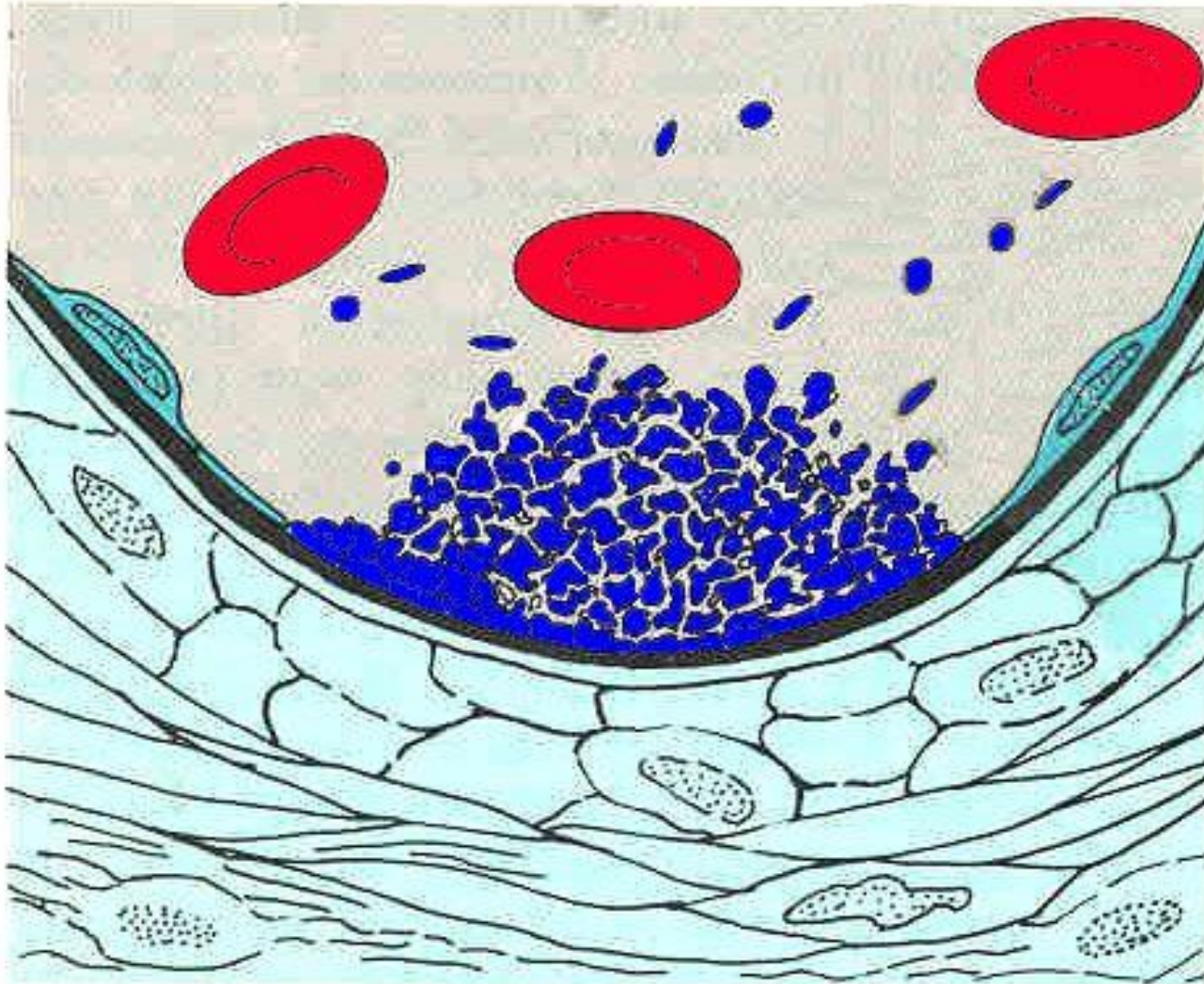
Normal blood vessel has continuous lining of endothelial cells.

Damaged Endothelial Cell Layer



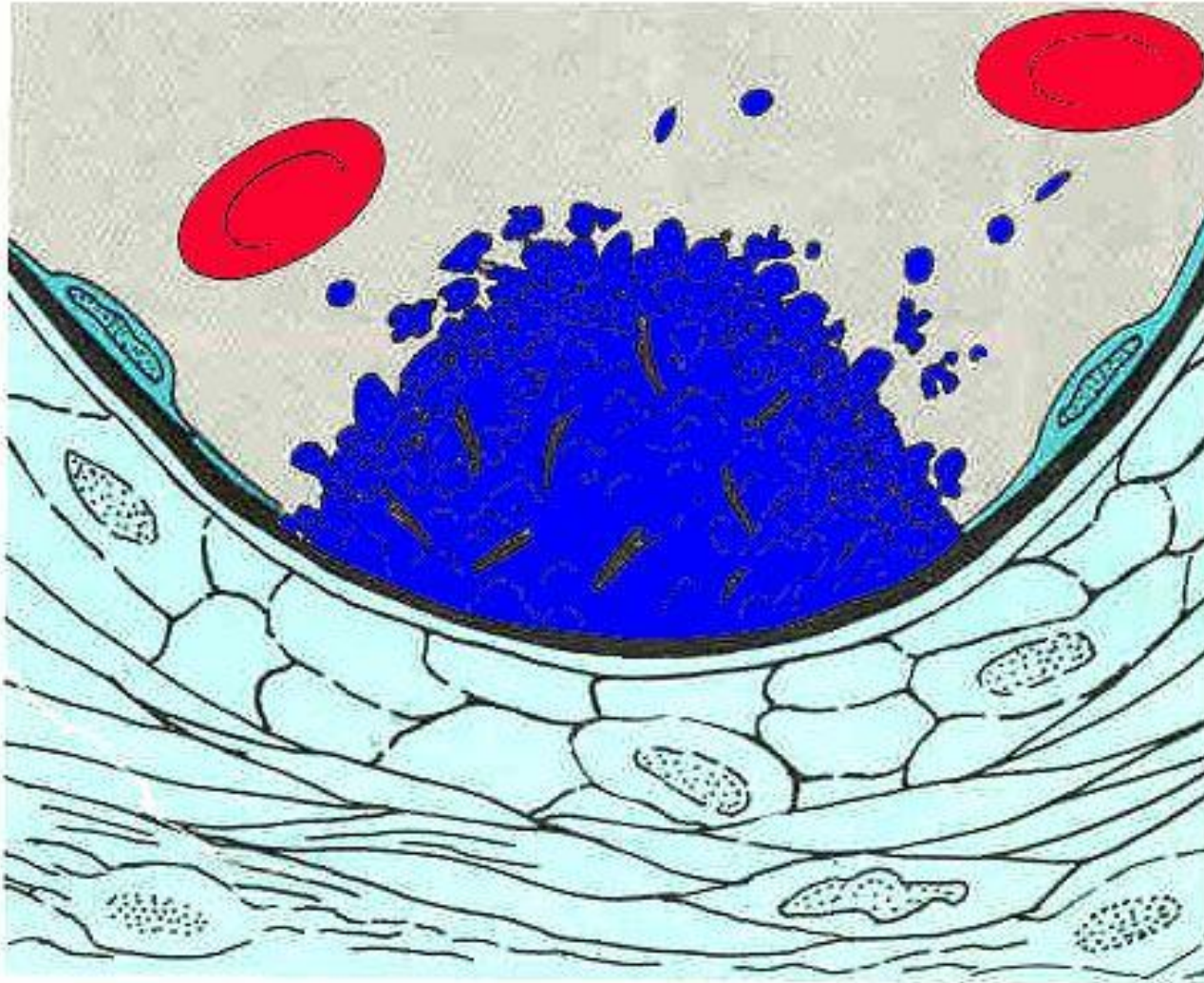
Platelets adhere at site of endothelial cell injury.

Platelet Plug Formation



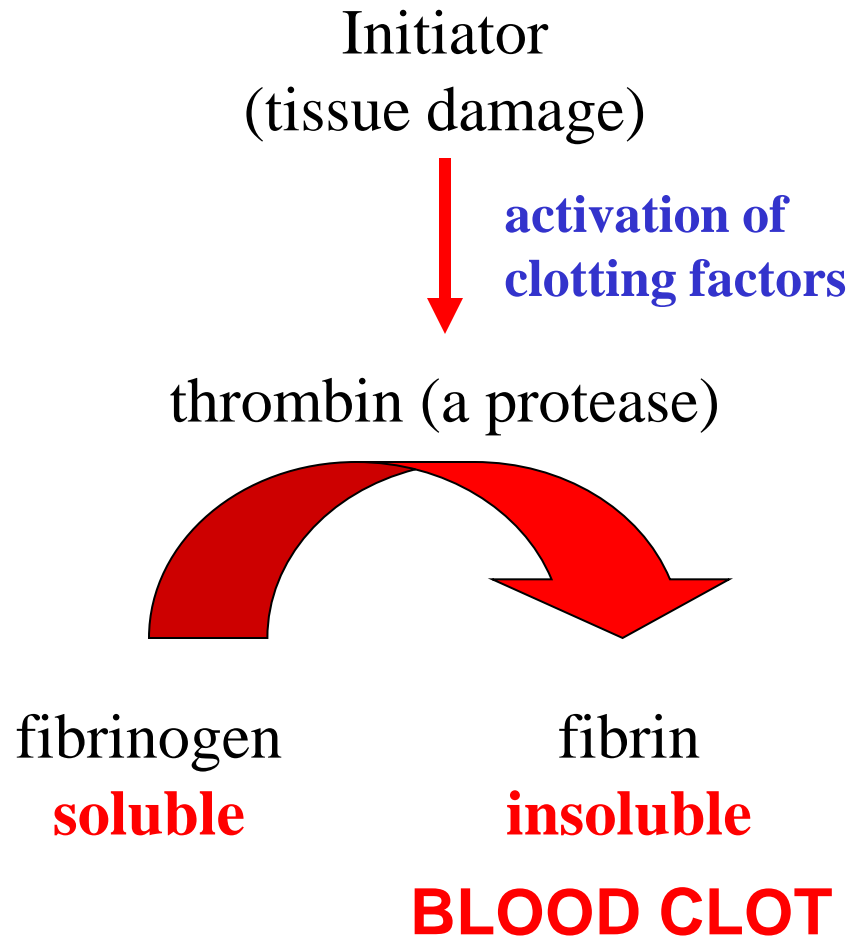
Platelet aggregation causes platelet mass to enlarge.

Fibrin Clot Formation



Fibrin deposition stabilizes the platelet thrombus.

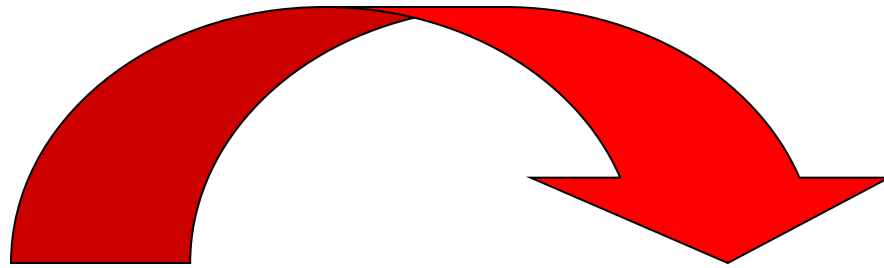
Blood Clot Formation



Blood Clot Dissolution

Fibrinolysis or Thrombolysis

plasmin (a **protease**)



fibrin polymer
insoluble
BLOOD CLOT

fibrin degradation products
soluble and cleared by liver

also called:

D_2E fragments or D-dimers
used as diagnostic marker of thrombosis

Challenges to Hemostasis

- Blood must remain fluid most of the time
- Blood clotting must occur rapidly
- Clot must be formed at site of injury
- Clot must be formed in the flowing blood
- Clot must be readily dissolved

Blood Clotting Cascade of Reactions

Intrinsic Pathway

Vascular injury Initiator

Tissue factor

Factor VII_a-Tissue factor

Factor VII-Tissue factor

Extrinsic Pathway

Factor XI

Factor XI_a

Factor IX

Factor IX_a

Factor IX

VIII_a

VIII

Factor X

Factor X_a

Factor X

Platelet membrane-bound

Common Pathway

Prothrombin

V_a

V

Fibrinogen

Thrombin

Initiator

Fibrin

Factor XIII

Factor XIII_a

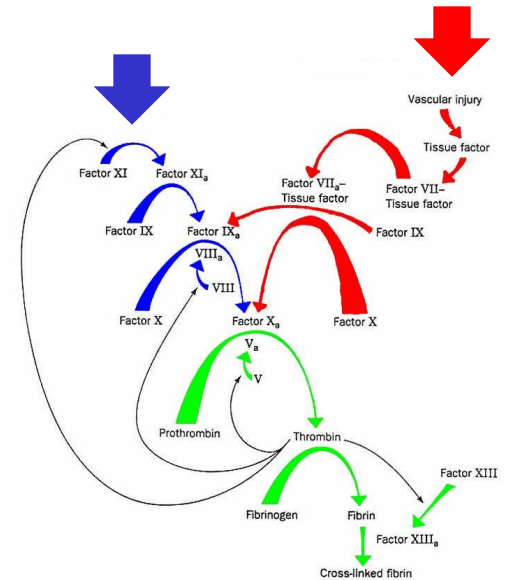
Cross-linked fibrin

Blood Clot

Propagation

Initiation of Cascade

- *in vivo*, clotting is initiated by **tissue damage**
- the released **tissue factor** activates **factor VII**
- used in the Prothrombin Time Assay & INR
- measures the **Extrinsic Pathway** and **Common Pathway**
- *in vitro*, clotting can be initiated by **negatively charged surfaces**
- glass or kaolin (a type of clay) activate factor XII
- used in the Activated Partial Thromboplastin Time Assay
- measures the **Intrinsic Pathway** and **Common Pathway**



What Stops Circulating Blood from Clotting?

- most clotting factors circulate in blood as inactive forms (zymogens)
- many are activated by **proteases** that cleave only 1-2 bonds of **zymogen** so that activation is very rapid

prothrombin (zymogen)	→	thrombin (protease)
factor IX (zymogen)	→	factor IXa (protease)
factor VIII (zymogen)	→	factor VIIIa (binding protein)

Clotting Factors

- many are **proteases** that cleave specific proteins:

thrombin (cleaves **fibrinogen**)

factor IXa (cleaves **factor X**)

- some are binding proteins:

factor VIIIa, factor Va

deficiency of either type of protein gives impaired clotting:

factor IX deficiency – Hemophilia B or Christmas Disease

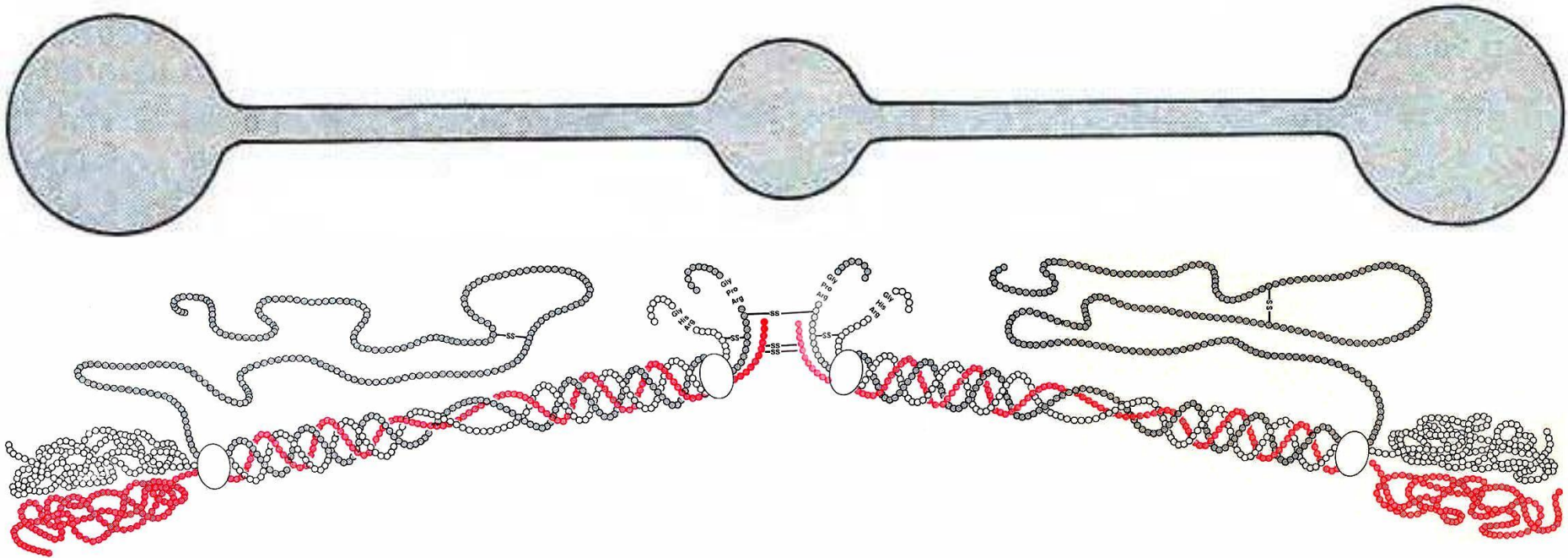
factor VIII deficiency – Hemophilia A or Classical Hemophilia

Fibrinogen

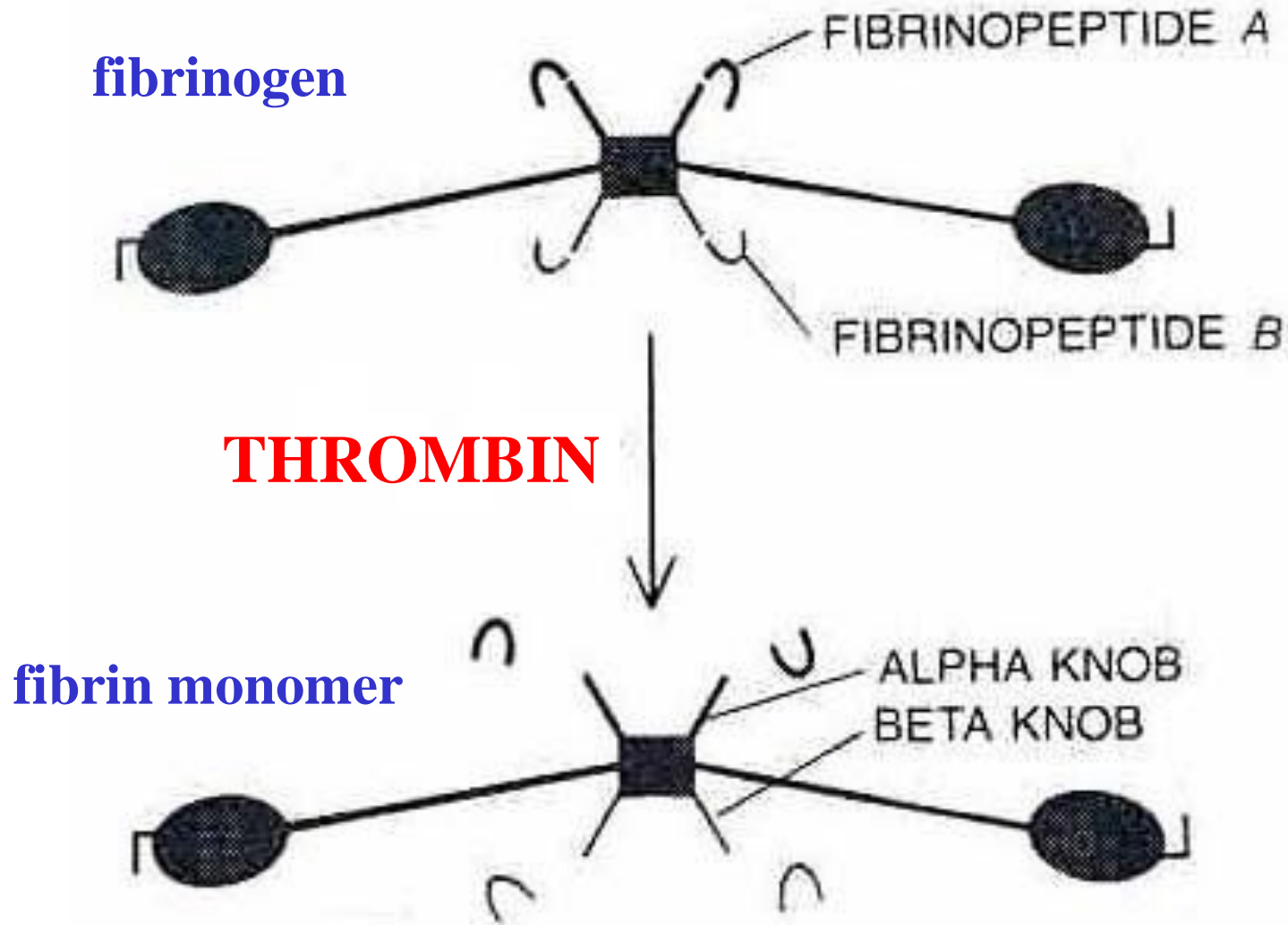
very complex protein made up of 6 polypeptides: $\alpha_2\beta_2\gamma_2$

shaped like a dumbbell

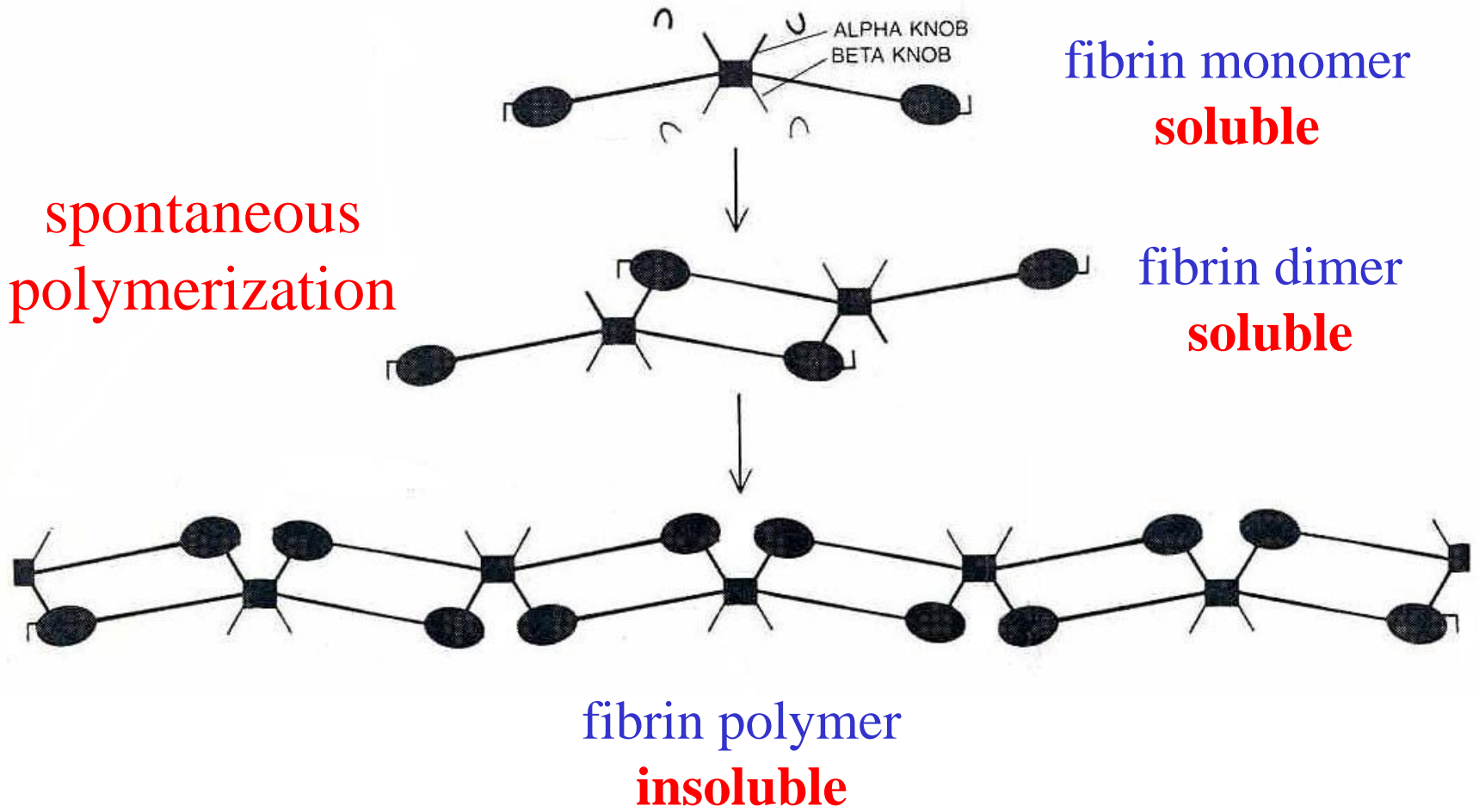
three globular domains are linked by triple helixes



Fibrin Formation: **Proteolysis**

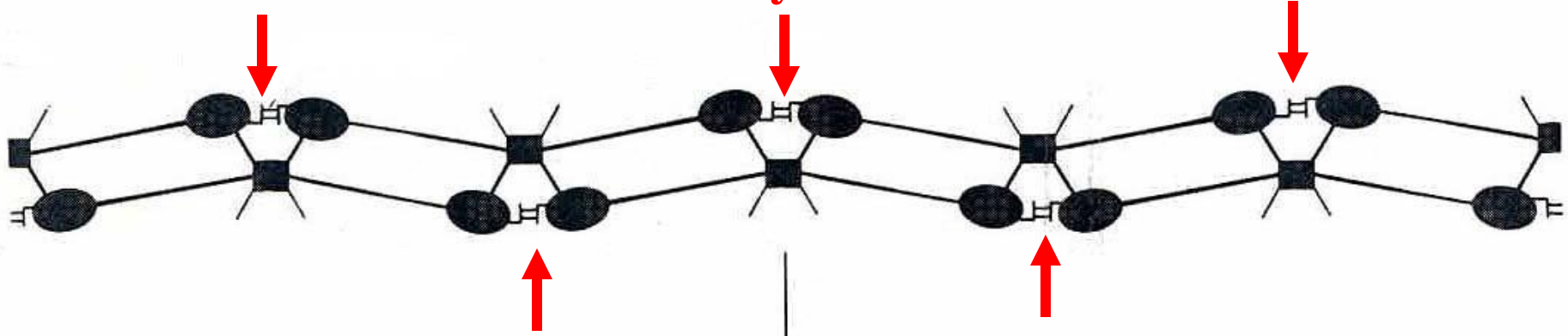


Fibrinogen Activation: Polymerization

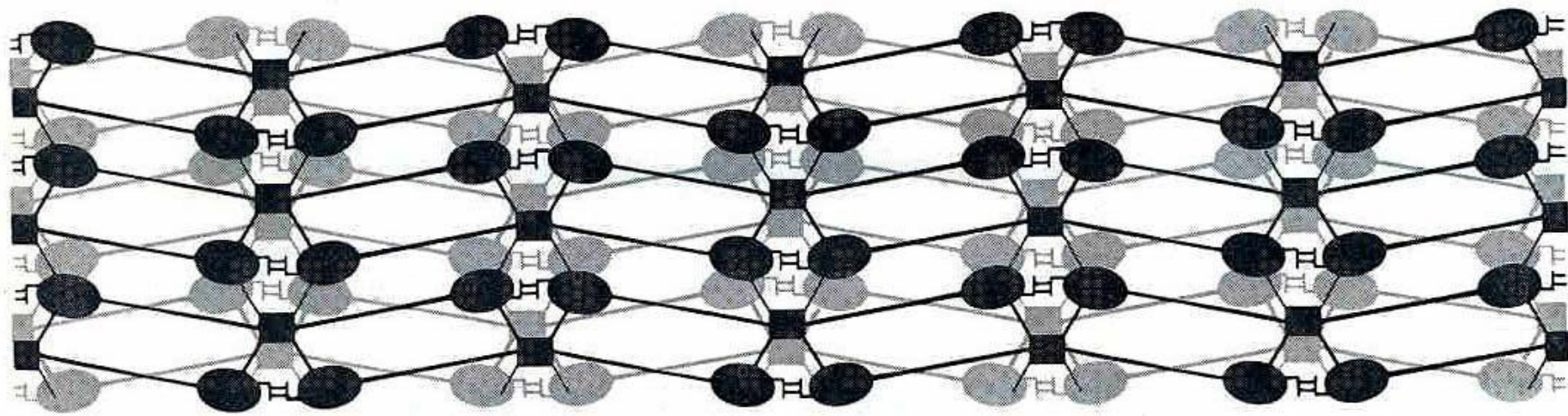


Fibrinogen Activation: **Cross-Linking**

introduced by factor XIIIa



Factor XIIIa introduces a covalent bond between Lys and Gln residues



fXIIIa also cross-links fibrin clot to surrounding tissue

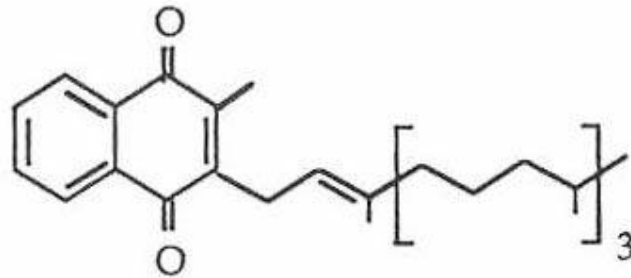
Fibrin Clot

© Dennis Kunkel Microscopy Inc.



Vitamin K

some clotting factors require vitamin K-dependent modification during their biosynthesis in the liver to be biologically active

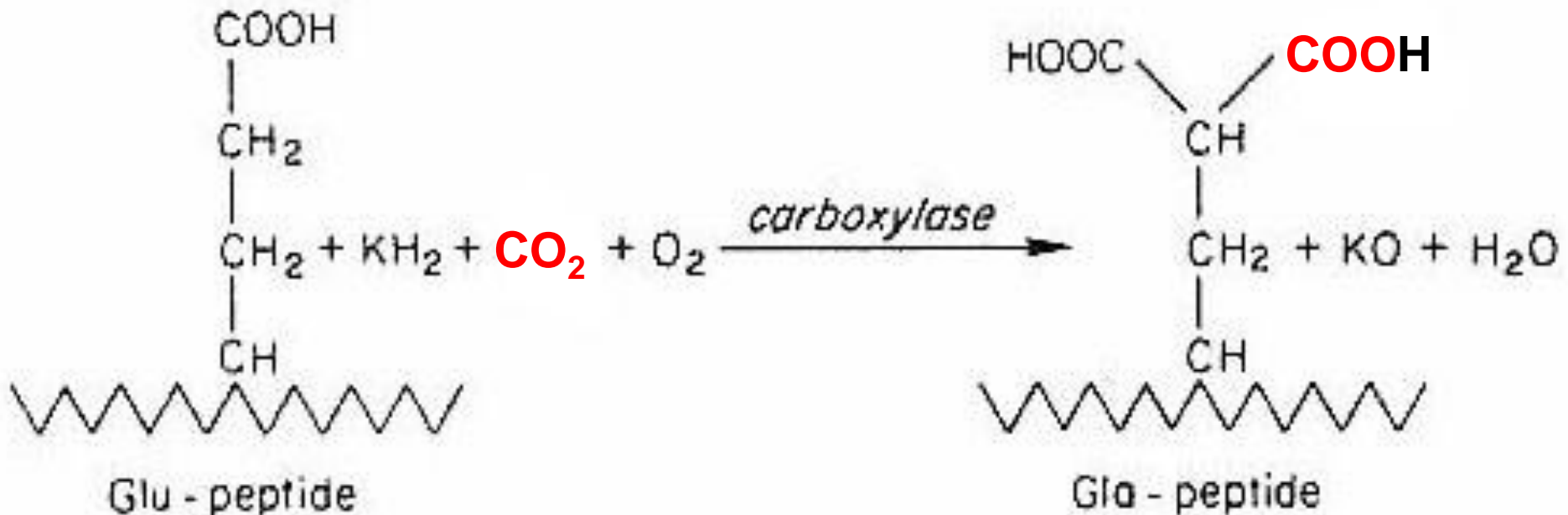


2-methyl-3-phytyl-1,4 naphthoquinone (vitamin K₁)

- vitamin K is a fat-soluble vitamin found in vegetable oils and green leafy vegetables – also synthesized by the gut flora
- normally, gut bacterial synthesis is sufficient
- in newborns (who have a poor gut flora), injection of vitamin K is normally given shortly after birth

Vitamin K-dependent carboxylation

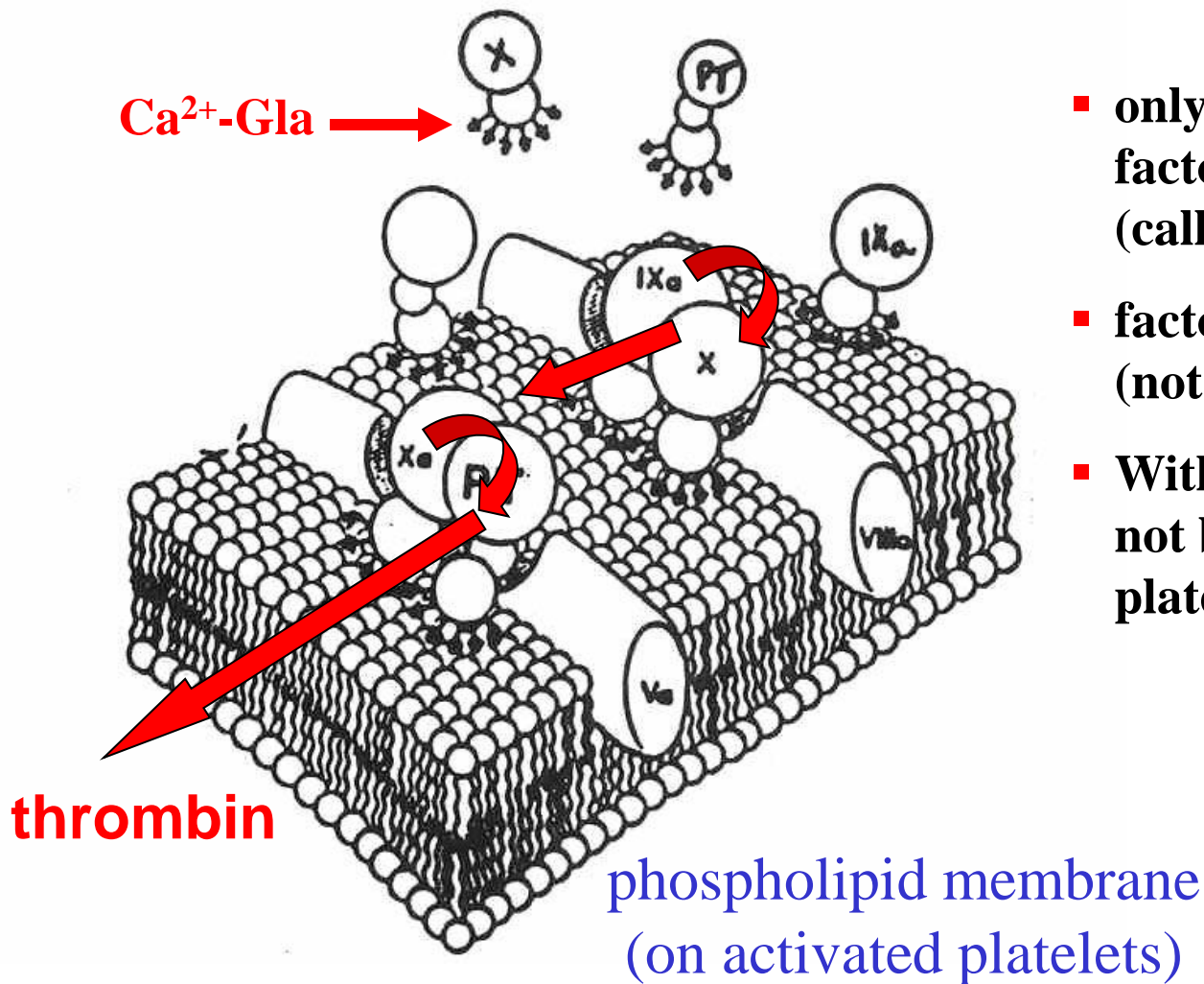
in the vitamin K-dependent clotting factors, some glutamic acid residues are carboxylated to γ -carboxyglutamic acid (Gla)



Gla proteins change shape

- Gla binds calcium ions leading to a shape change
- Ca^{2+} -Gla-proteins can bind to phospholipid membranes (supplied by activated platelets at the site of injury)
- ensures that fibrin formation occurs at the injury site instead of in the flowing blood
- the Gla is absolutely required for the membrane binding
- non-carboxylated vitamin K-dependent proteins are **biologically inactive**

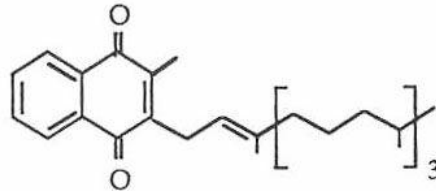
Gla-proteins bind to the Platelet Plug



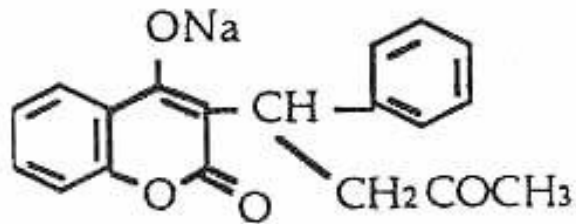
- only Ca^{2+} -Gla proteins bind: factor VII, X, IX, prothrombin (called the vitamin K proteins)
- factors Va and VIIIa also bind (not via Gla though)
- Without Gla, the proteins do not bind to the activated platelet membrane

Effect of Coumarol Drugs

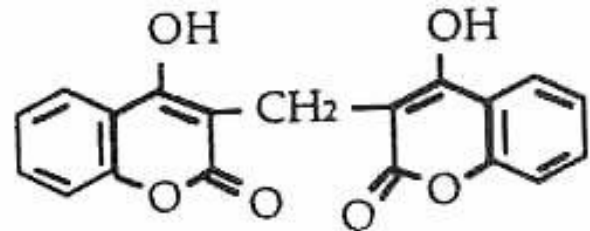
dicoumarol discovered when cows bled after eating sweet clover



2-methyl-3-phytyl-1,4 naphthoquinone (vitamin K₁)



Warfarin (sodium salt)



Dicoumarol

- Analogs of vitamin K - warfarin is a synthetic version
- Coumarol drugs inhibit the action of vitamin K in the liver
- Non-Gla cannot bind to the membrane at the site of injury
- Clotting is inhibited
- Reversed by vitamin K
- Long term anticoagulants that work on liver synthesis

Overview of Clotting

PROCOAGULATION



ANTI-COAGULATION

Thrombin production
leading to fibrin formation

Inhibition of **thrombin**
production

Termination of Clotting

- dilution in the flowing blood
- trapping of clotting factors in growing fibrin clot
- plasma protease inhibitors such as antithrombin

binds to active site of thrombin and forms inactive complex
antithrombin deficiency leads to risk of thrombosis

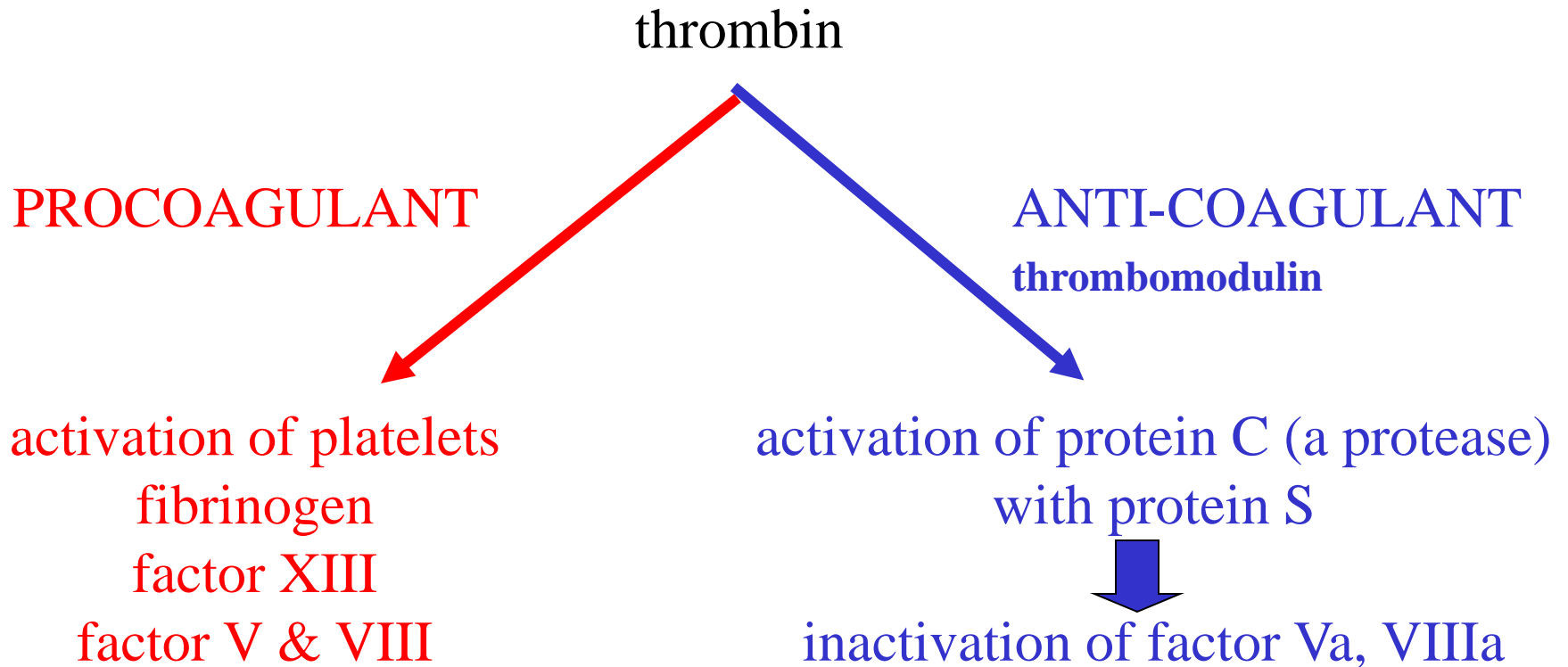
heparin accelerates the antithrombin-thrombin reaction

heparin is an anticoagulant

- thrombomodulin - protein C - protein S pathway

Thrombomodulin - Protein C - Protein S Pathway

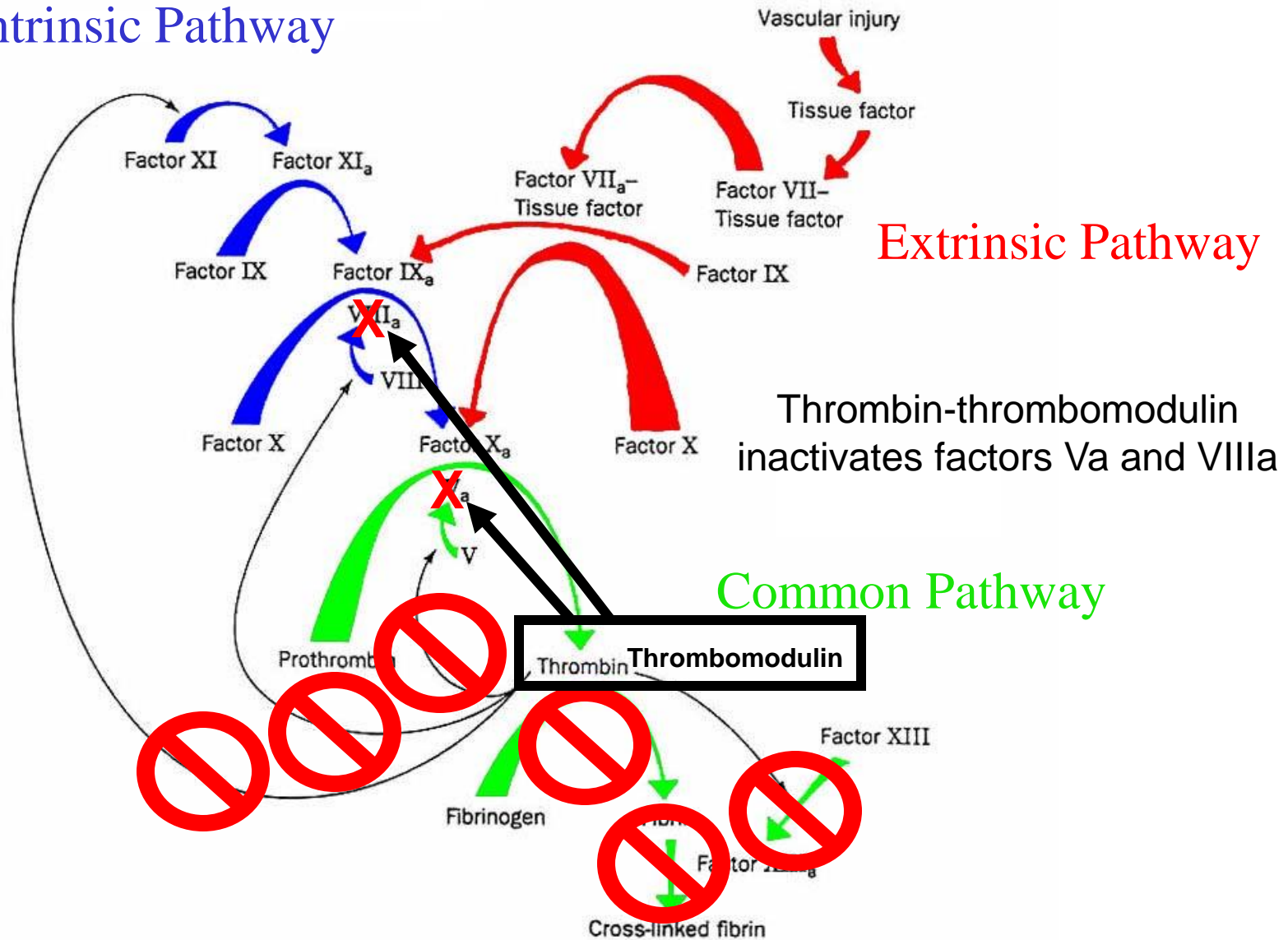
thrombomodulin appears on endothelial cell plasma membranes
converts thrombin from a **procoagulant** to an **anti-coagulant**:



Modulation of Thrombin Activity

Pro-coagulant to Anti-coagulant

Intrinsic Pathway



Anticoagulants

- metal chelators such as EDTA and citrate

strip Ca^{2+} from Gla proteins and their shape changes
non- Ca^{2+} Gla proteins cannot bind to phospholipids
reversible by adding Ca^{2+} - anticoagulant for blood collection

- coumarol drugs such as Warfarin

inhibit liver carboxylation of Gla proteins
cannot be reversed as non-Gla proteins appear in blood plasma
replacement of drug with vitamin K gives new liver protein synthesis
used clinically in thrombosis-risk patients

- heparin

affects thrombin by accelerating antithrombin inactivation
used clinically for medium term anticoagulation

Overview of Clotting

PROCOAGULATION



ANTI-COAGULATION

Thrombin production
leading to fibrin formation

Inhibition of **thrombin**
production

What can cause bleeding?

Too little clotting

Lack of clotting factors

Factor VIII – hemophilia A

Factor IX – hemophilia B

Too much anticoagulation

Heparin, coumarol drugs

What can cause thrombosis?

Too much clotting

Stasis

Immobility (including anesthesia, long leg fracture or other limb immobilization, paralysis)

Acquired

Cigarette smoking, birth control pill, surgery, malignancy

Unknown

???

What can cause thrombosis?

Too much clotting

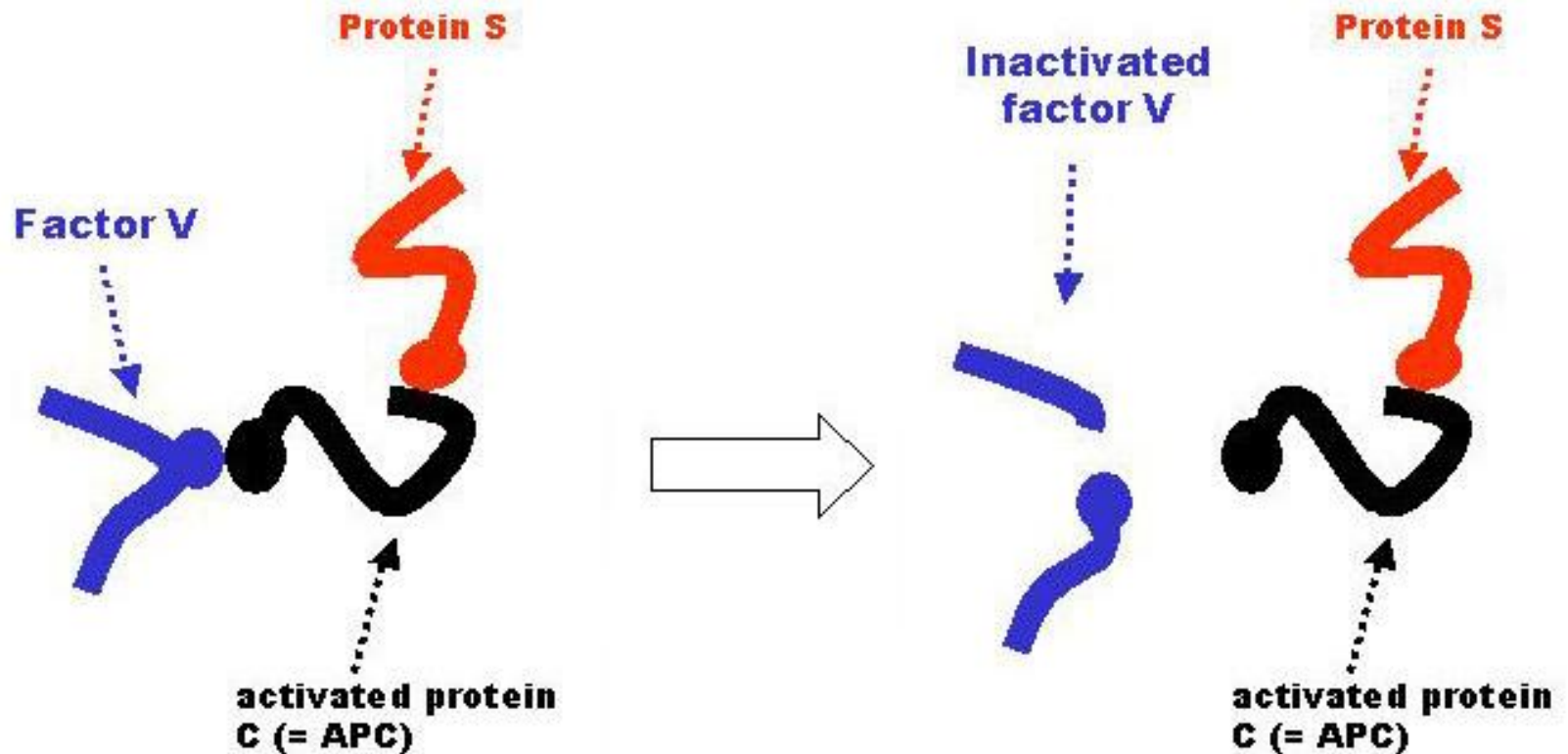
Inherited deficiency of anticoagulant protein

Antithrombin deficiency or protein C deficiency

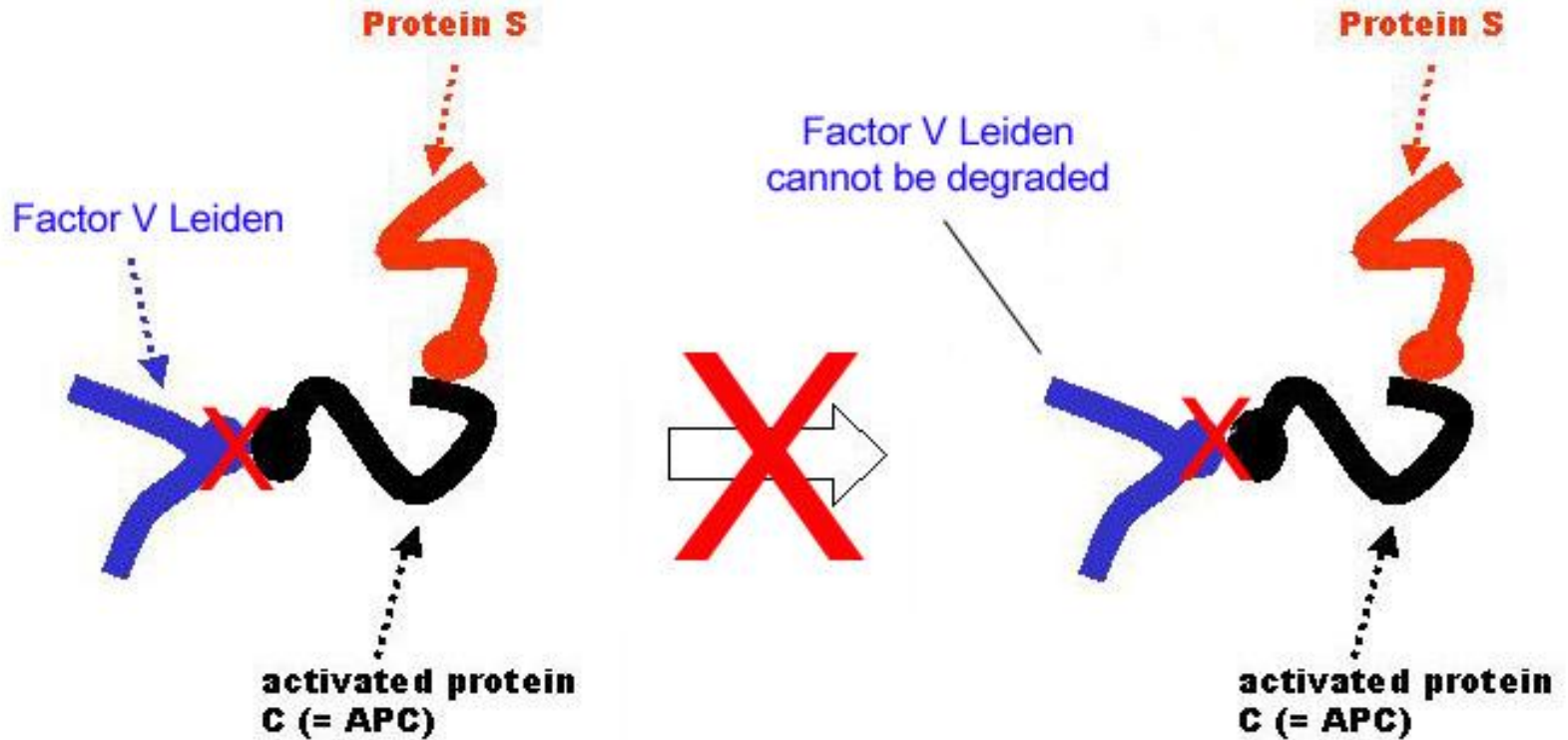
Mutation in procoagulant protein

Factor V Leiden

Normal Factor V Inactivation



Factor V Leiden



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© Stephan-Hölz, M.D.

Factor Va Leiden remains active so more thrombin is produced

Overview of Clotting & Thrombolysis

CLOTTING

tissue damage leading to
fibrin clot formation



THROMBOLYSIS

tissue repair and fibrin
clot dissolution

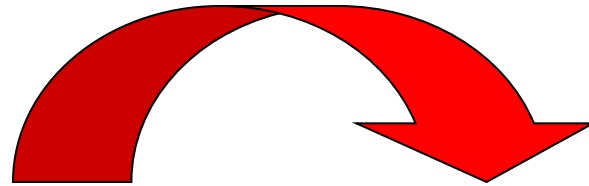
Plasminogen Activation

Plasminogen activator (a protease)

Tissue-type plasminogen activator (tPA)

Urokinase (uPA)

Streptokinase/Staphylokinase (bacterial)

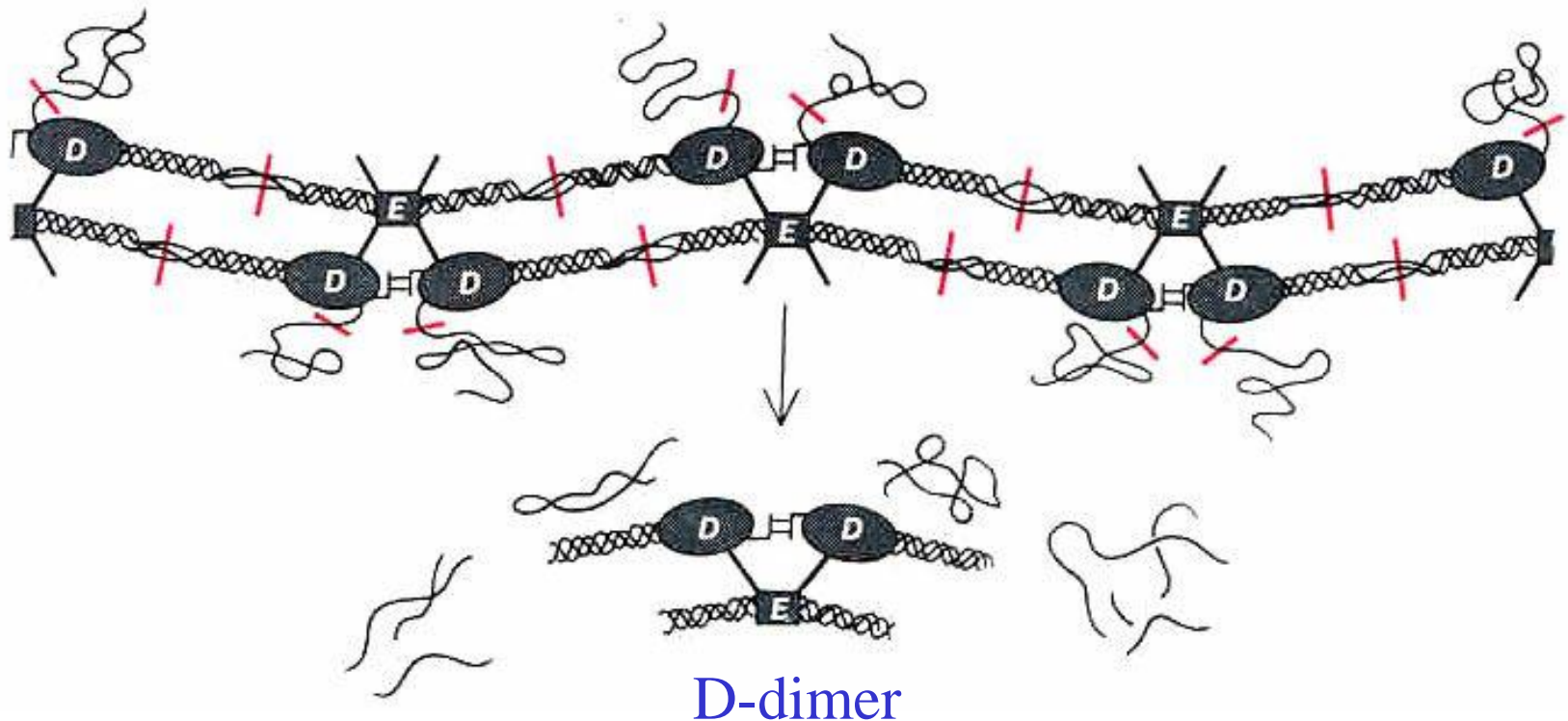


Plasminogen
(Inactive)

Plasmin
(Enzyme)

Fibrinolysis / Thrombolysis

plasmin (a protease) cleaves the triple helix of the fibrin polymer
D-dimers (D₂E fragments, fibrin degradation products) are soluble
and cleared by the liver (diagnostic marker for thrombosis)



Meeting the Challenges to Hemostasis

- Blood must remain fluid most of the time
 - Clotting factors circulate as inactive zymogens
- Blood clotting must occur rapidly
 - clotting factor zymogens are activated by cleavage of 1-2 bonds
- Clot must be formed at site of injury
 - Clotting occurs where tissue factor is released and platelet plug occurs
- Clot must be formed in the flowing blood
 - Clotting factor complexes assemble on platelet plug membrane
- Clot must be readily dissolved
 - Plasmin cleaves few bonds in fibrin to dissolve fibrin clot

Laboratory Tests

Initiate clotting at a specific place

Measure time it takes for plasma to clot

Compare to pooled plasma from population

Activated Partial Thromboplastin Time

blood + citrate

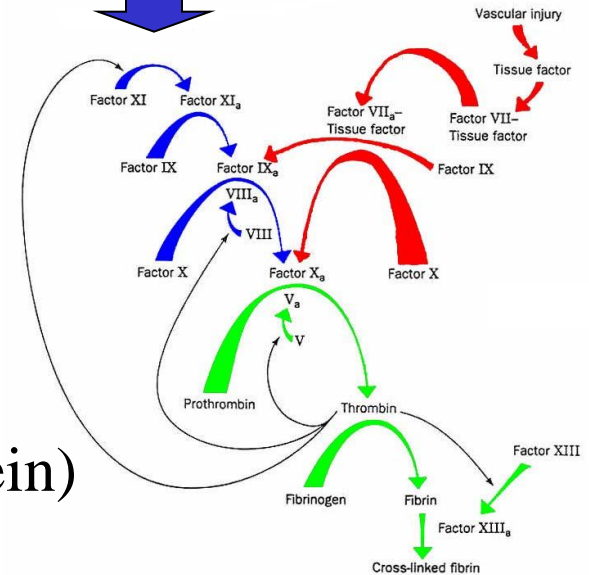
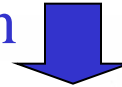


plasma

1. incubate with kaolin
2. add partial thromboplastin (phospholipid but no protein)
3. add Ca^{2+}

measure clotting time
~ 24-37 s but varies

activation



measures **intrinsic** and
common pathways

Prothrombin Time

International Normalized Ratio (INR)

blood + citrate

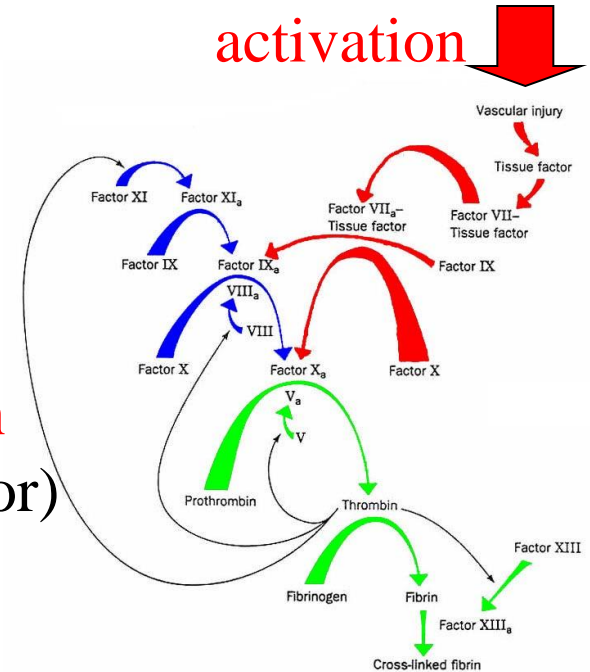


plasma

1. incubate with **thromboplastin** (phospholipid and tissue factor)
2. add Ca^{2+}

measure clotting time
~ 10-13 s but varies

activation



measures **extrinsic** and **common** pathways

International Normalized Ratio (INR)

- Prothrombin time (in seconds) for a normal individual will vary with the type of analytical system employed.
- Due to the variations between different batches of tissue factor used in the reagent to perform the test
- The INR was devised to standardize the results
- Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture
- ISI value indicates how a particular batch of tissue factor compares to an internationally reference tissue factor
- ISI is usually between 1.0 and 2.0

International Normalized Ratio (INR)

$$\text{INR} = \left(\frac{\text{PT}_{\text{TEST}}}{\text{PT}_{\text{NORMAL}}} \right)^{\text{ISI}}$$

For normal individuals, $\text{INR} = 1$

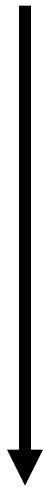
Individuals on coumarol drugs, $\text{INR} > 1$

Thrombin Time

blood + citrate



plasma

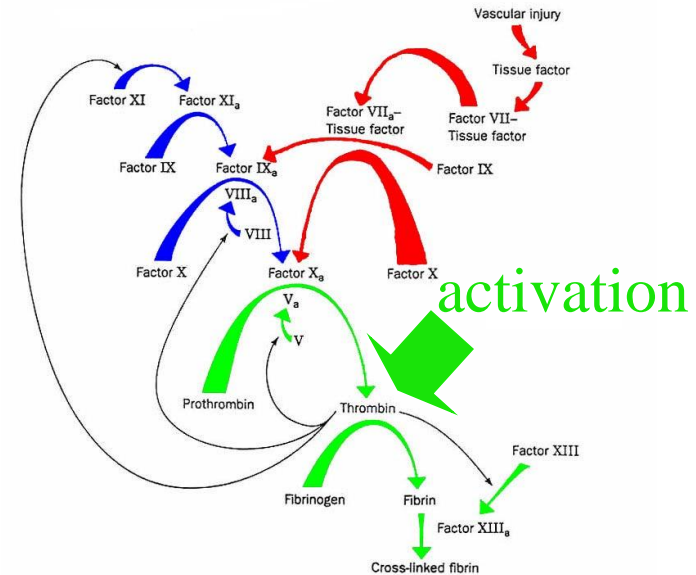


measure clotting time

~ 9-11 s but varies

incubate with thrombin

measures fibrinogen function only



Conclusions

Hemostasis involves blood vessels, platelets and blood proteins

Blood coagulation occurs quickly locally – procoagulant phase

Blood coagulation is inhibited – anti-coagulation phase

Tissue damage is repaired and blood clot is removed (thrombolysis)

Inherited or acquired conditions that upset the balance can cause bleeding problems (hemophilias) or excess blood clots (thrombosis)

Commonly used laboratory tests can determine the procoagulant/anti-coagulant status of a patient's blood sample