Platelets and Hemostasis: Part 3

PHYSIOLOGY 210

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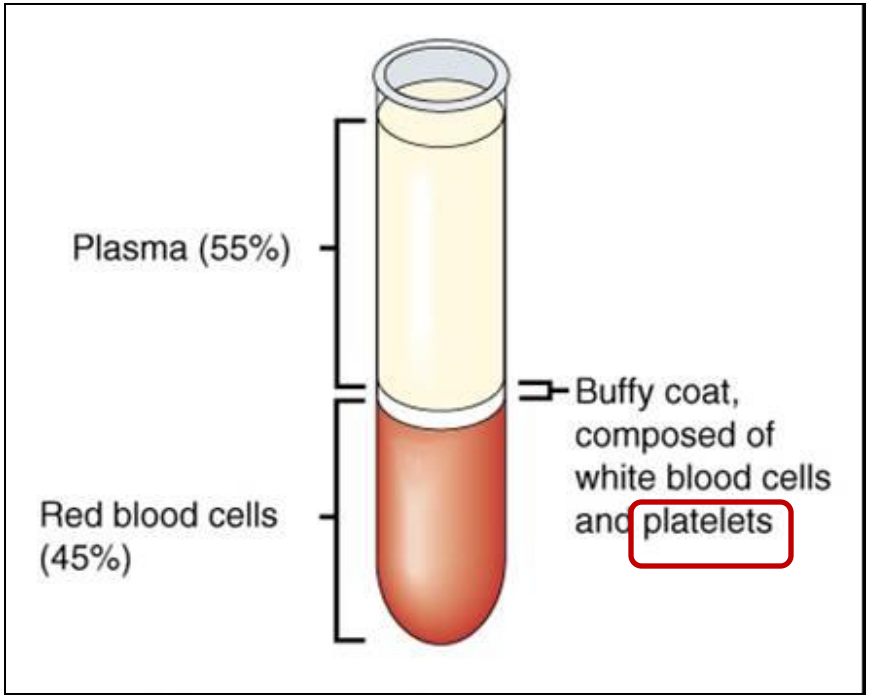
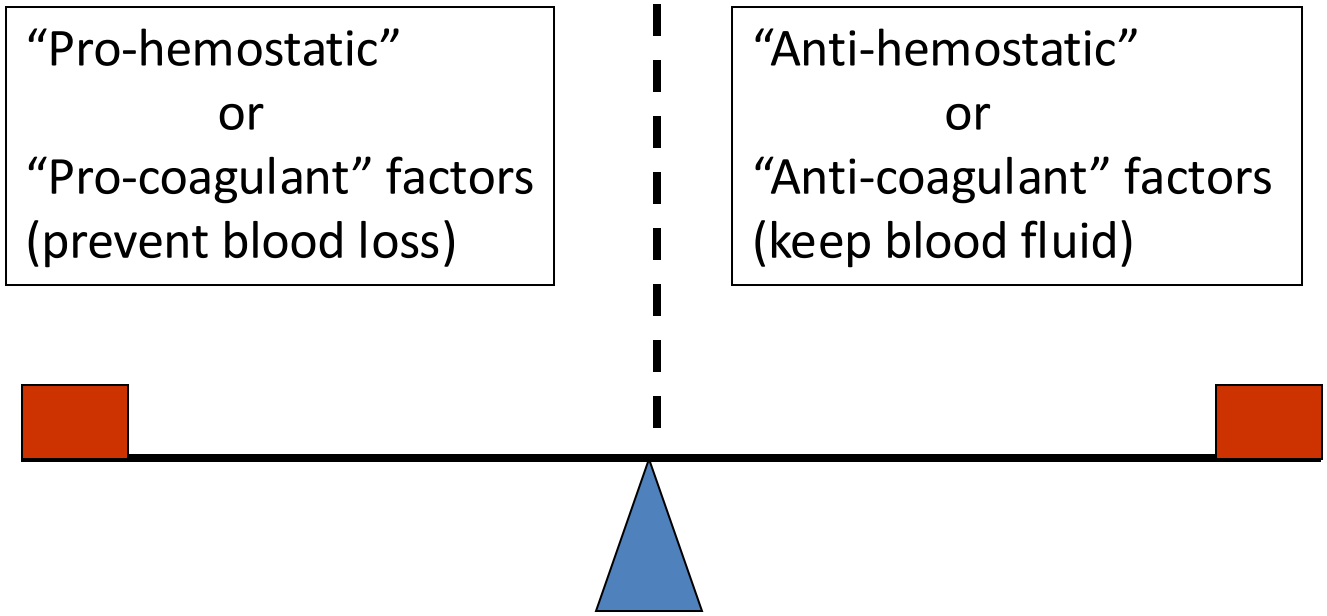
Introduction to hemostasis and platelets: Part 3a
Topic outline

- What is hemostasis?
  - (What is homeostasis?)
- Why is hemostasis necessary?
- Where does hemostasis occur?
- How is hemostasis achieved?
- What role do platelets play?



Why is hemostasis necessary?



Key steps of hemostasis

1. Vasoconstriction
2. Primary hemostasis or **platelet plug** formation ("white thrombus")
3. Secondary hemostasis or **blood clotting** or blood coagulation ("red thrombus")

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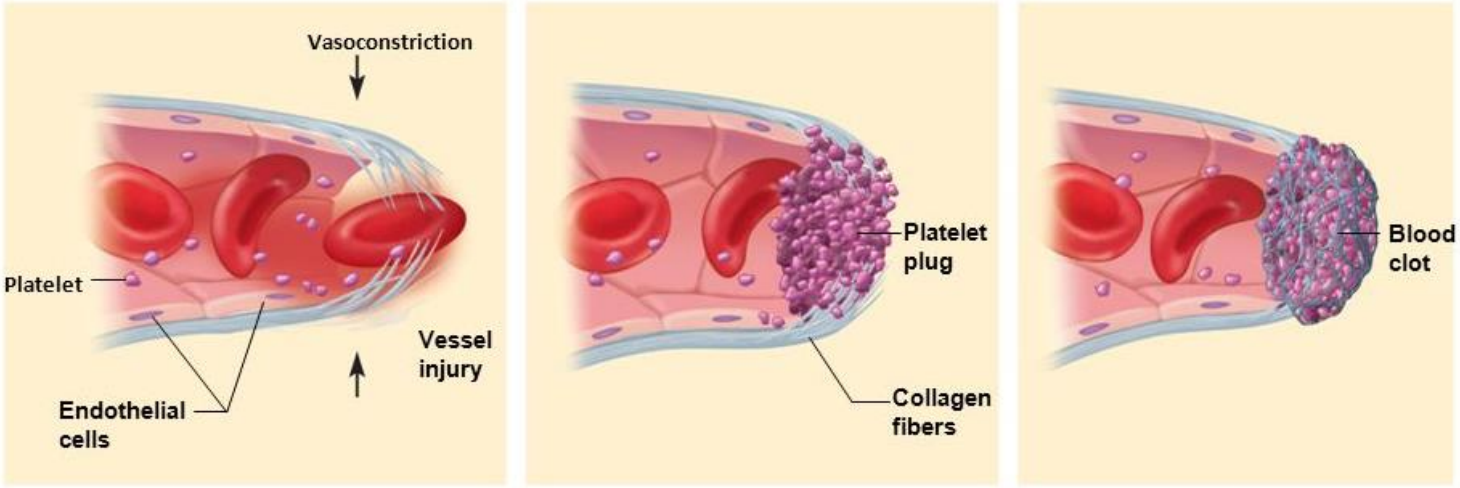
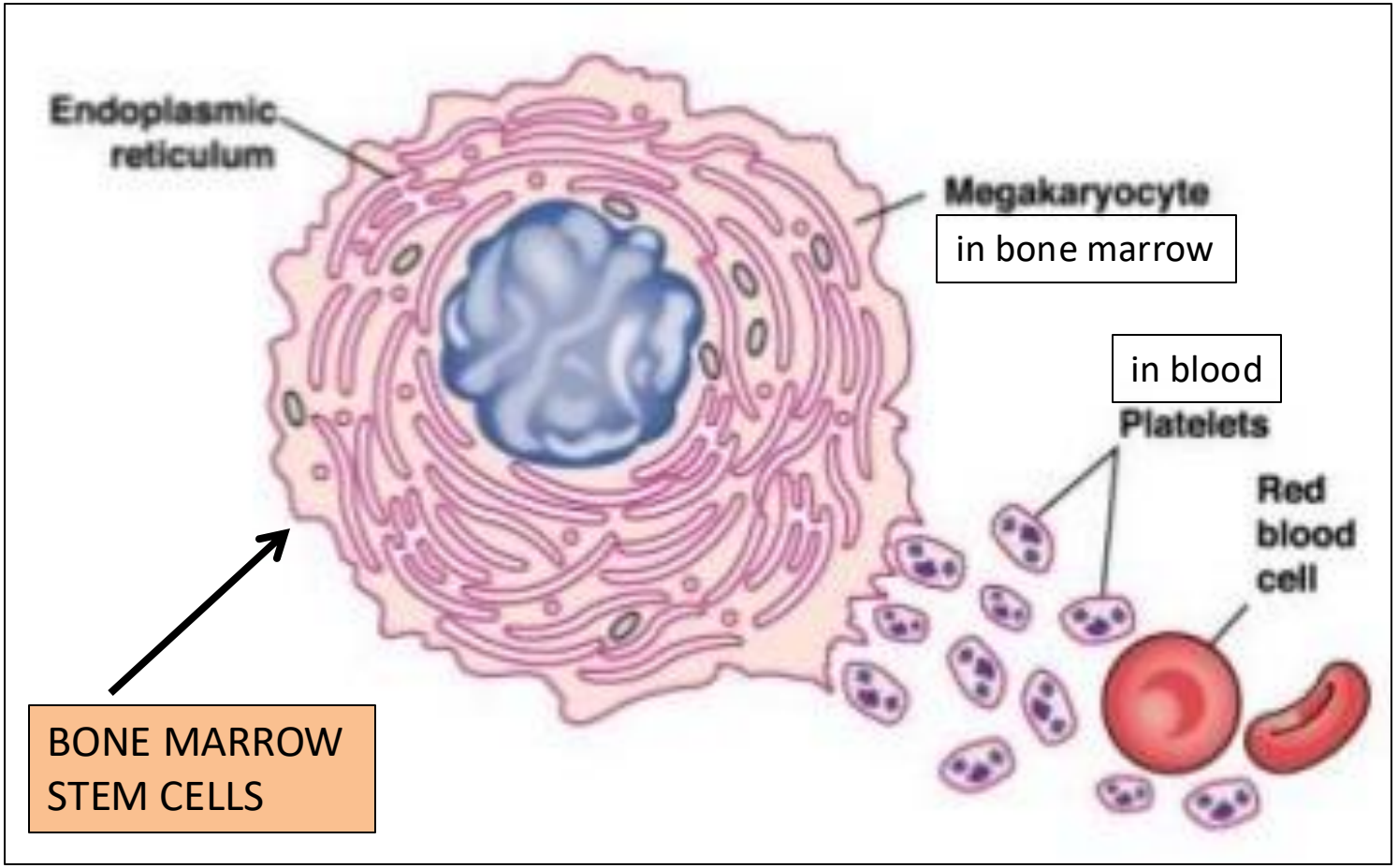
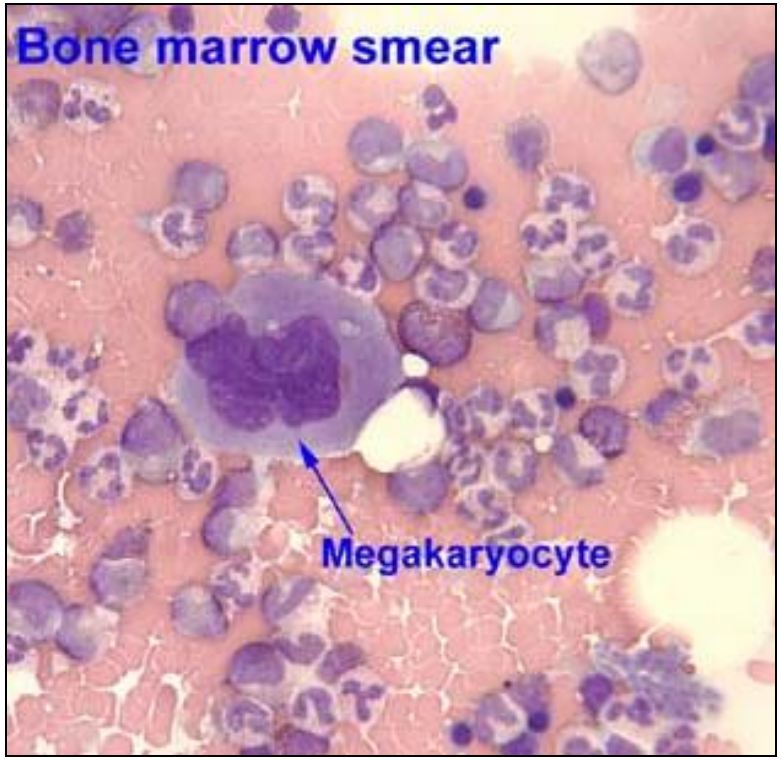
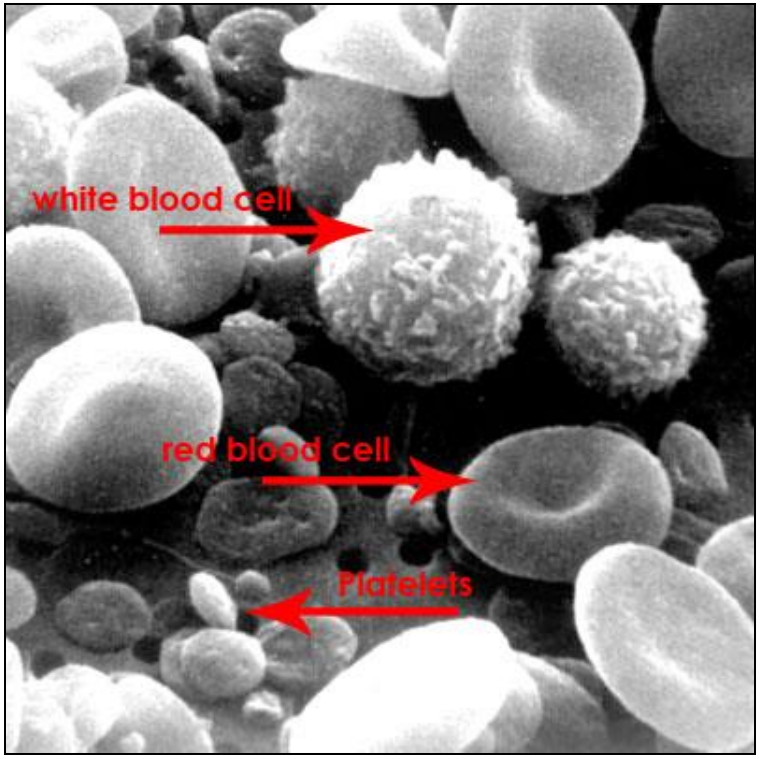


Where do platelets come from?



Megakaryocytes and platelets





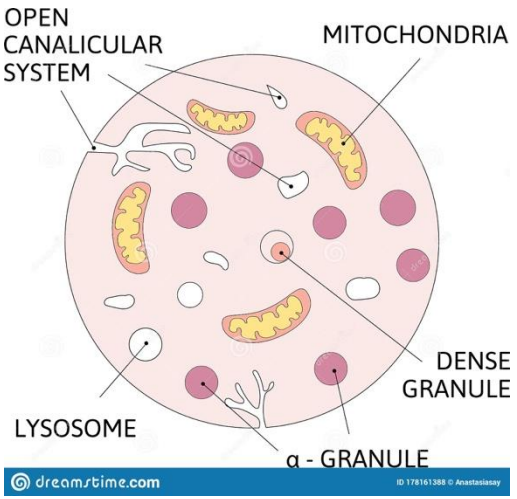
http://www.ouhc.edu/histology

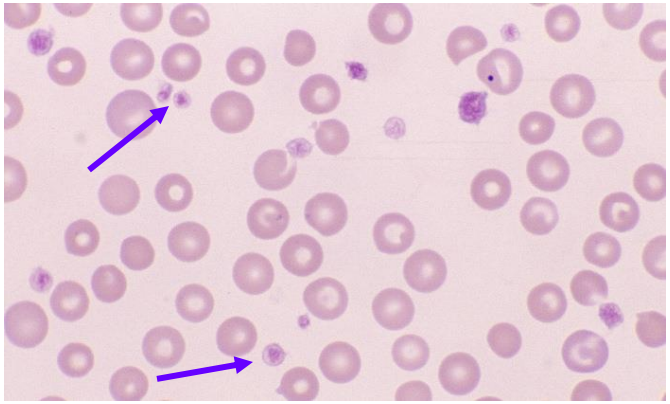
Key words: Part 3 a

- Hemostasis, homeostasis
- Buffy coat
- Platelet plug, white thrombus, primary hemostasis
- Blood clot, red thrombus, secondary hemostasis
- Megakaryocytes, platelets

Platelet structure and function: Part 3 b
Topic outline

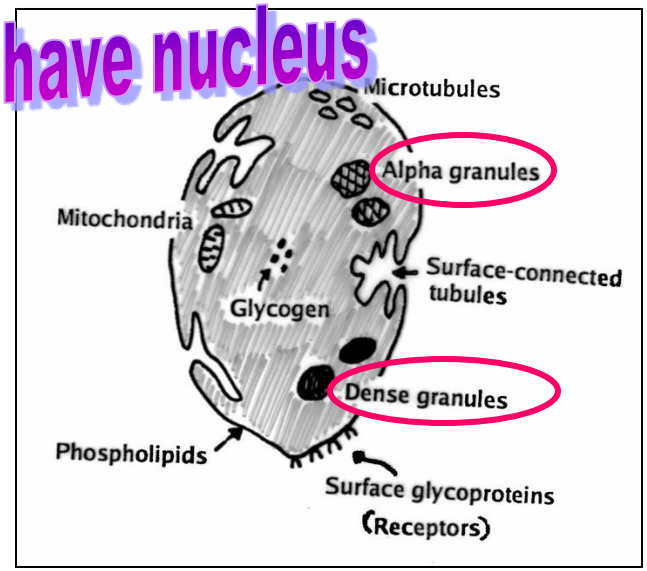
- Structure of platelet
- Platelet granules and their contents
- Formation of platelet plug
- Role of <u>activated</u> platelets

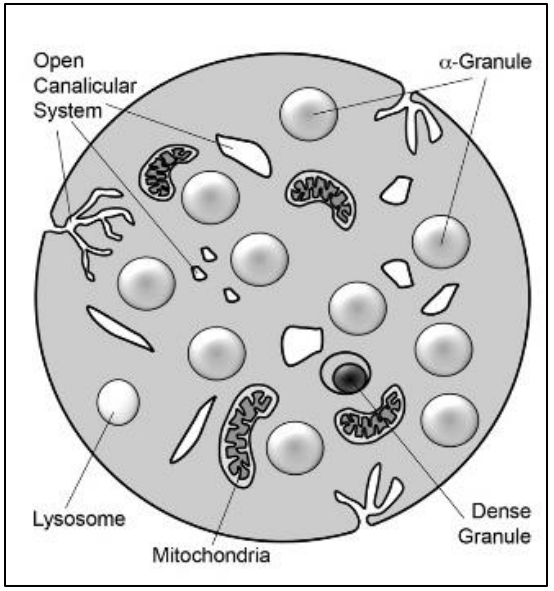


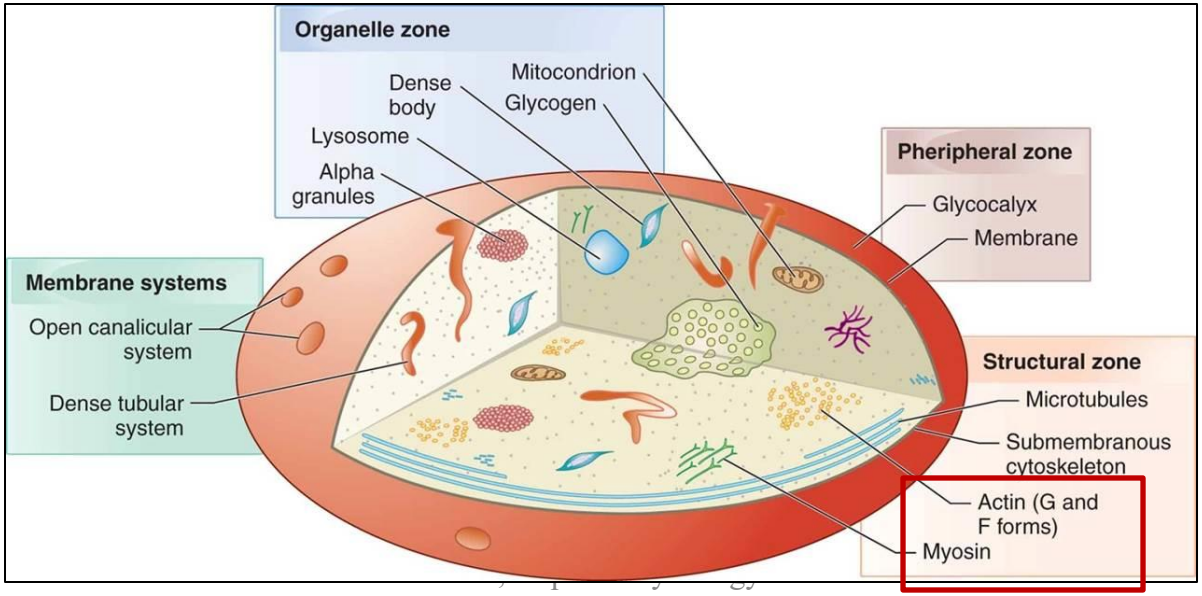


Structure of platelets

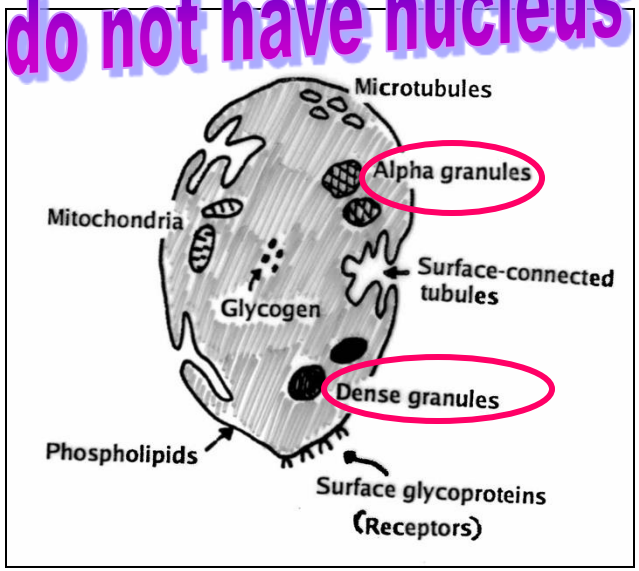
do not have nucleus



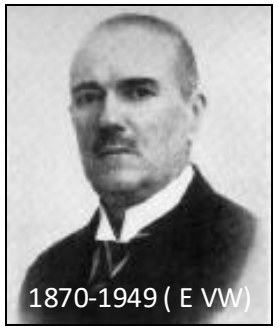


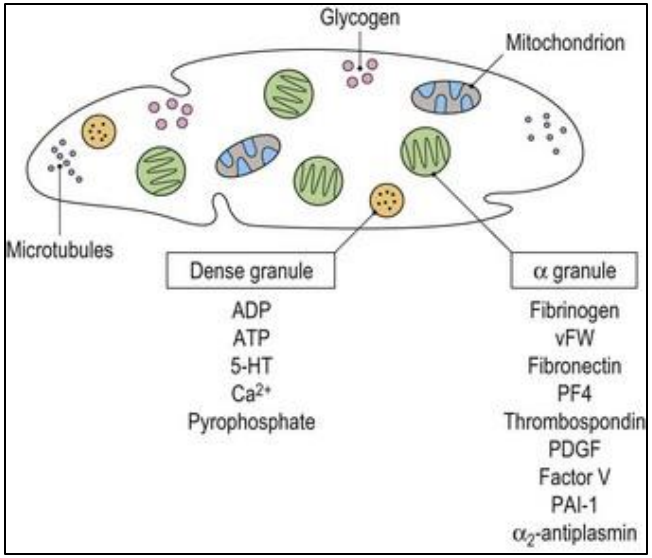


Content of platelet granules



1. Alpha granules contain relatively large molecules:
   - Adhesion molecules such as von Willebrand factor (vWF)
   - Growth factors
   - Some clotting factors
   - Cytokines





2. Dense granules contain relatively small molecules

   ADP and ATP

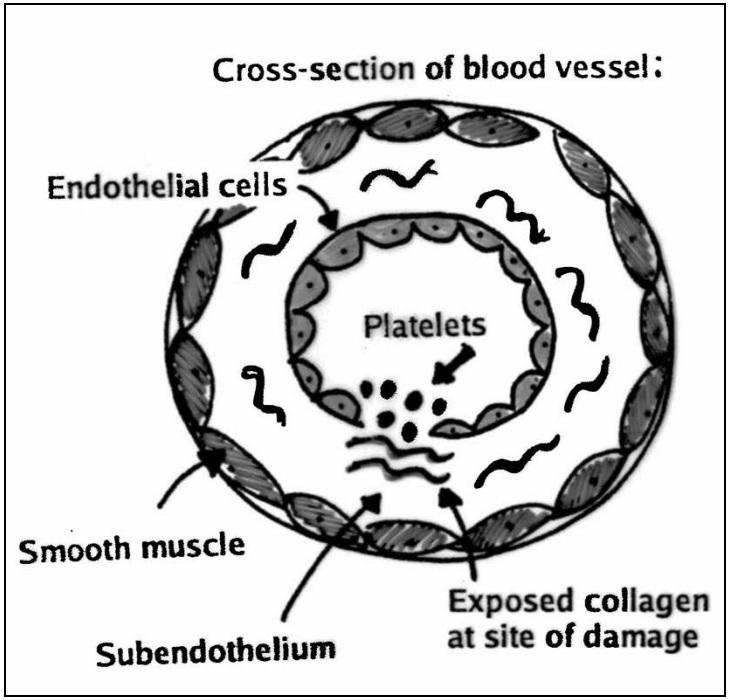
   5 hydroxytryptamine (5HT) or serotonin

   Ca^{++}

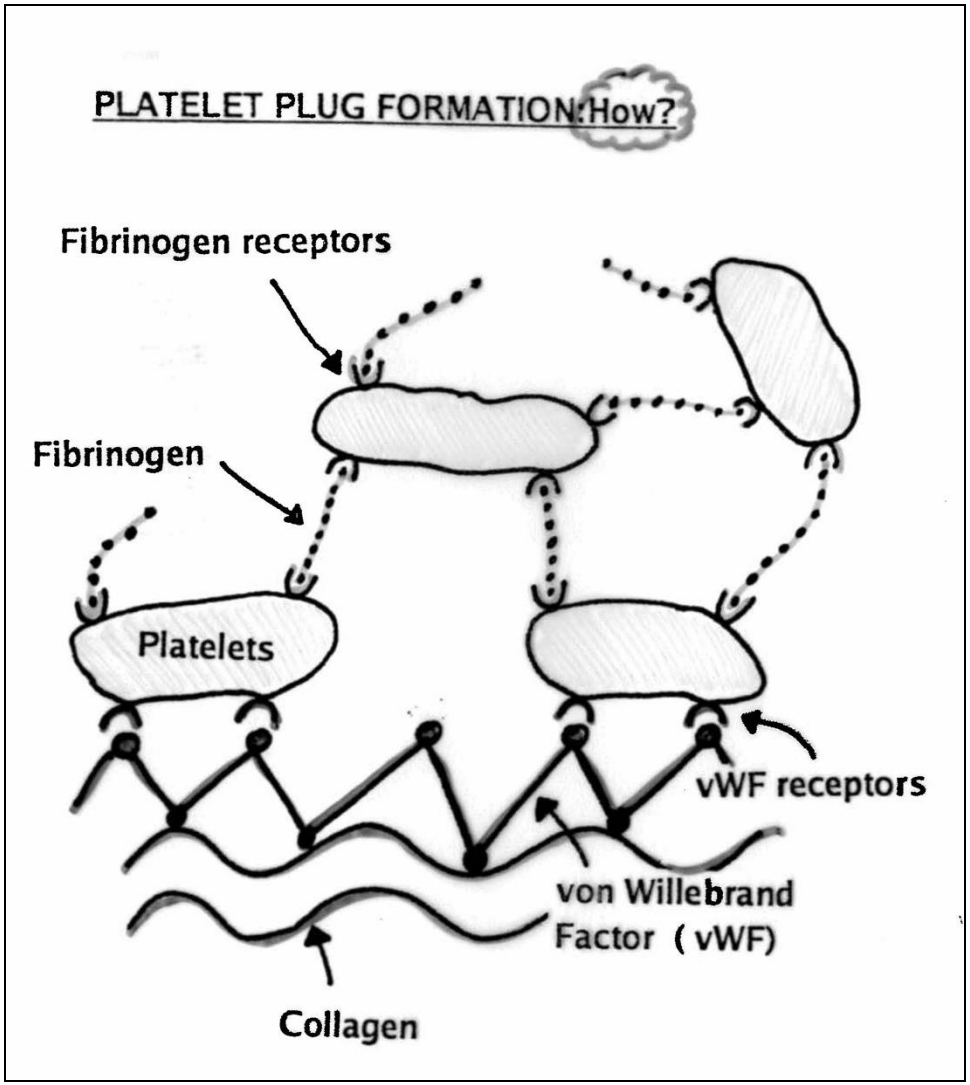
http://clinicalgate.com/acquired-disorders-affecting-megakaryocytes-and-platelets/

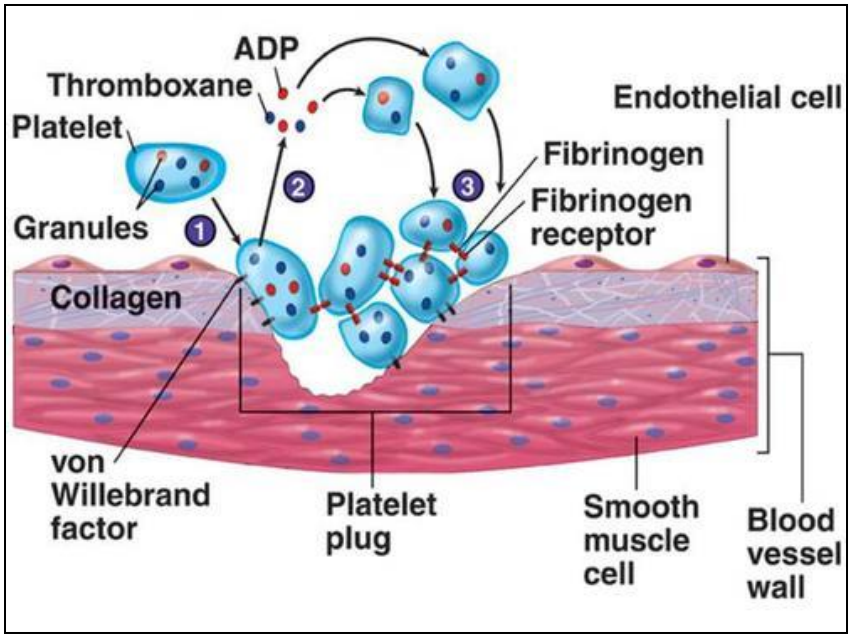
Platelet plug formation (primary hemostasis)

1. ADHESION of platelets
   - they stick to damaged vessel wall
2. ACTIVATION of platelets
   - they change shape, express various receptors and secrete various substances
3. AGGREGATION of platelets
   - they stick to each other and form a plug

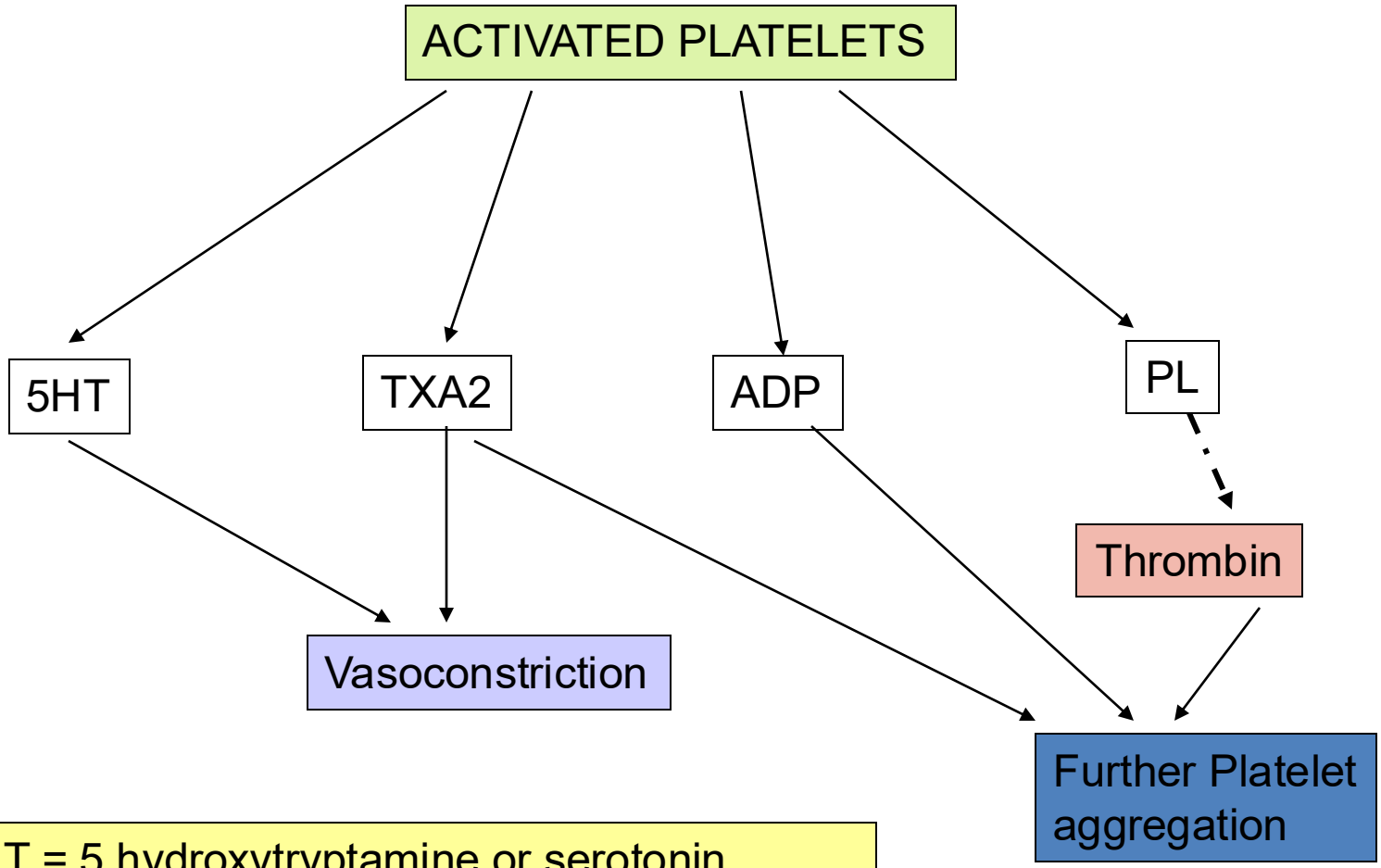


Mechanism of platelet plug formation





Roles of activated platelets



5HT = 5 hydroxytryptamine or serotonin
TXA2 = thromboxane A2
ADP = adenosine diphosphate
PL = phospholipid exposed on platelet surface

Key words: 3b

- Alpha granule, dense granule
- von Willebrand's factor (vWF)
- Fibrinogen
- Collagen
- 5 hydroxytryptamine (5HT), Thromboxane A2
- Endothelial cells

What do you remember?

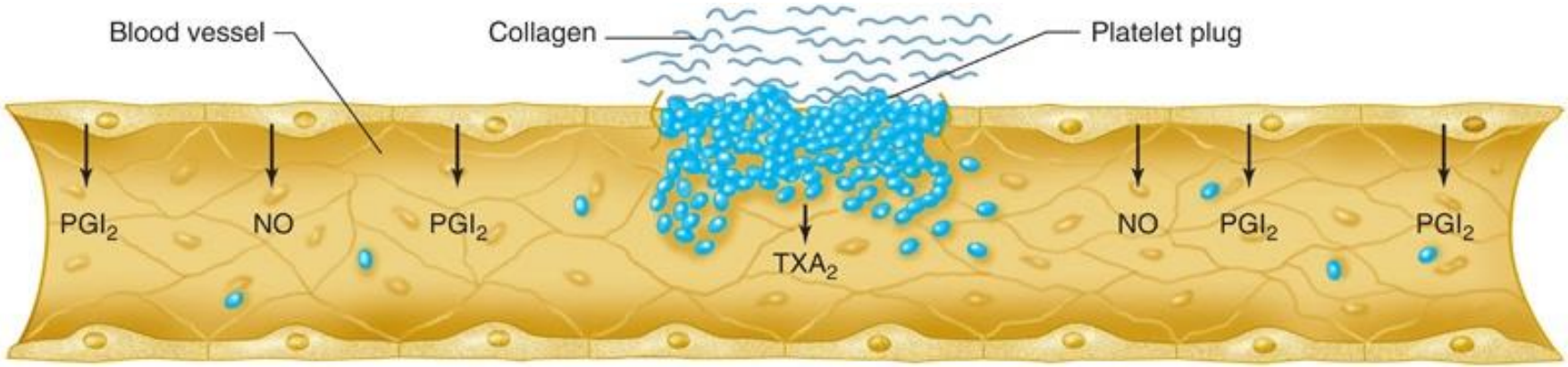
What is the **function** of Von Willebrand's factor?

a. Release Ca^{++} essential for blood clotting.
b. Act as an adhesive protein in binding platelets to the exposed collagen.
c. Act as a source of serotonin.

Factors affecting platelet plug formation: Part 3c
Topic outline

- Prevention of the spread of platelet plug
- Biochemical pathways controlling platelet plug formation
- Role of Aspirin in hemostasis

Why does platelet plug not continuously expand?



Signaling mediates responses to damage in a blood vessel:

adjacent endothelial cells are a source of chemical signals that influence platelet aggregation and alter blood flow and clot formation at the affected site.

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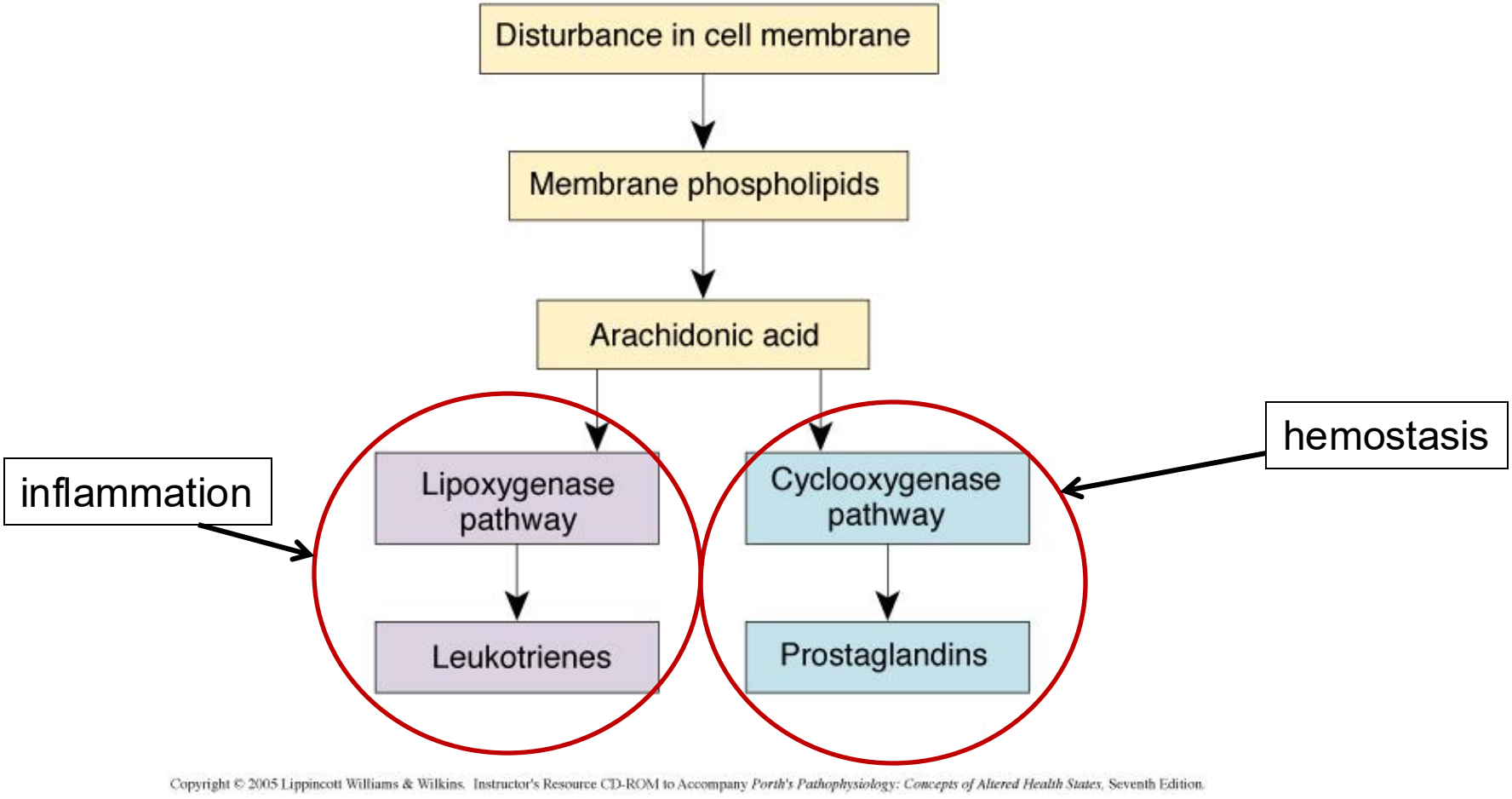
Effects of Arachidonic acid metabolites



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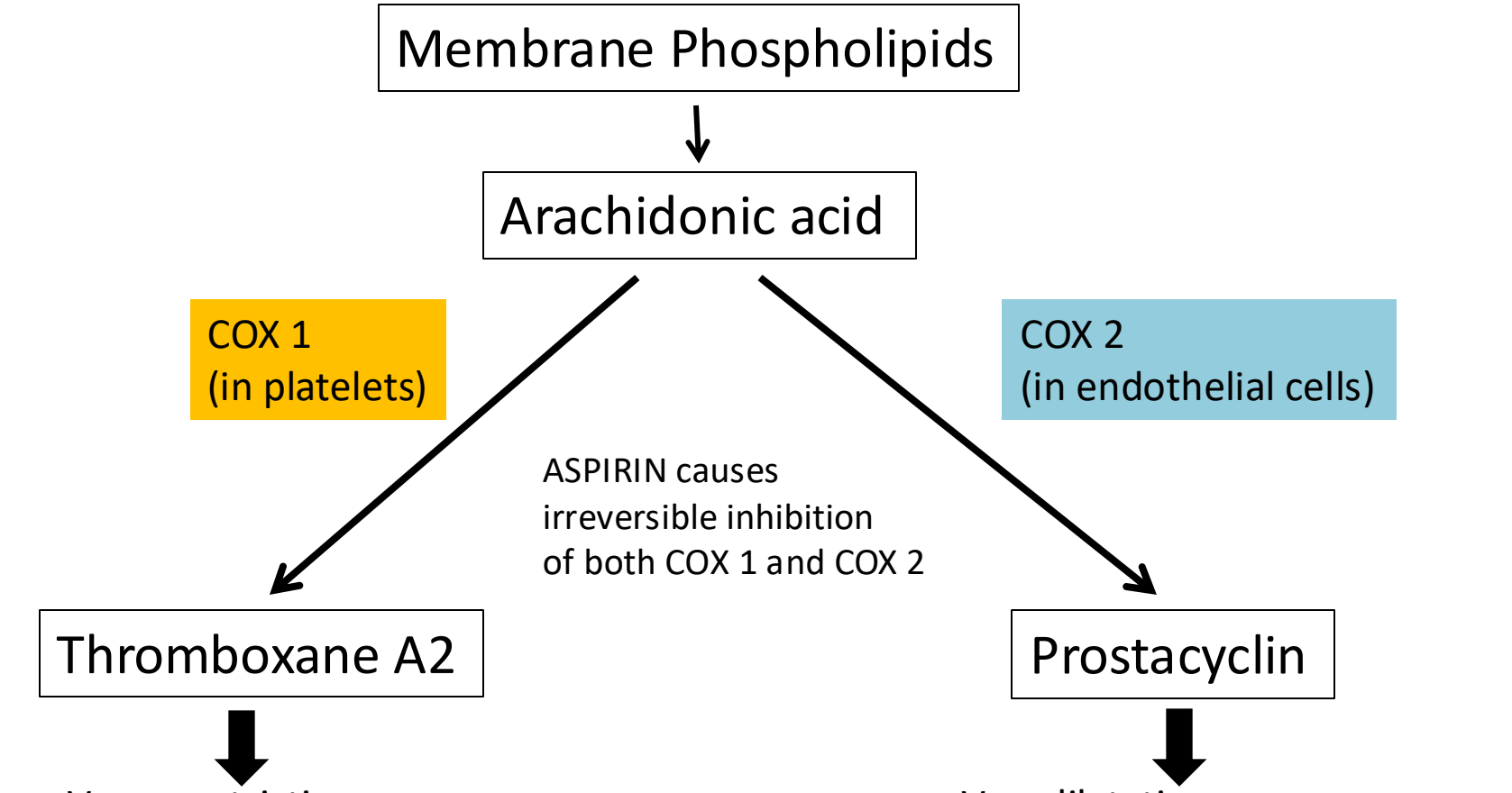
Cells involved: platelets, endothelial cells

Effect of Aspirin on Hemostasis



Net effect of **low** dose Aspirin: anti-hemostasis

Key words: Part 3c

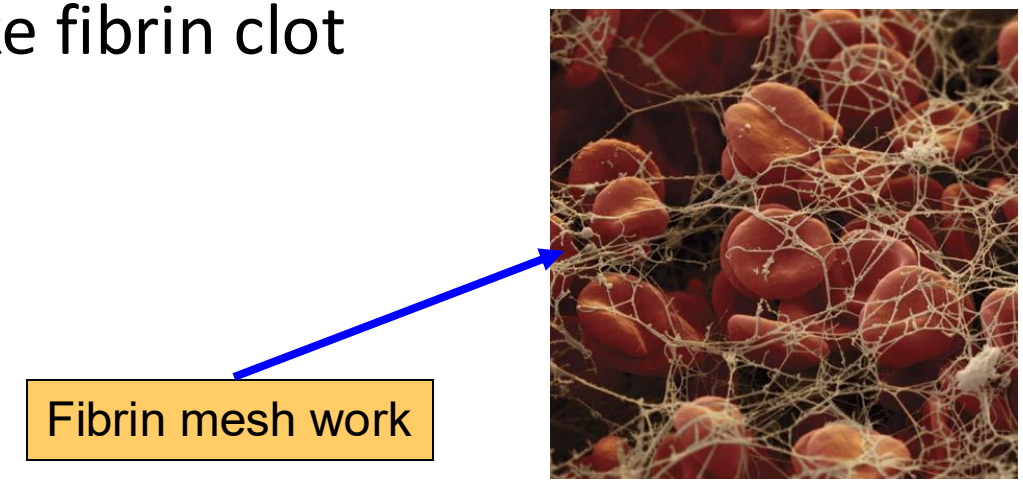
- Arachidonic acid, leukotrienes, prostaglandins
- Lipoxygenase, Cyclooxygenase (COX)
- Vasodilation, vasoconstriction
- Nitric oxide (NO), prostacyclin

Secondary hemostasis - Formation of blood clot
Part: 3d
Topic outline

- Secondary hemostasis or initiation of clotting/coagulation
- Factors involved in blood clotting
- Physical appearance of a blood clot
- Role of platelets in clot formation
- Formation of blood clot

Secondary Hemostasis

- Occurs following a platelet plug formation
- Involves a cascade of enzyme (clotting factors) activation
- Activation of enzymes occur by proteolytic cleavage
- Formation of gel-like fibrin clot

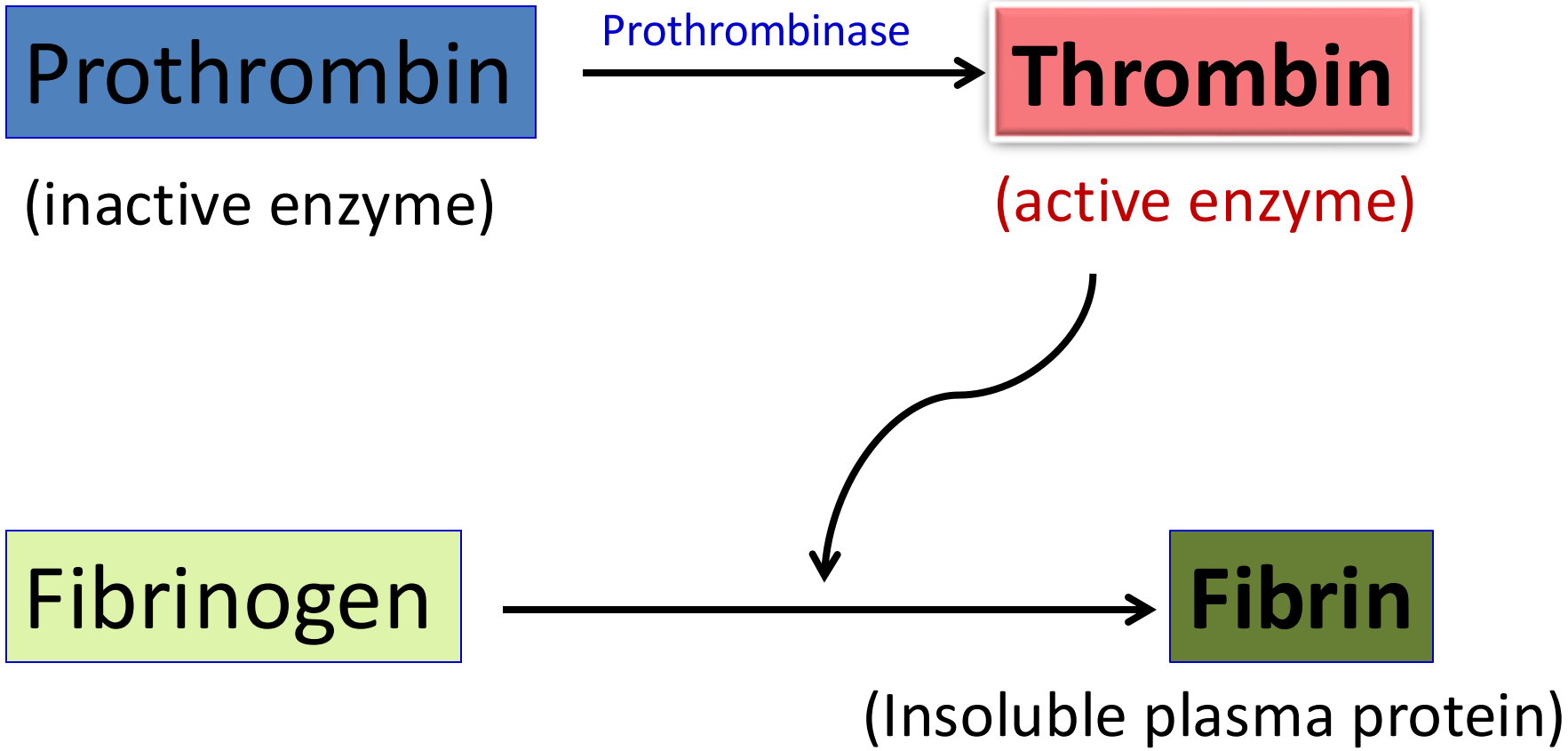


Factors involved in Blood clotting

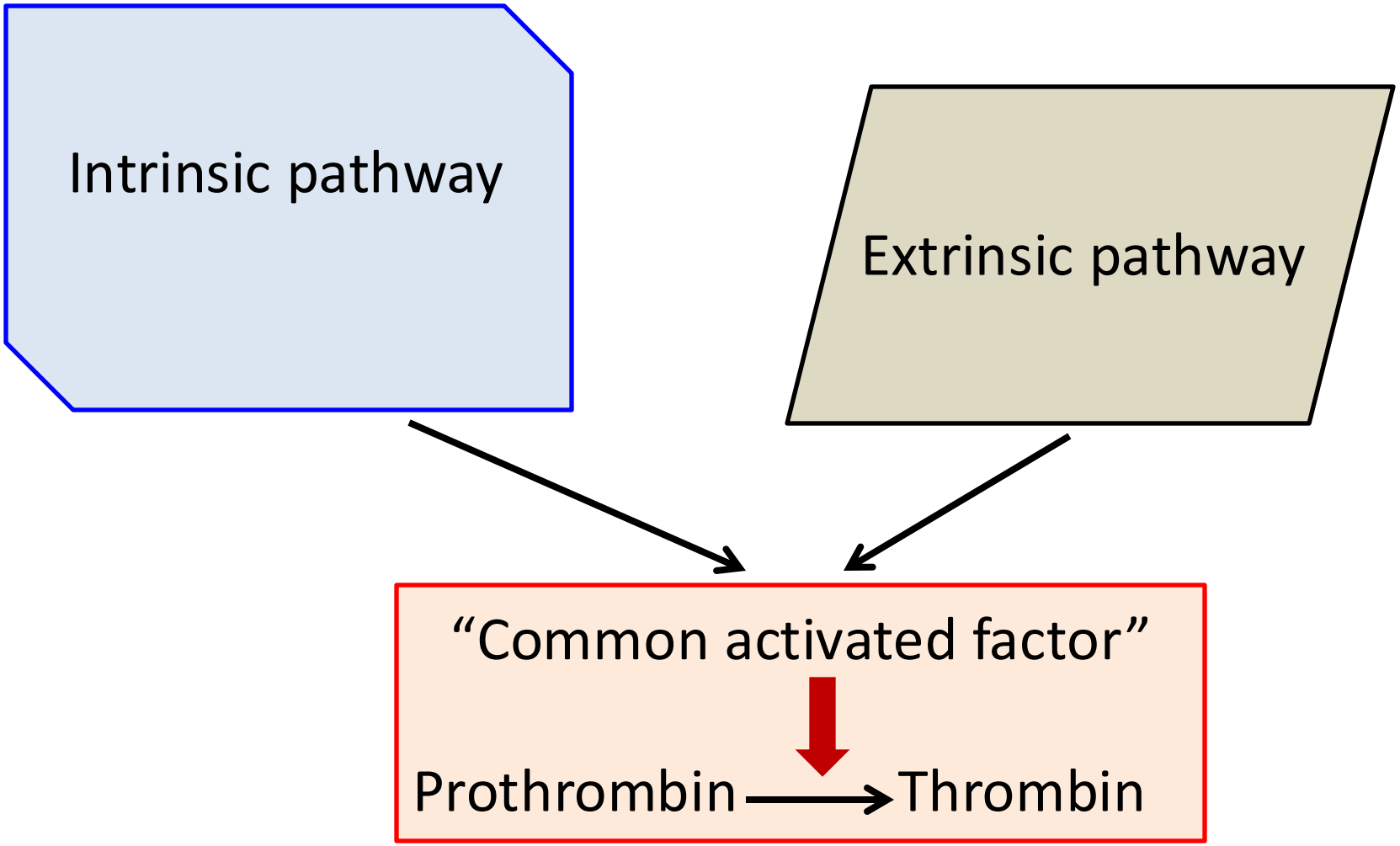
- Clotting/coagulation factors are plasma proteins
- Mostly made in the liver (except factor IV)
- Factors are usually represented by Roman numerals (I, II, and X etc.)
- * = need vitamin K for synthesis
- Factors activated by proteolytic cleavage are represented by a suffix “a”
- Factors V and VIII act as cofactors

Factors	Names
I	FIBRINOGEN
II*	PROTHROMBIN
III	TISSUE FACTOR
IV	CALCIUM
V	Proaccelerin
(VI)	Not used
	Proconvertin
	Anti-hemophilic factor A AHF –A)
IX*	Christmas factor (AHF-B)
X*	Stuart-Prower factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilizing factor

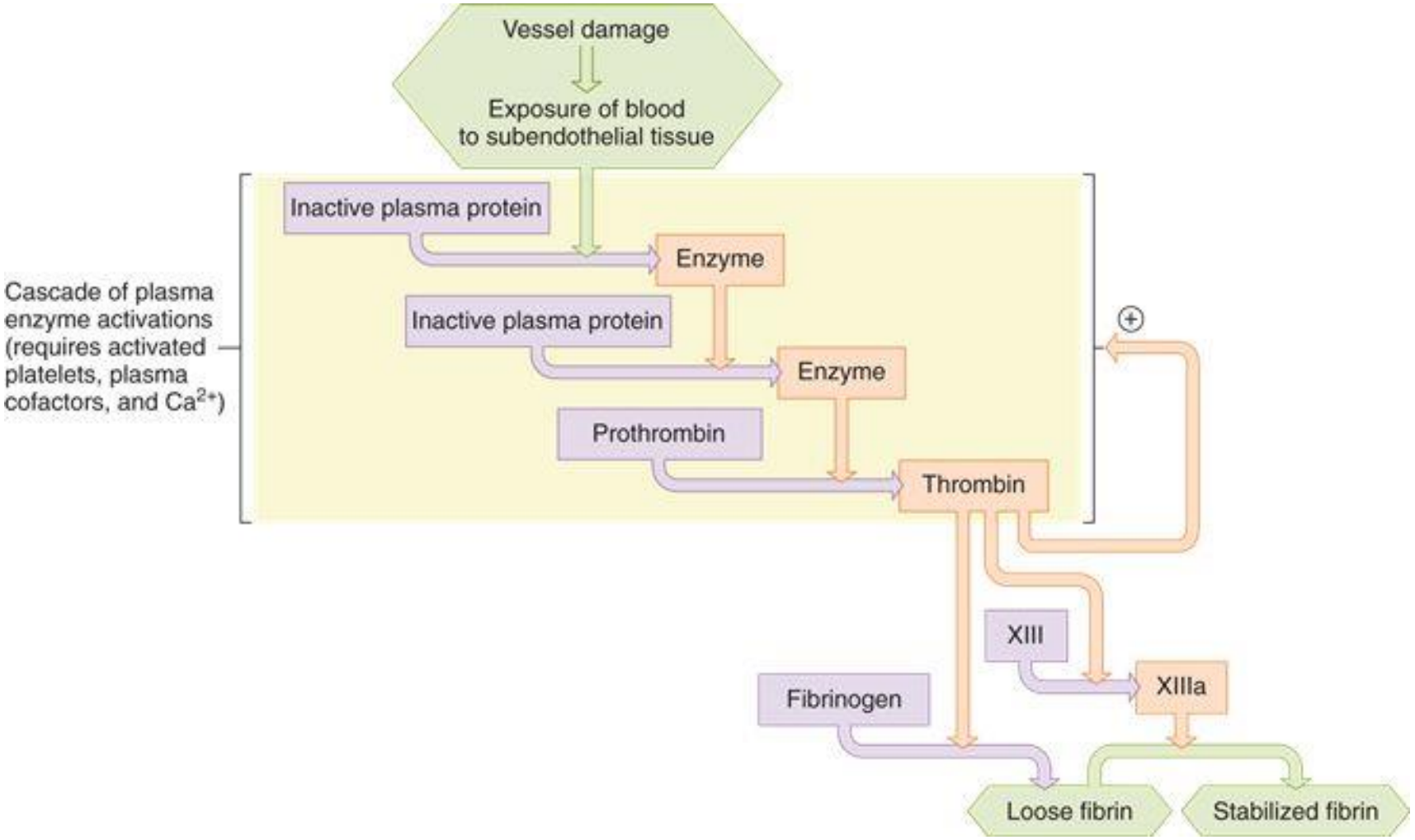
Blood clotting: key step



Activation of thrombin



Cascade of enzyme activation



Key words : Part 3d

- Secondary hemostasis, Fibrin clot
- Clotting factors
- Intrinsic pathway, Extrinsic pathway, Common pathway
- Prothrombin, thrombin
- Enzyme cascade

Source: Wikipedia.org

Pathways leading to formation of blood clot: Part 3e

Topic outline

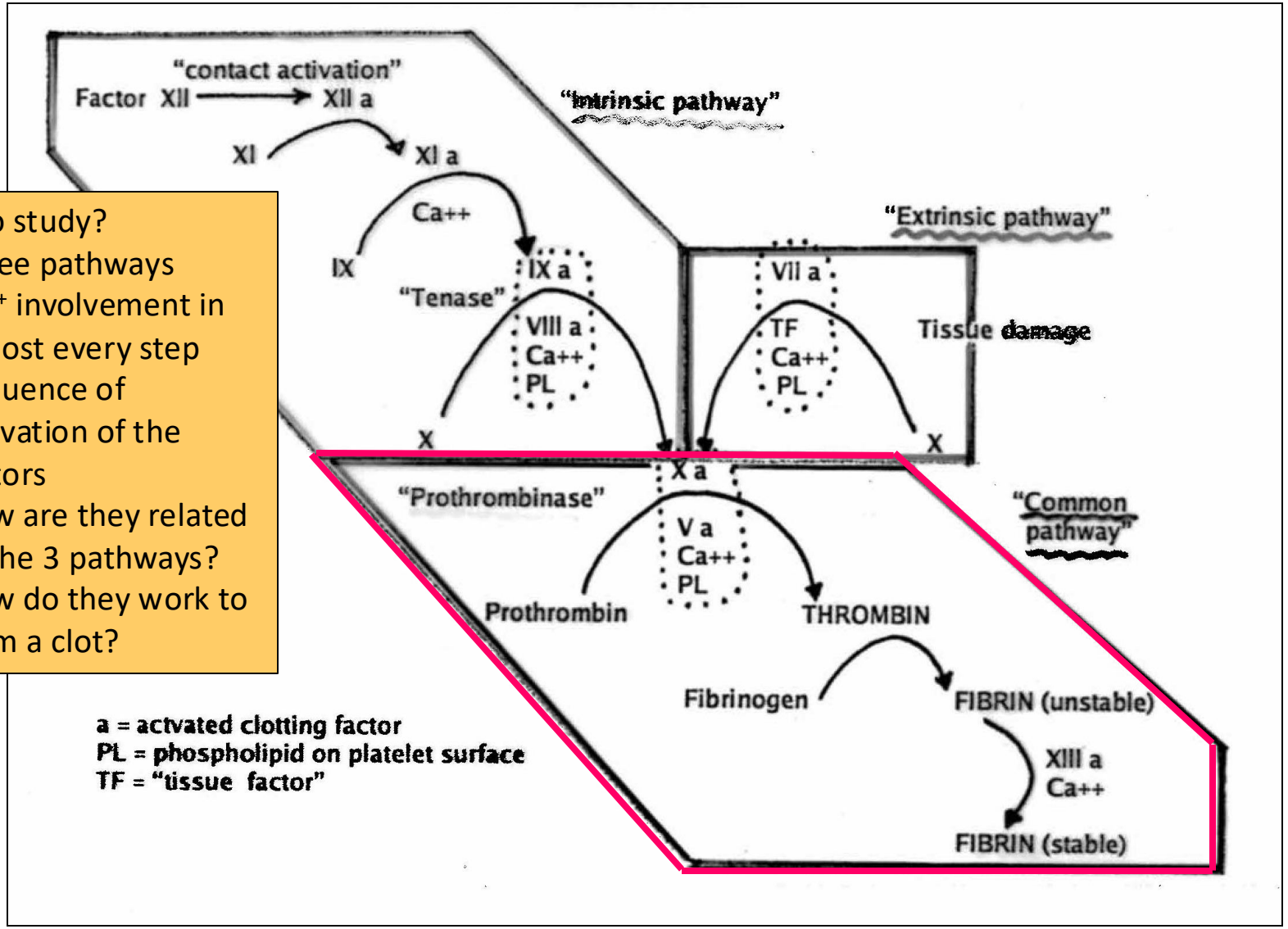
- Interaction of intrinsic, extrinsic and common pathways
- Bleeding disorders associated with deficiency of clotting factors
- Central role of thrombin

❖ https://www.sciencemag.org/news/2009/10/case-closed-famous-royals-suffered-hemophilia

Traditional/Classical mechanism of blood clotting

What to study?

a) Three pathways
b) Ca^{++} involvement in almost every step
c) Sequence of activation of the factors
d) How are they related to the 3 pathways?
e) How do they work to form a clot?



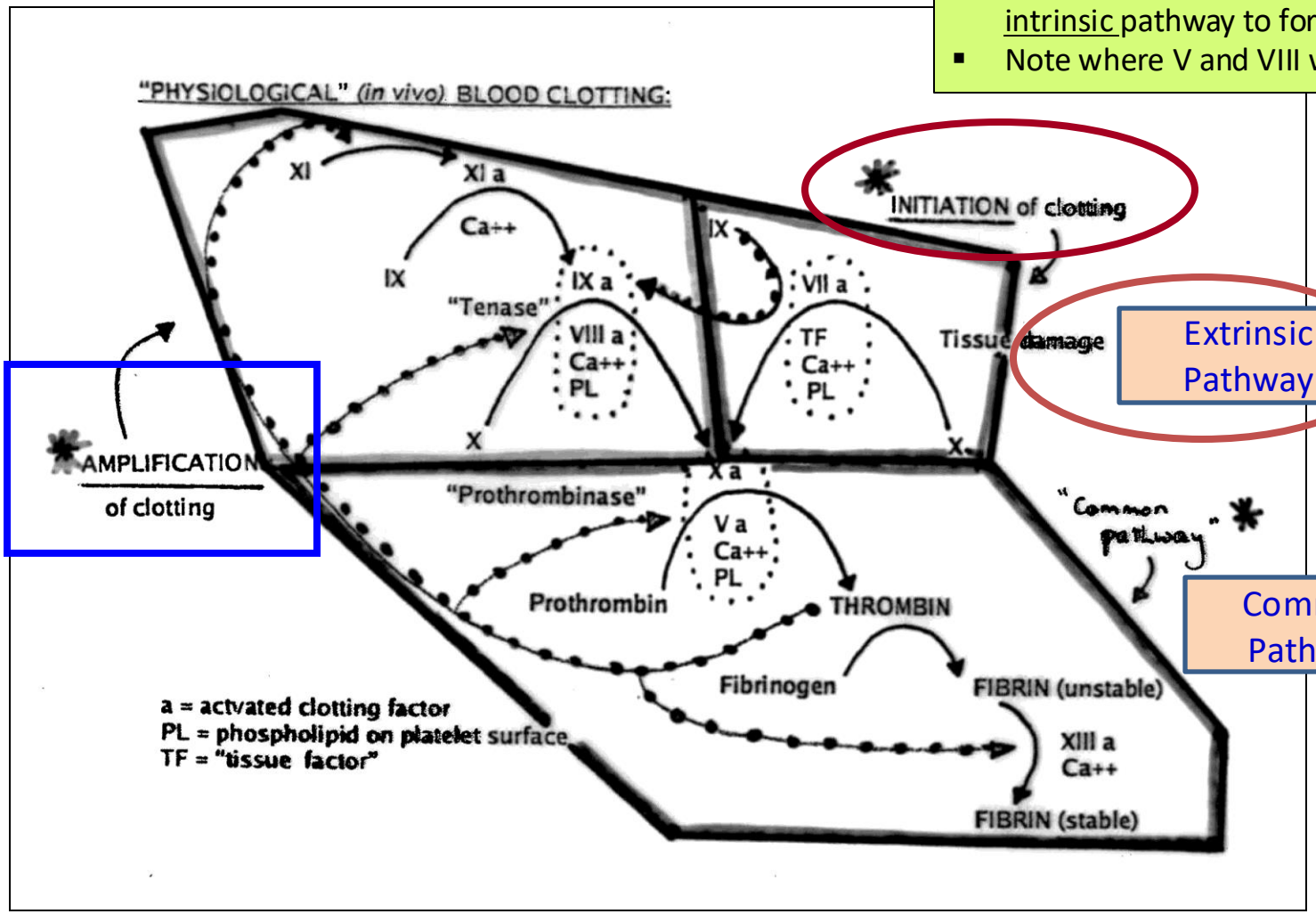
Effect of various clotting factor deficiencies on clotting

Deficiency of factors:	RESULT
VII	Severe bleeding
VIII	Severe bleeding
XI	Moderate bleeding
XII	No bleeding problem in vivo; failure to clot in vitro

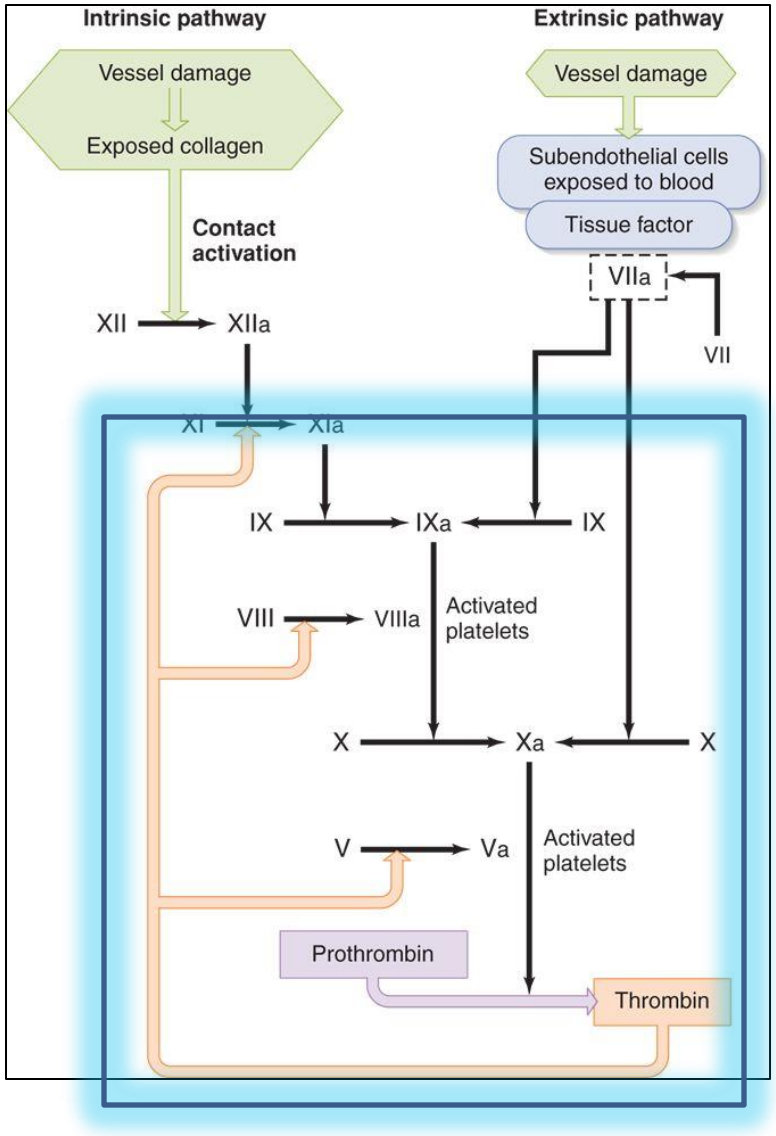
Blood from a healthy normal individual can clot when placed in a glass test tube. Blood will have a delayed clot formation in a silicone-coated test tube.

Physiological mechanism of blood clotting in vivo

- Remember that the pathways are named for our benefit of understanding
- Activation of the extrinsic and intrinsic pathways happen in a sequential manner
- Initiation happens at extrinsic pathway, small amounts of thrombin amplify the intrinsic pathway to form a large clot
- Note where V and VIII works in the pathway



Amplification of clotting protein activity by thrombin



1. Activation of factor XIII by thrombin **not** shown.
2. Steps requiring calcium is **not** shown.

For self –study purpose

What does Thrombin do in the clotting pathway?

- **Summary**

Part 3b Slide 6

1. Activation of platelets

Part 3e Slide 4

2. Conversion of soluble fibrinogen to insoluble fibrin
3. Activation of several other clotting factors (factor V, VIII,XI, and XIII)

Part 3f Slide 5

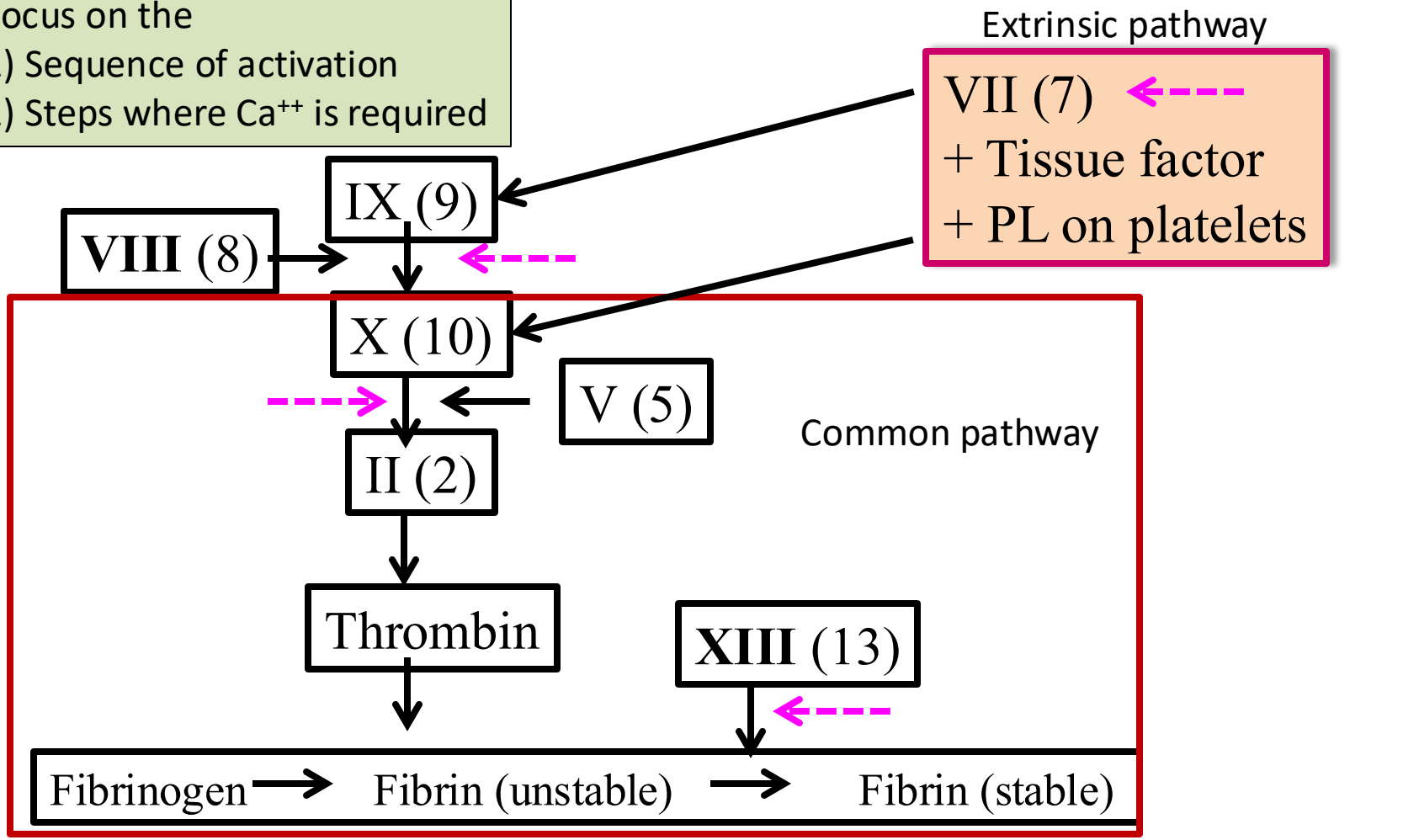
4. Activation of "protein C" (anticoagulant activity)

Sequence of activation following the extrinsic pathway

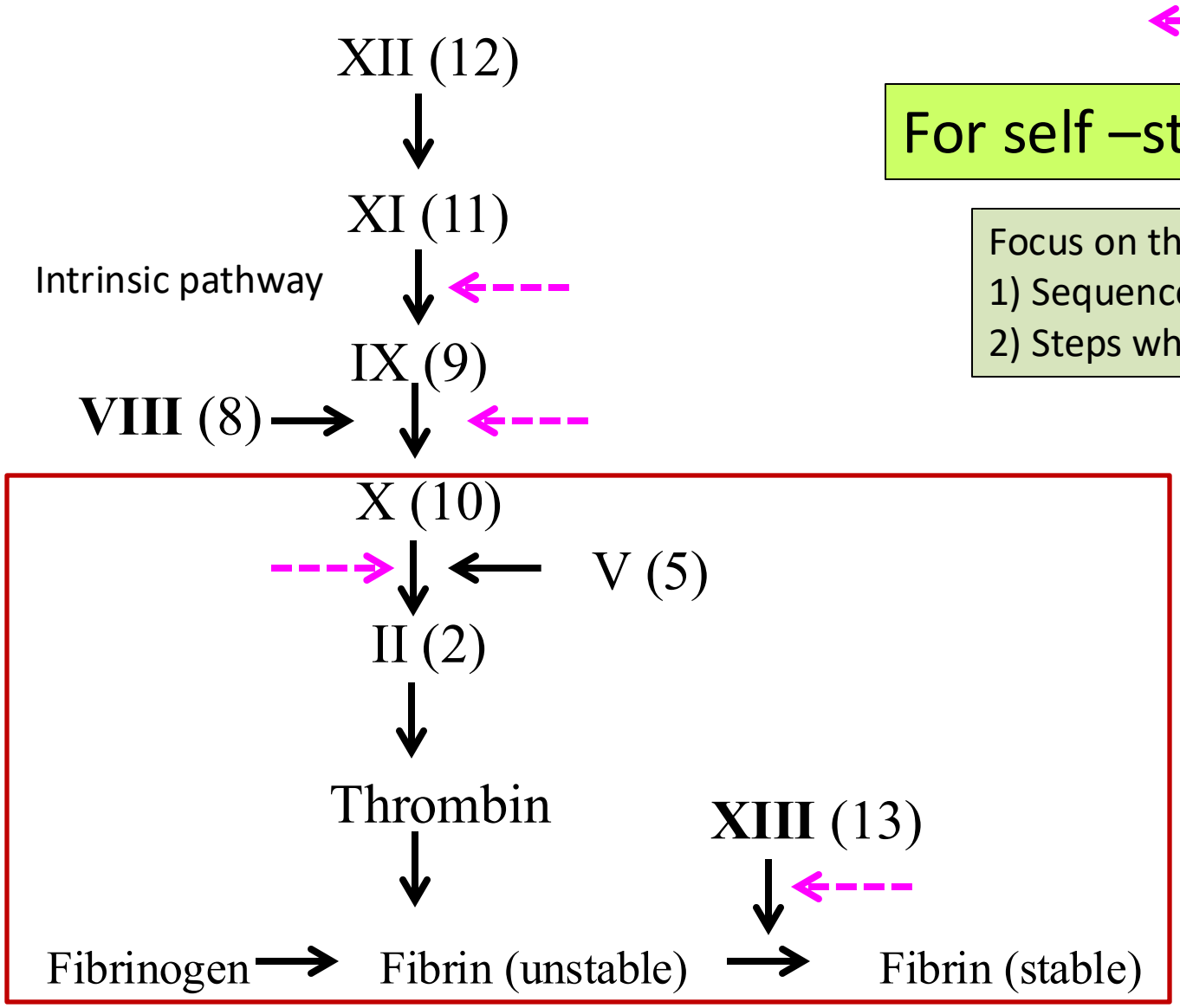
For self –study purpose

Ca++ requirement

Focus on the
1) Sequence of activation
2) Steps where Ca^{++} is required



Sequence of activation in the intrinsic pathway



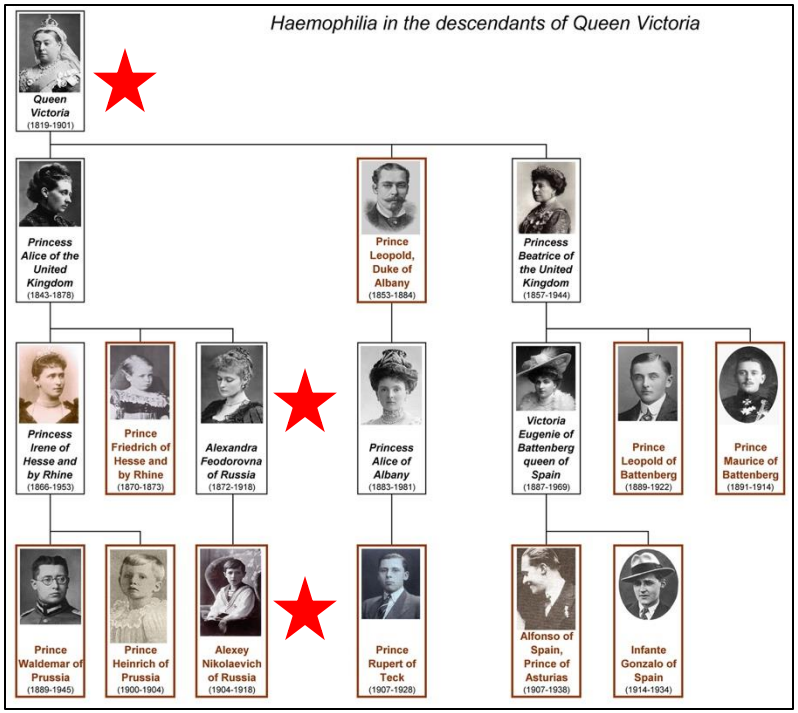
For self –study purpose

Focus on the
1) Sequence of activation
2) Steps where Ca^{++} is required

Hemophilia B: X-linked recessive gene

- Deficiency of factor IX (of Hemophilia B)
- Less common than Hemophilia A (due to deficiency of Factor VIII)
- Expressed in males
- No treatment available

Just for Interest



Genotypes for Males

- X^HY = normal blood clotting
- X^hY = male with hemophilia

- Tsarevich Alexei of Russia had hemophilia

Key words: Part 3e

- Extrinsic, intrinsic , common pathway
- Initiation and amplification of clotting pathway
- Thrombin, Tissue factor (TF)
- Bleeding disorders, Hemophilia

Mechanisms of anticoagulation and clot breakdown: Part 3f Topic outline

- Prevention of clot formation
- Natural anticoagulants and their actions
- Clinical anticoagulants and their actions
- Fibrinolysis
- Abnormal hemostasis and their outcomes

Regulation of blood clotting

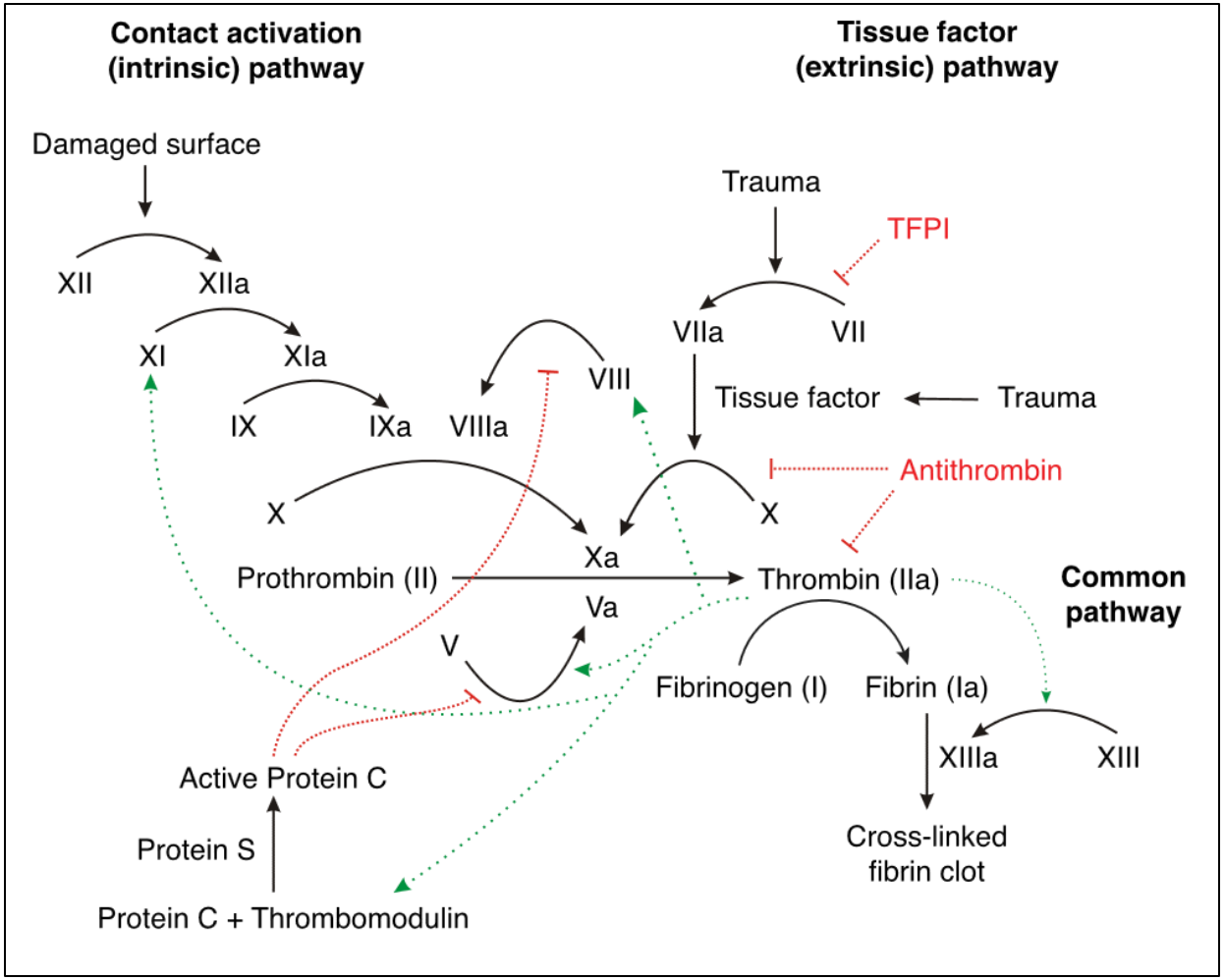
1. Prevention of clot formation where and when it is not required
   - Role of various anticoagulants
2. Breakdown of clot as tissue repair occurs
   - Role of **fibrinolytic** system

Prevention of clot formation: natural anticoagulants

Anticoagulant	How it works?
TFPI (Tissue factor pathway inhibitor)	Inhibits X a and VII a
Antithrombin 3	Inhibits thrombin etc
Thrombomodulin	Changes thrombin activity
Protein C and S	Inhibit V a and VIII a

Site of action: natural anticoagulants

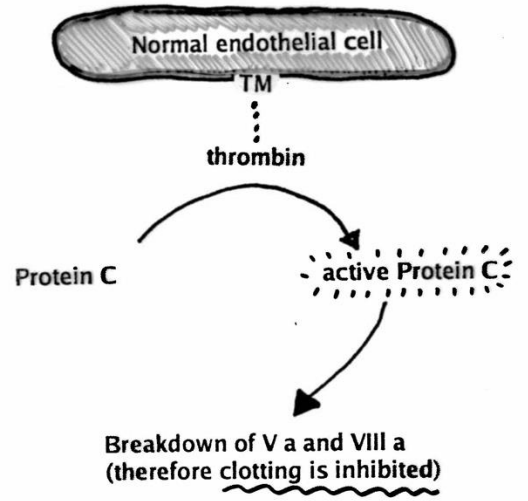
This slide is to be used for self-study purpose.
Focus on the:
1)Sequence of activation
2) Steps where anticoagulants work



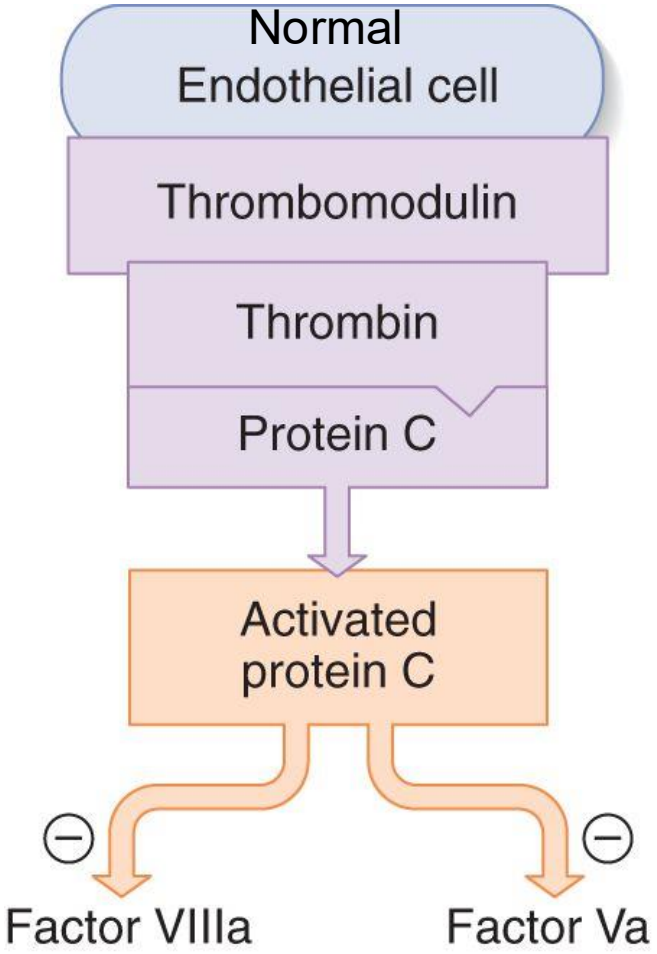
Actions of thrombin as an anticoagulant

ROLE OF THROMBOMODULIN:

1. Remember that THROMBIN (not bound to thrombomodulin,) has PRO-coagulant activity; it increases platelet activation, increases fibrin formation etc.
2. However, THROMBIN bound to THROMBOMODULIN has ANTI-coagulant activity.



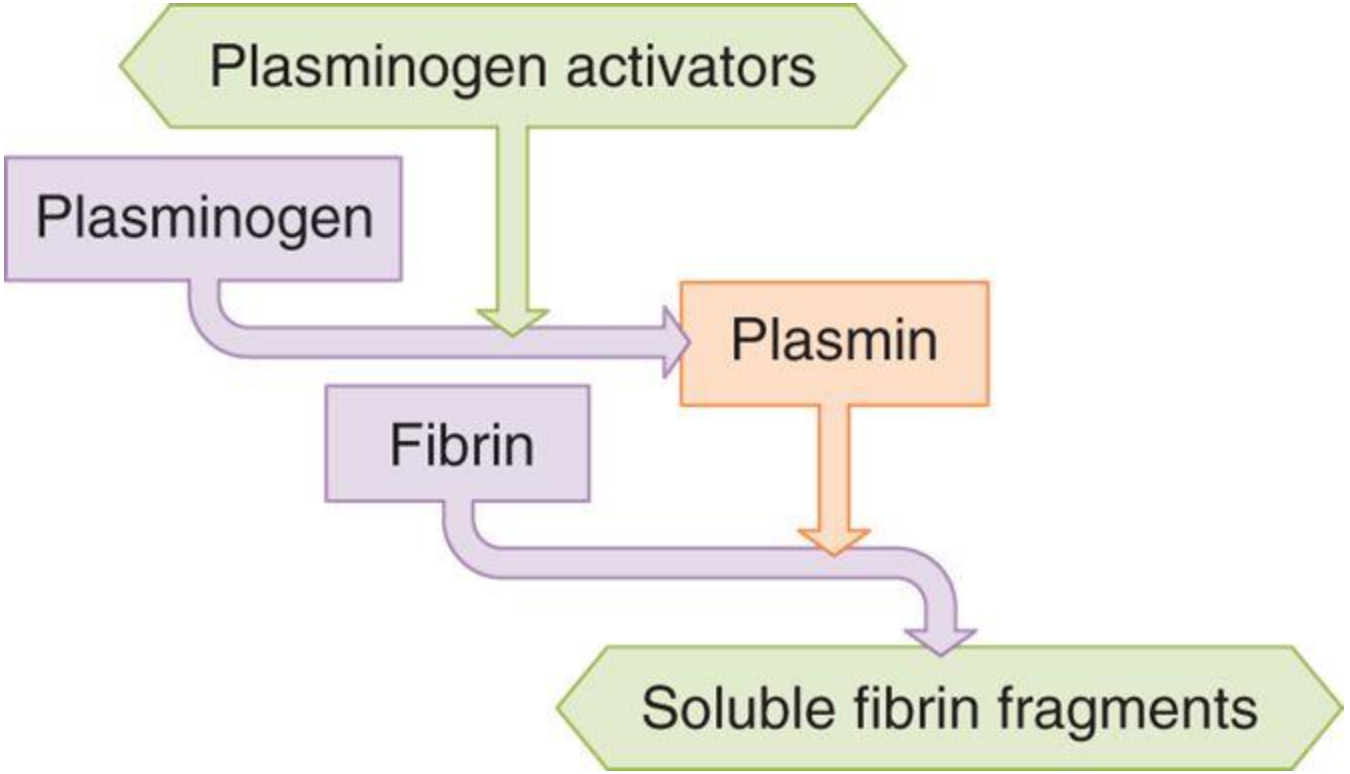
[TM = thrombomodulin]



Prevention of clot formation: "Clinical Anticoagulants"

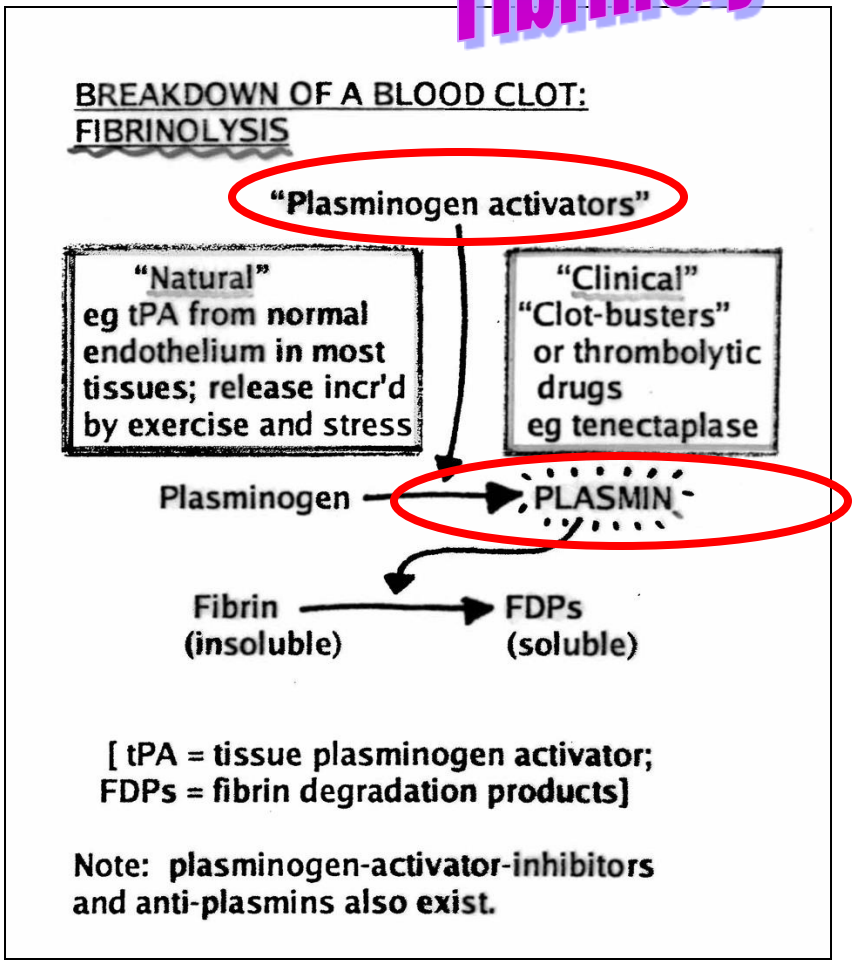
Anticoagulant	How it works	Where it works
Calcium chelators (eg. Na citrate)	Remove ionized Ca^{++}	In vitro
Heparin	Increases effect of antithrombin 3	In vitro and in vivo
Antagonists of vitamin K	Inhibit synthesis of II, VII, IX and X in liver	In vivo

Breakdown of Fibrin Clot: Fibrinolysis



Fibrinolysis

- Natural Plasminogen activator
  - tissue plasminogen activator
  - released from endothelial cells
  - release increased by exercise
- Clinical clot busters or thrombolytic drugs
  - used to treat patients with heart attacks
  - eg., tenectaplase



Abnormal hemostasis: imbalance of PRO- and ANTI- hemostatic factors

Excessive bleeding/hemorrhage

- Failure of hemostatic mechanisms when they **ARE** required

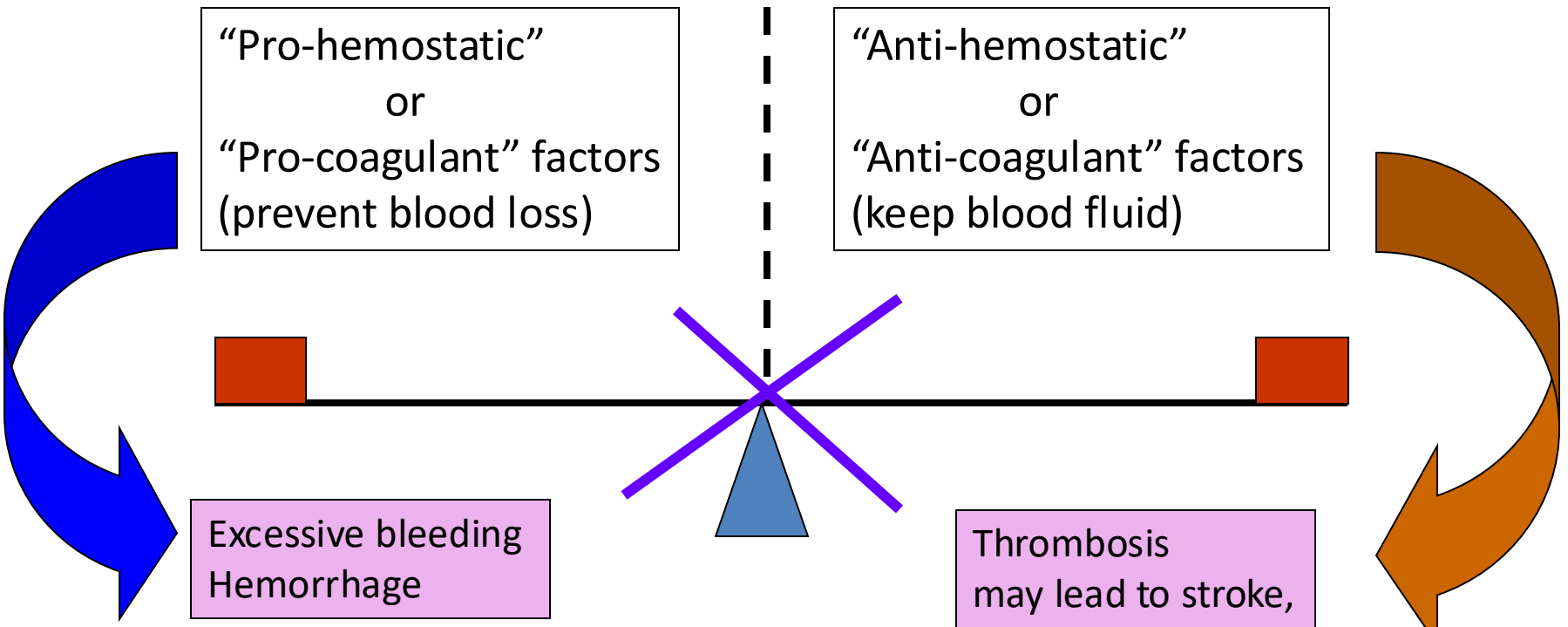
1. problems with platelets
   a) not enough platelets (thrombocytopenia)
   b) abnormal platelet function (eg., deficiency of vWF)
2. problems with clotting factors
   a) hereditary deficiencies (eg. hemophilias)
   b) acquired deficiencies (eg. due to vit K deficiency)

Thrombosis

- Formation of blood clot when they are **NOT** required

1. hereditary disorders: (deficiency of natural anticoagulants and fibrinolytic factors)
2. acquired disorders: decreased /sluggish blood flow, damage to the blood vessel wall, etc

Abnormal hemostasis when this fine balance is broken…..



platelets=thrombocytes

thrombosis

Key words: Part 3f

- Anticoagulants
- TFPI, Antithrombin, Thrombomodulin, Protein C
- Calcium chelator, Heparin
- Fibrinolysis
- Plasminogen activator, Plasminogen, Plasmin
- Thrombolytic drugs

Study guidelines: Part 3

1. What is hemostasis? Is it different from *homeostasis*?
2. Why is hemostasis necessary?
3. What do platelets contribute towards platelet plug formation?
4. What is a primary plug? How is a platelet plug formed?
5. What is a secondary plug? What is the difference between the intrinsic and extrinsic pathways of clot formation? How are the two pathways involved during a successful clot formation?
6. What is fibrinolysis? Can unwanted blood clotting be prevented *in vitro* and *in vivo*? What do the terms *in vitro* and *in vivo* mean?
7. What factors can contribute to excessive bleeding and what are the effects of excessive bleeding?
8. What can contribute to excessive clotting and what are the effects observed with excessive clotting?