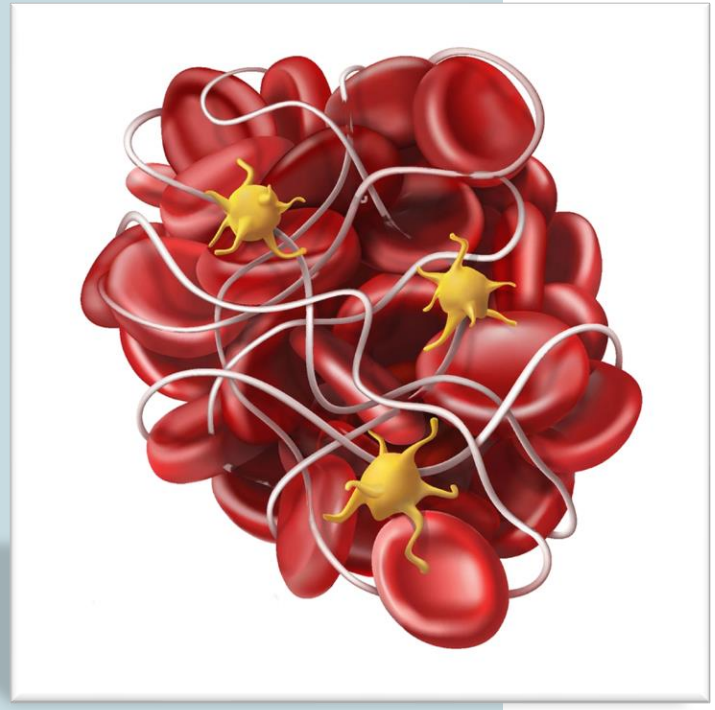

A CLOT OF FUN!

An Overview of Hemostasis and Coagulation

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Program in Medical Laboratory Science

CLPC Seminar – Fall 2024

OBJECTIVES

1. Examine the role of platelets in hemostasis.
2. Discuss the formation of fibrin in coagulation.
3. Briefly discuss antiplatelet and anticoagulant medications.
4. Describe how basic clinical laboratory tests are used to assess hemostasis.

OVERVIEW

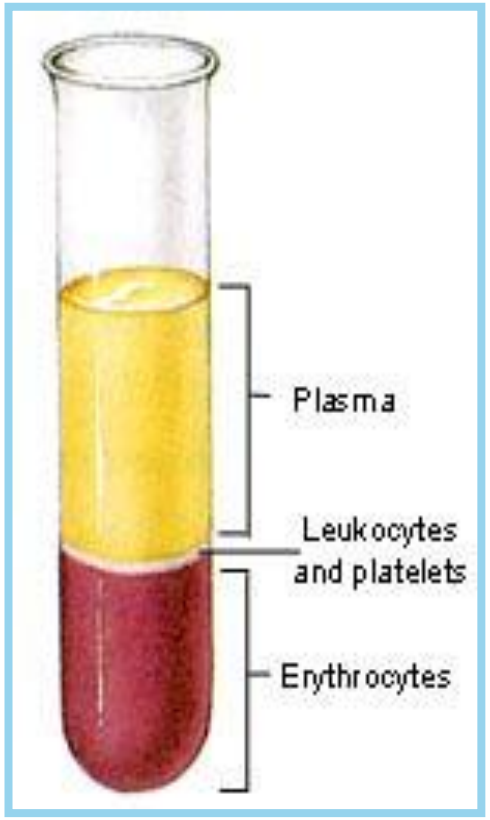
THE BIG
PICTURE

A LITTLE HISTORY…

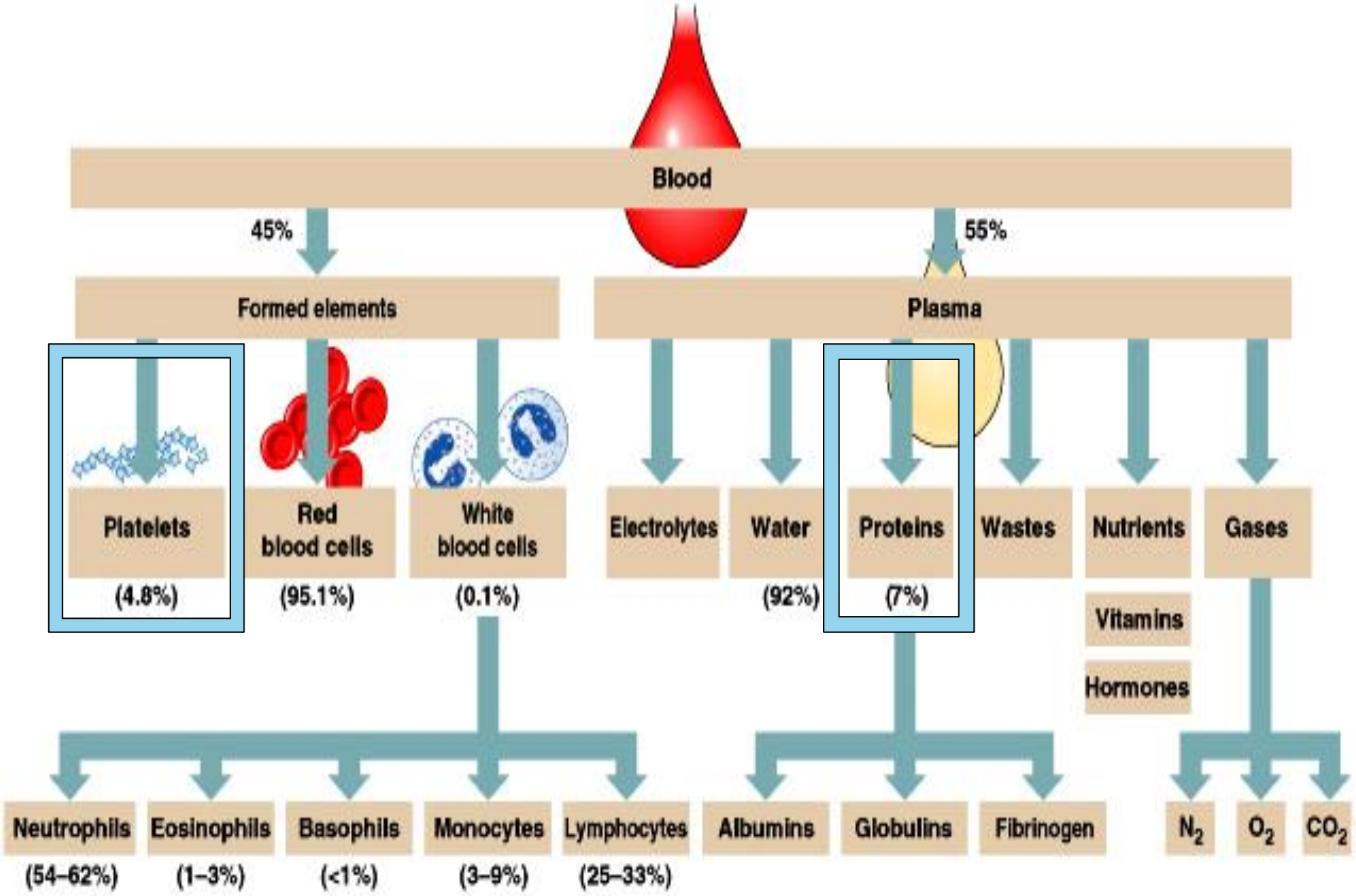
- **Ancient Greek warriors**: excessive bleeding = death
- **Hippocrates – 400BC**
  - Blood of a wounded soldier congealed as it cooled.
  - Bleeding from a small wound stopped quickly as “skin” covered the blood.
  - Bleeding resumed if “skin” were removed.

HISTORY, CONT.

- 1600s – Clots observed in veins at **BODY** temperature.
- 1770 – demonstration that semi-solid mass could be generated from liquid portion of blood, without cells.
- Over time, realization that clotting process is very complex.
- Lab tests were eventually developed to measure various parts of the clotting process.
- Drugs were also developed to target specific clot-forming actions.

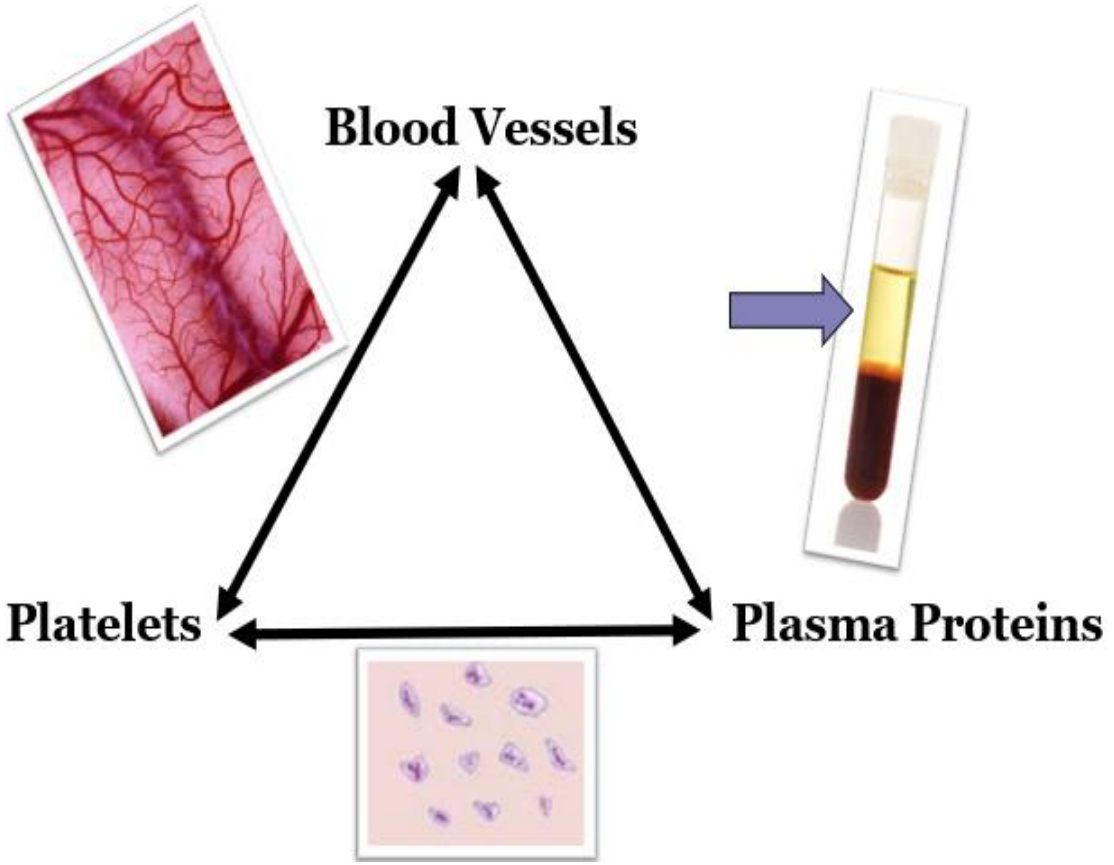


SUMMARY: WHAT'S IN BLOOD?



TERMS

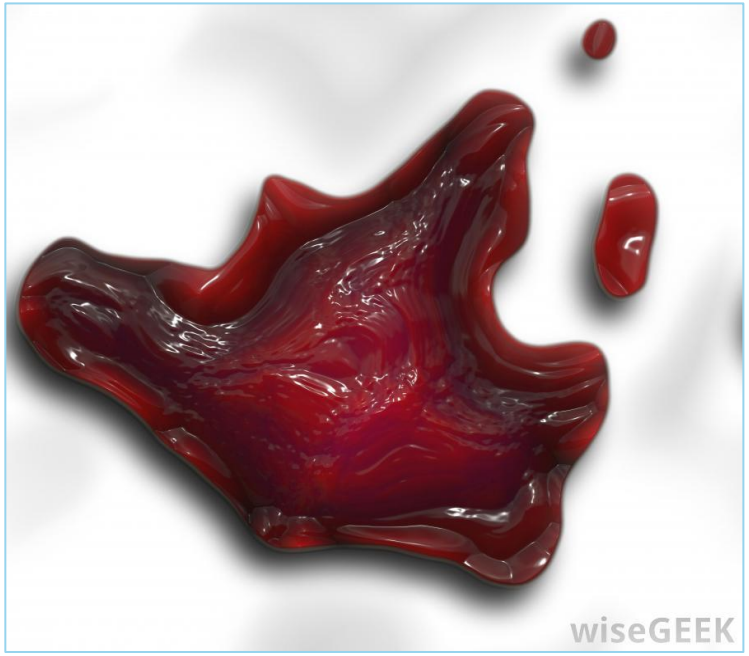
- **Hemostasis –**
  - Highly regulated process that contributes to maintaining blood in a fluid state when contained within intact blood vessels
    - Prevents spontaneous hemorrhage
    - Halts bleeding after injury
  - Requires balance between actions of:
    - **Blood vessels (vasoconstriction)**
    - **Platelets (platelet plug formation)**
    - **Coagulation factors (fibrin formation)**
    - **Fibrinolysis (fibrin breakdown)**



TERMS, CONT.

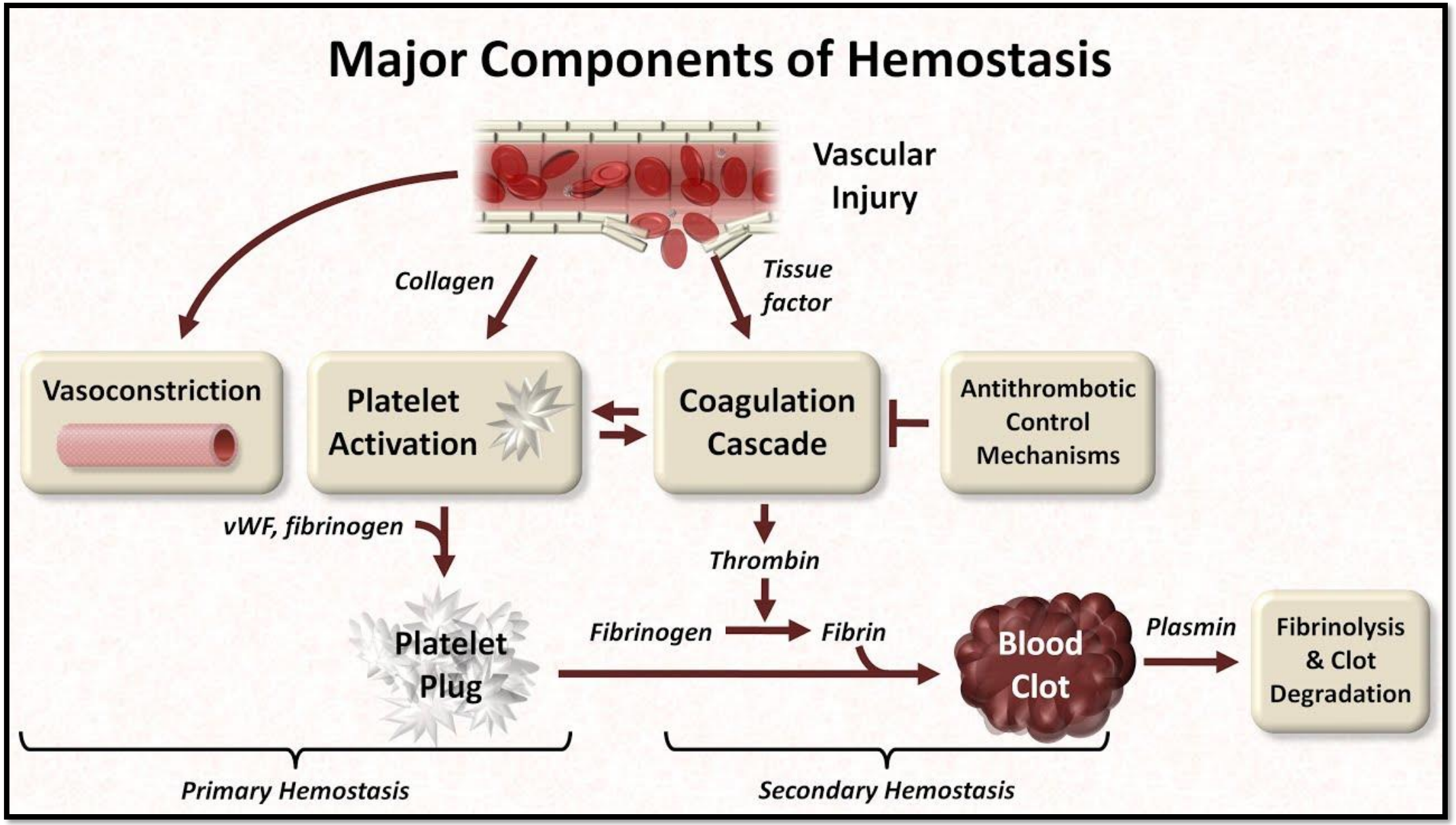
- **Coagulation –**
  - Process by which soluble plasma proteins are converted to an insoluble fibrin “clot”
  - Can occur with or without the actions of blood vessels and platelets (in a test tube)

- **Blood Clot –**
  - A gelatinous or semi-solid mass of coagulated blood, comprised of red blood cells, platelets, and fibrin
  - Called a “thrombus” when formed *within* a vessel or organ
  - Thromboembolism – dislodged thrombus



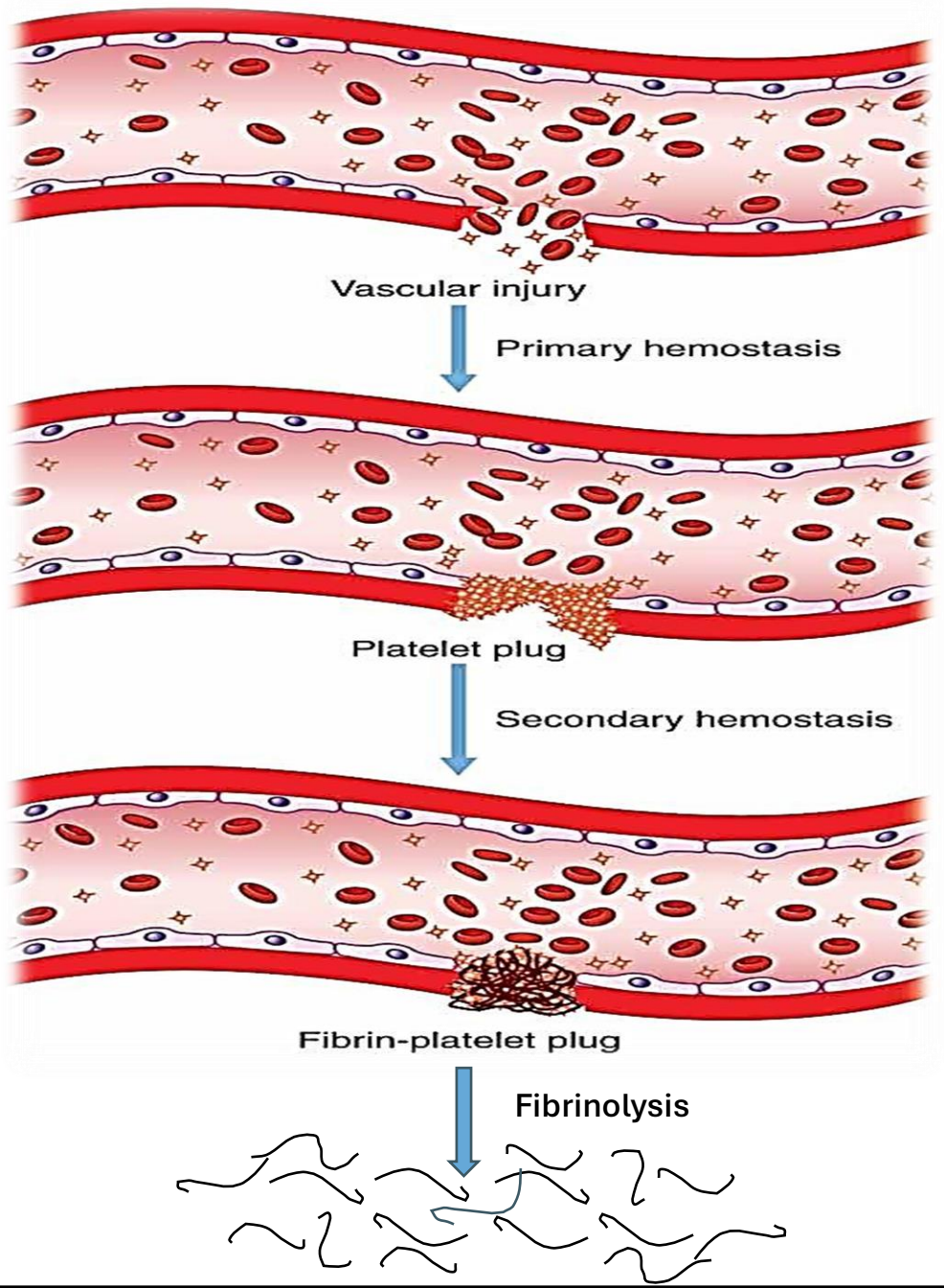
OVERVIEW OF NORMAL HEMOSTASIS

- Normally, blood flows freely through blood vessels, controlled by physiologic processes.
- Injury severs vessel(s), causes bleeding.
- Body forms a clot to stop bleeding.
- As healing takes place, clot is broken down when no longer needed.
- Normal blood flow is restored.


Major Components of Hemostasis
Vascular Injury
Collagen
Tissue factor
Vasoconstriction
Platelet Activation
Coagulation Cascade
Antithrombotic Control Mechanisms
vWF, fibrinogen
Thrombin
Platelet Plug
Fibrinogen
Fibrin
Blood Clot
Plasmin
Fibrinolysis & Clot Degradation
Primary Hemostasis
Secondary Hemostasis

HEMOSTASIS IN THREE EASY STEPS

1. **Primary hemostasis** – actions of the blood vessels and platelets
   - Formation of the platelet plug, to rapidly stop/slow blood loss
2. **Secondary hemostasis** – actions of protein coagulation factors
   - Formation of fibrin, to stabilize platelet plug
3. **Fibrinolysis** – breakdown of hemostatic plug
   - Degradation of fibrin clot, as healing begins after bleeding cessation



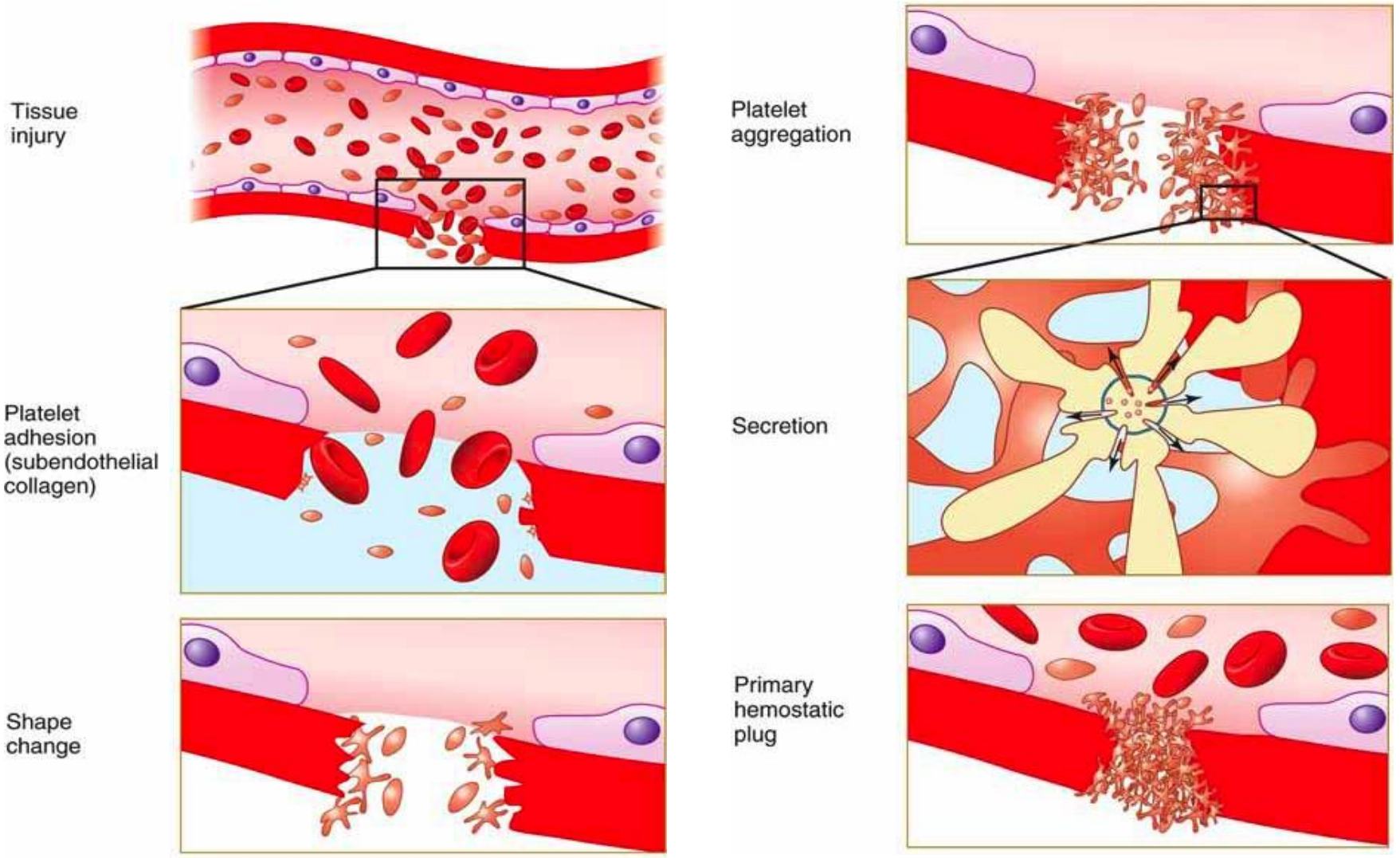
PRIMARY HEMOSTASIS

PLATELET
PLUG
FORMATION

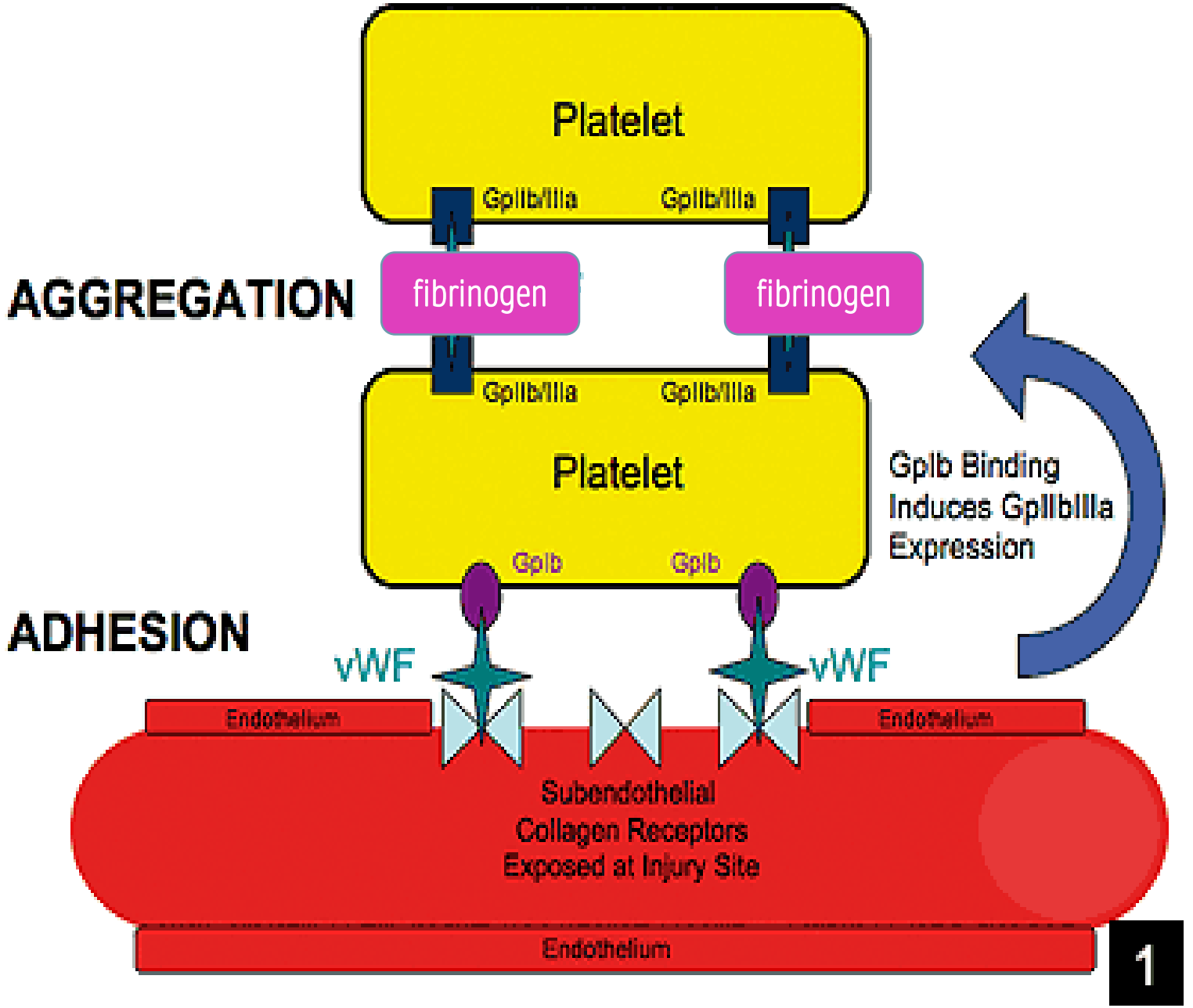
PRIMARY HEMOSTASIS:
KEY OUTCOMES

1. ***Adhesion*** – Platelets attach to injured vessel via exposed collagen, in a monolayer
2. Activation – Platelets shift from resting to active, accompanied by shape change
3. Secretion – Platelets release internal granules, which contain platelet agonists, to recruit more platelets
4. ***Aggregation*** – Platelets attach to each other to form a temporary and unstable plug, which fibrin will stabilize.

FORMATION OF PRIMARY HEMOSTATIC PLUG



Clinical Laboratory Hematology, 4th ed.



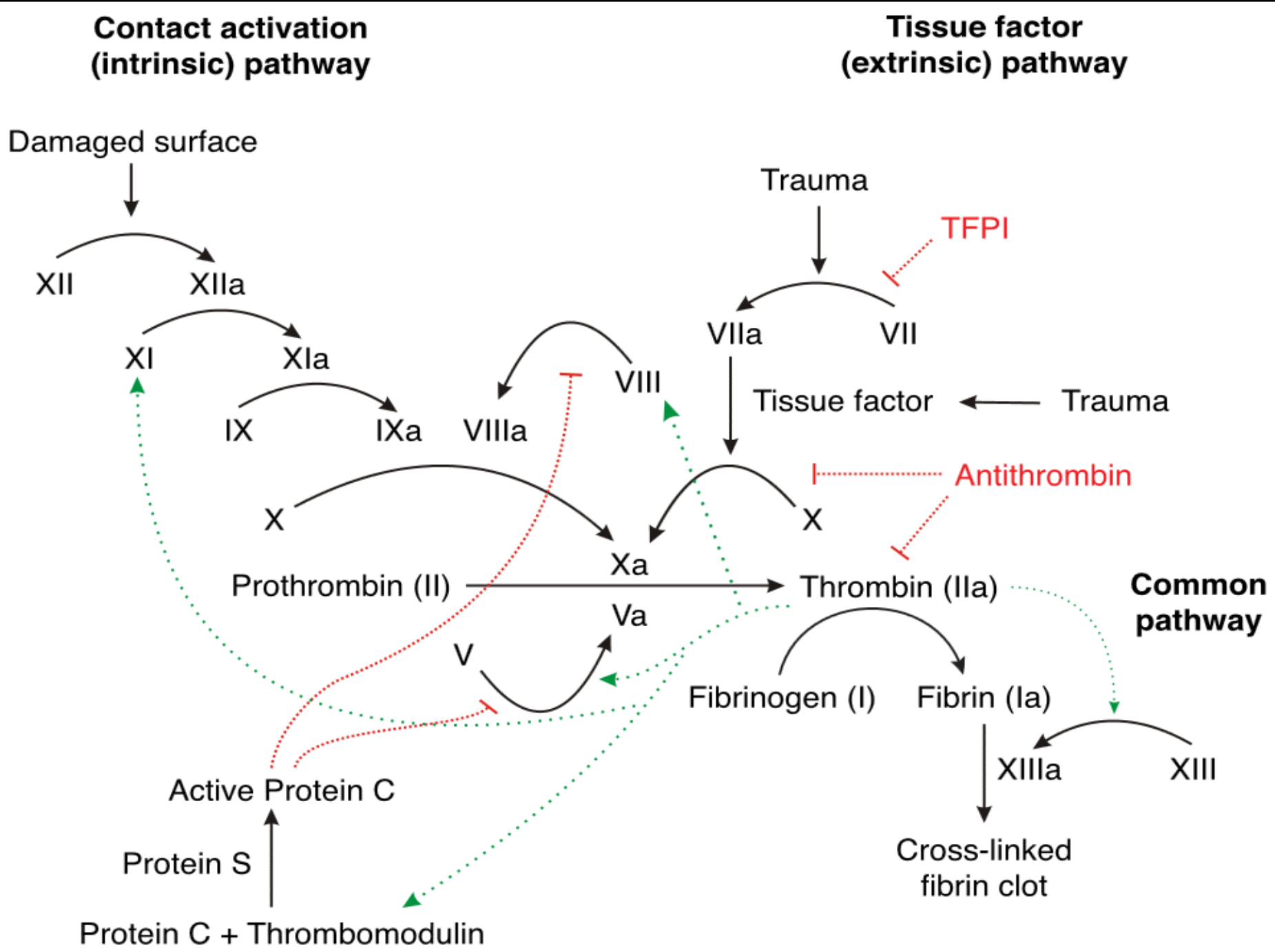
- **von Willebrand factor (vWF)** – "bridge" between exposed collagen and platelets (for adhesion)
- **GPIb** – platelet receptor that binds to vWF
- **Fibrinogen** – "bridge" between platelets (for aggregation)
- **GPIIb/IIIa** – platelet receptor that binds to fibrinogen

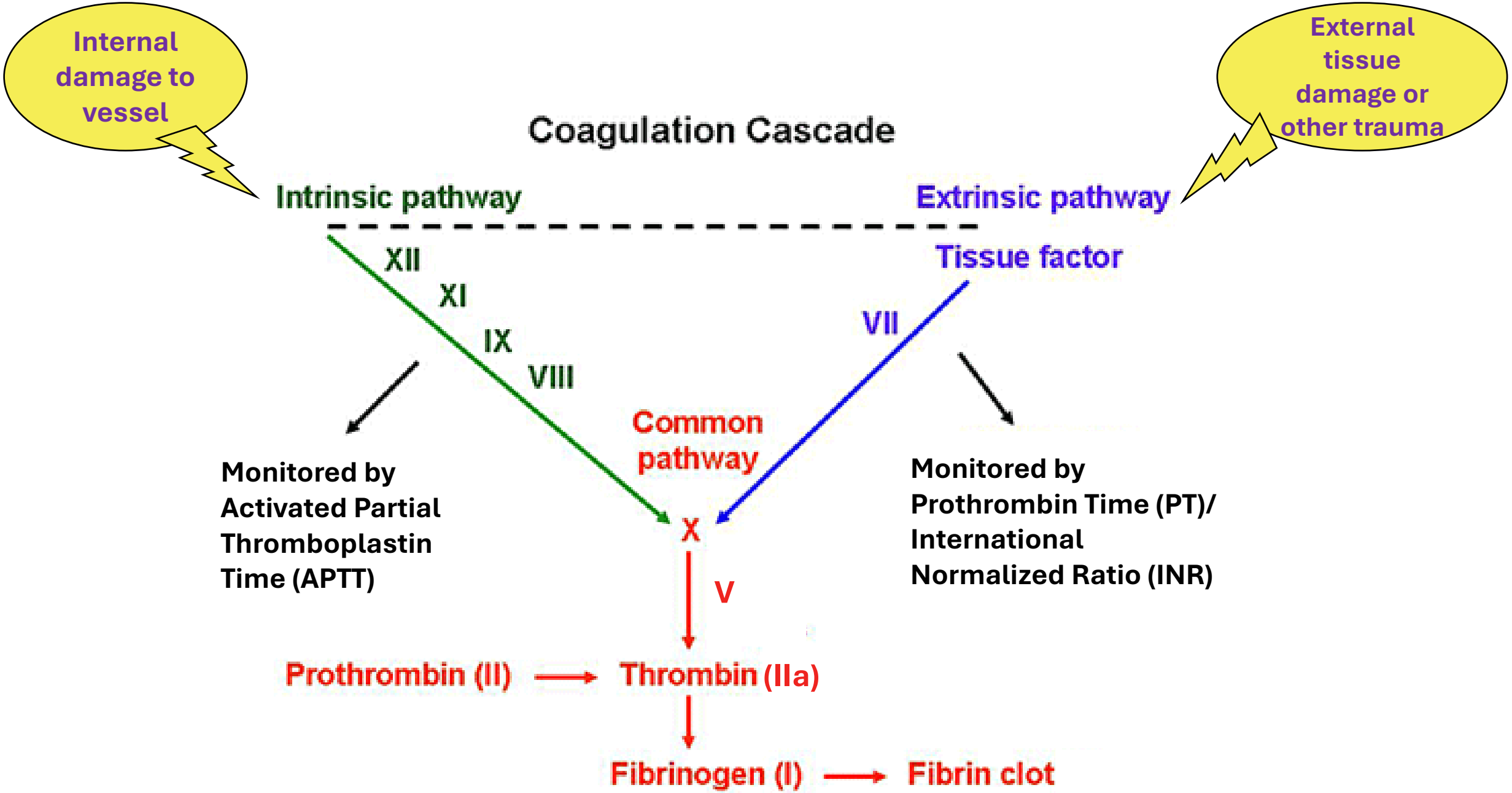
SECONDARY HEMOSTASIS

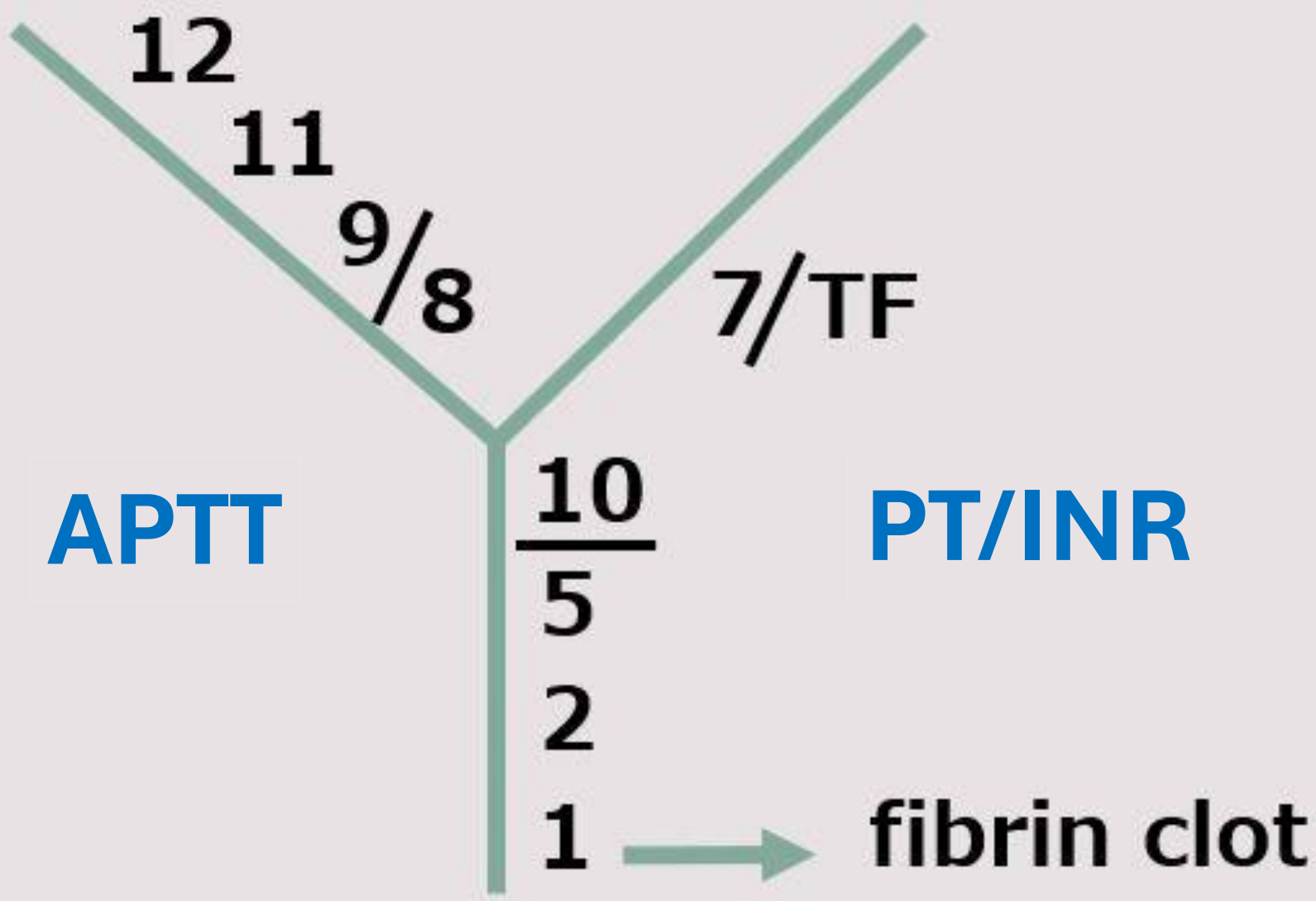
FIBRIN FORMATION

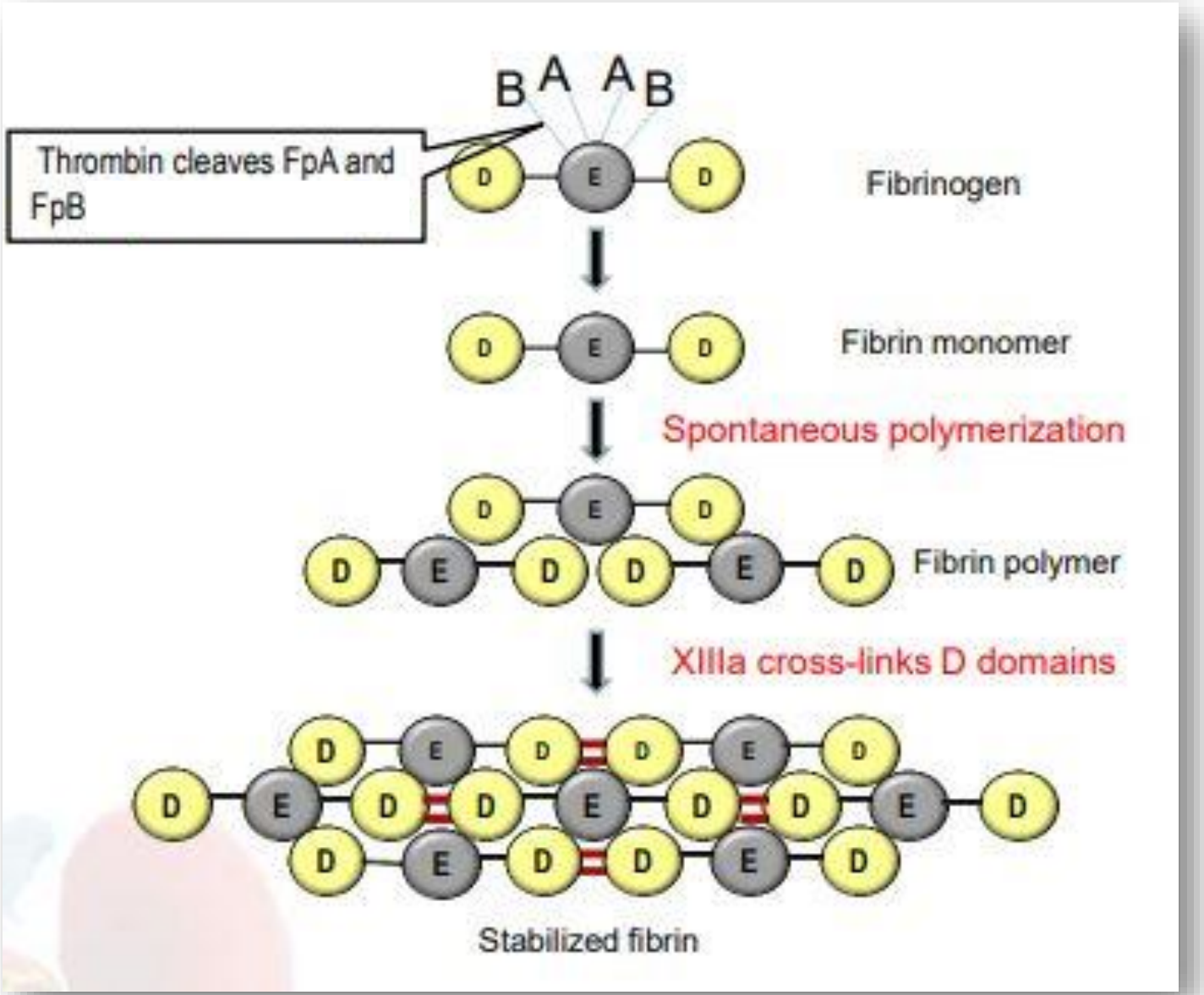
SECONDARY HEMOSTASIS: KEY OUTCOMES

1. Inactive factors become activated enzymes in a “cascade” of reactions.
2. Most important enzyme, **thrombin** (factor IIa), converts soluble plasma fibrinogen into insoluble “sticky” fibrin.
3. Fibrin polymers are stabilized when an enzyme called factor XIII forms covalent bonds between “D” regions of fibrin.
4. Stabilized fibrin reinforces primary platelet plug, forming a clot.


Contact activation
(intrinsic) pathway
Tissue factor
(extrinsic) pathway
Damaged surface
XII
XIIa
XI
XIa
IX
IXa
VIII
VIIIa
X
Xa
Prothrombin (II)
Thrombin (IIa)
Va
V
Fibrinogen (I)
Fibrin (Ia)
XIIIa
XIII
Cross-linked
fibrin clot
Common
pathway
Trauma
TFPI
VIIa
VII
Tissue factor
Trauma
Antithrombin
X
Active Protein C
Protein S
Protein C + Thrombomodulin


Internal damage to vessel
External tissue damage or other trauma
Coagulation Cascade
Intrinsic pathway
Extrinsic pathway
XII
XI
IX
VIII
Tissue factor
VII
Common pathway
Monitored by Activated Partial Thromboplastin Time (APTT)
Monitored by Prothrombin Time (PT)/ International Normalized Ratio (INR)
X
V
Prothrombin (II)
Thrombin (IIa)
Fibrinogen (I)
Fibrin clot


12
11
9/8
7/TF
APTT
10
5
2
1
PT/INR
fibrin clot


B A A B
Thrombin cleaves FpA and FpB
D E D
Fibrinogen
D E D
Fibrin monomer
Spontaneous polymerization
D E D
D E D D E D
Fibrin polymer
XIIIa cross-links D domains
Stabilized fibrin

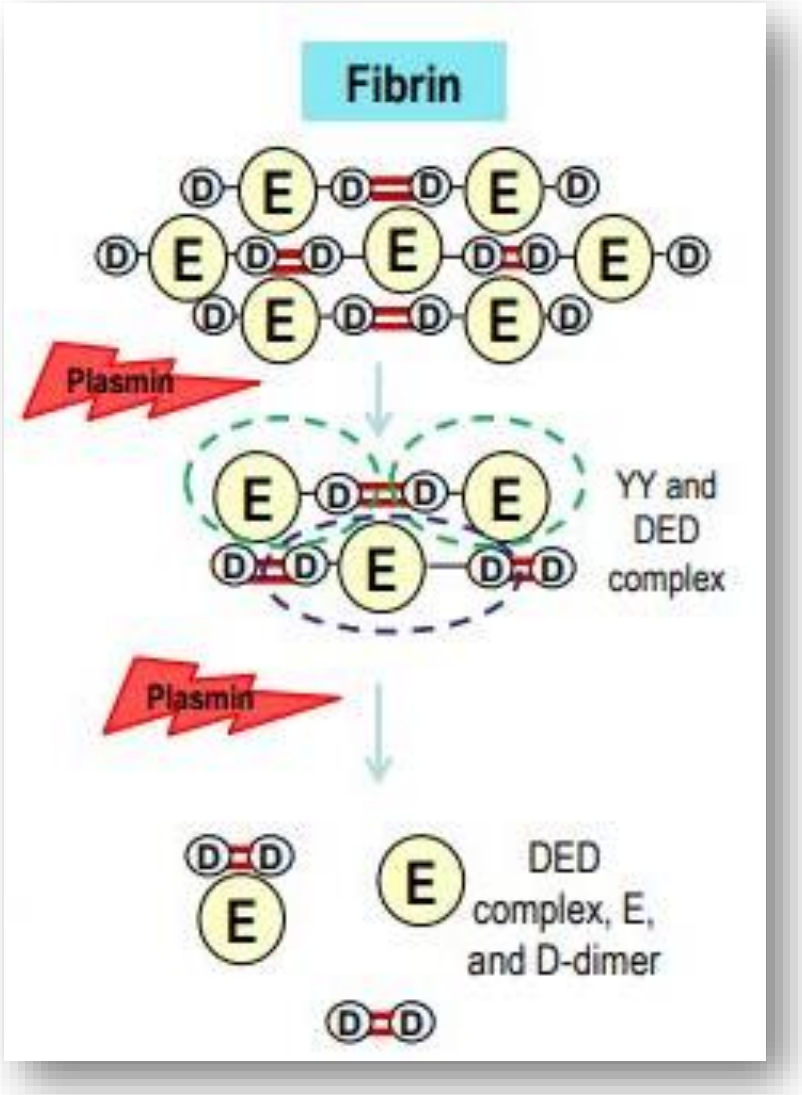
FIBRINOLYSIS

CLOT
BREAKDOWN

FIBRINOLYSIS:
KEY OUTCOMES

1. Enzyme plasmin becomes activated around the time clotting begins.
2. Plasmin digests fibrin, which is cleared by the liver.
3. Fibrin breakdown stays localized to the site of the clot.
4. Normal blood flow resumes as healing takes place.

BREAKDOWN OF FIBRIN BY PLASMIN



DRUGS AND CLOTTING

FOR
TREATMENT
AND
PREVENTION

TYPES OF CLOTS

ARTERIAL

Fast blood flow

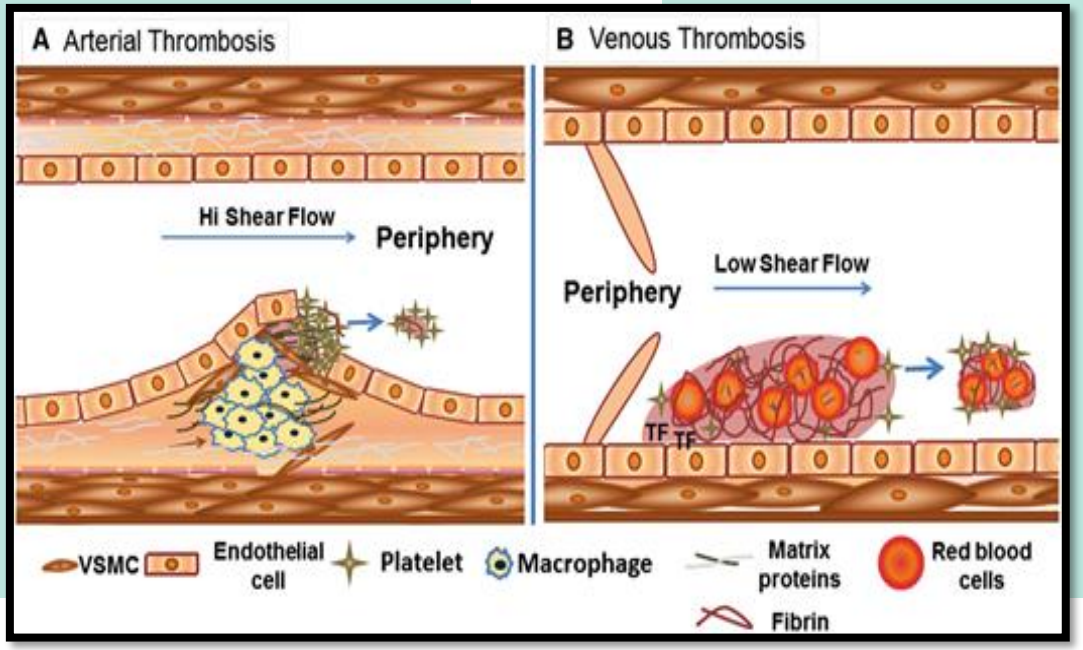
Associated with atherosclerotic plaques and risk factors for CVD

Plaques rupture and cause damage to vascular endothelium, which then attracts platelets.

Platelet aggregates (plus fibrin) may cause embolism or complete vessel occlusion.

- Stroke
- Heart attack

Anti-platelet meds for those at risk



VENOUS

Slow blood flow

Commonly occur in lower extremities

Associated with chronic vascular damage, malignancy, surgery, inactivity, and procoagulant DNA mutations

RBCs become trapped in fibrin mesh as it forms when blood flow is slow or even static.

May embolize or cause complete vessel occlusion

- Pulmonary embolism
- Deep Vein Thrombosis

Anticoagulant meds for those at risk

TWO TYPES OF DRUGS THAT IMPAIR HEMOSTASIS

ANTIPLATELET

Inhibit various steps in the platelet aggregation process, ultimately preventing (or decreasing) platelet activity and primary hemostatic plug formation

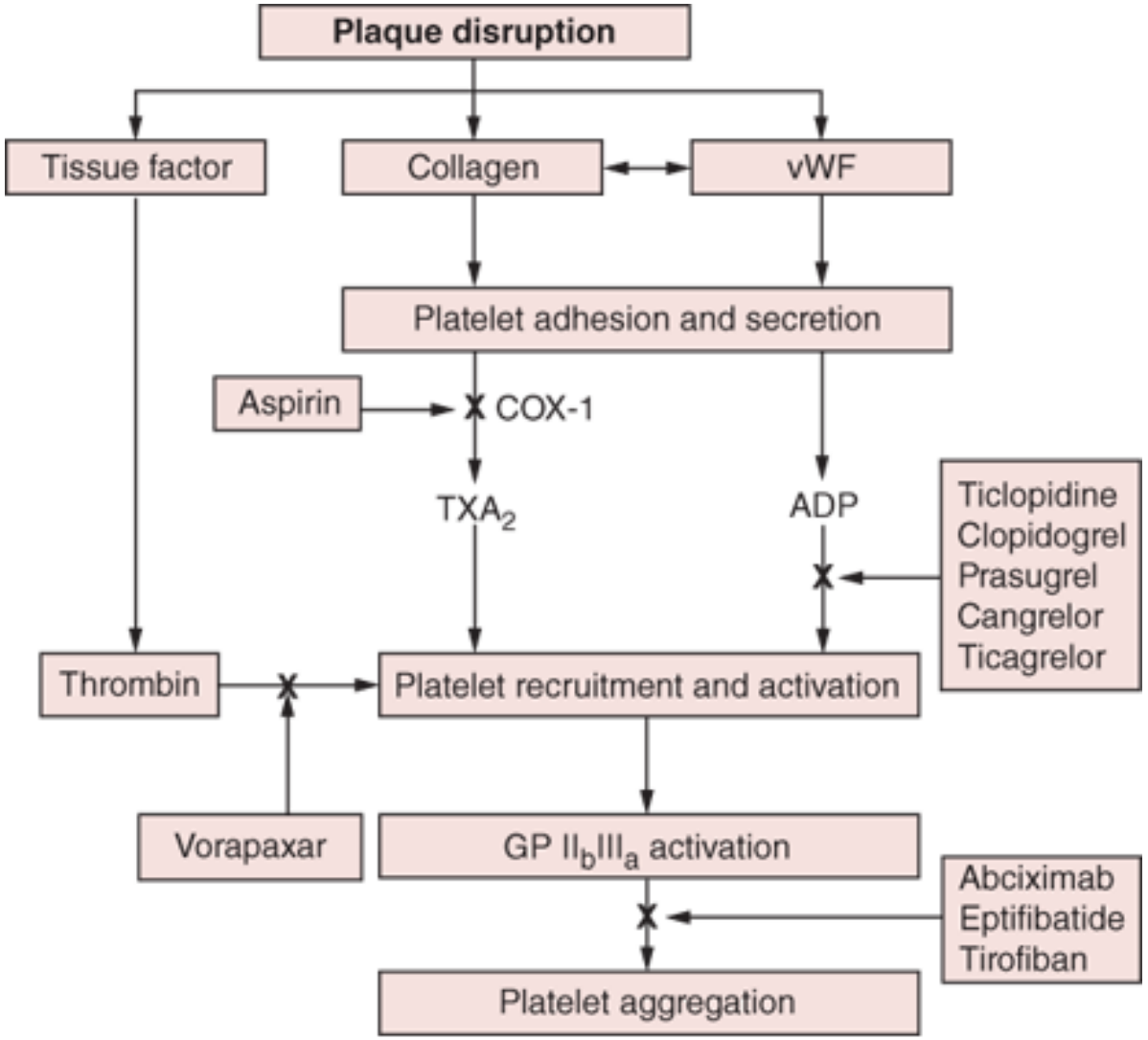
- **Decrease** internal platelet agonist production
- **Decrease** activation of platelets by thrombin

ANTICOAGULANT

Prevent fibrin formation and subsequent stabilization of primary platelet plug

- **Enhance** activity of naturally occurring anticoagulants
- **Prevent** formation of active coagulation factor enzymes
- **Directly inhibit** certain coagulation enzymes

ANTIPLATELET DRUGS



Chapter 3 Antiplatelet Therapy, Crawford MH. CURRENT Diagnosis & Treatment: Cardiology, 6e; 2023.

ANTICOAGULANT DRUGS

Table 1. Anticoagulants categorized by mechanism of action.

Anticoagulation Category	Medication Name(s)	Mechanism of Action	Route(s) of Administration
Vitamin K Antagonists	Warfarin, Acenocoumarol, Phenprocoumon	Inhibition of vitamin K epoxy reductase to decrease the synthesis of vitamin K-dependent coagulation factors	Oral
Heparin (Unfractionated)	Heparin	Inhibition of thrombin and several activated coagulation factors (including Xa) by binding to and enhancing the activity of antithrombin III	Intravenous or Subcutaneous paretneral injection
Heparin (Low Molecular Weight)	Enoxaparin, Dalteparin, Tinxaparin, Nadroparin	Binds to antithrombin III and inhibits thrombin to a much lesser extent than unfractionated heparin; primarily inhibits factor Xa	Subcutaneous parenteral injection
Factor Xa Inhibitors	Fondaparinux *, Rivaroxaban, Apixaban, Edoxaban, Betrixaban	Prevents the cleaving of prothrombin by factor Xa to form thrombin	Fondaparinux- Subcutaneous parenteral injection Rivaroxaban, apixban, edoxaban, betrixaban- Oral
Factor IIa Inhibitors (Direct Thrombin Inhibitors)	Dabigatran, Bivalirudin, Argatroban	Directly binds to and inhibit thrombin	Dabigatran- Oral Bivalirudin- Intravenous Argatroban- Intravenous or Subcutaneous parenteral injection

* Fondaparinux, while technically a synthetic low molecular weight heparin, is considered an indirect factor Xa inhibitor.

McRae, H.L.; Militello, L.; Refaai, M.A. Updates in Anticoagulation Therapy Monitoring. Biomedicines 2021, 9, 262. https:// doi.org/10.3390/biomedicines9030262

LAB TESTING

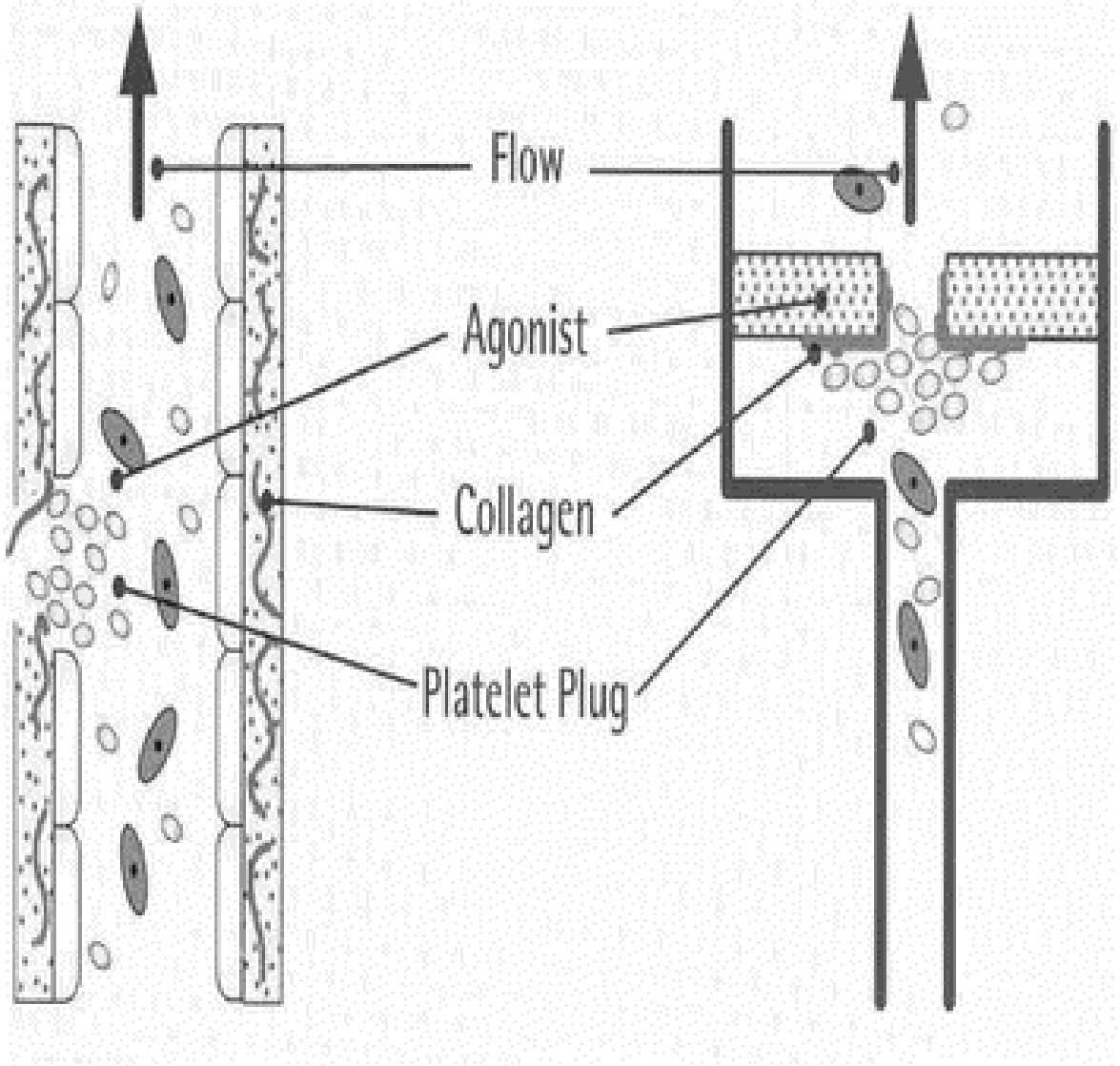
FOR
DETECTION
AND
MONITORING

LAB TESTING FOR HEMOSTASIS

- **PFA** – platelet function assay
- **PT** – prothrombin time
- **INR** – international normalized ratio
- **APTT** – activated partial thromboplastin time
- **ACT** – activated clotting time
- **D=D** – D-Dimer

PLATELET FUNCTION ASSAY (PFA)

- AKA **closure time** assay
  - *In vitro* measurement of primary hemostasis as a whole
  - Has replaced the bleeding time
  - Blood is pulled through a small aperture in a cartridge that is coated with collagen and other **agonists** that simulate a wound and trigger primary hemostasis (platelet adhesion, aggregation, etc)
  - Aperture on analyzer gets blocked by platelet plug formation and “closure time” is recorded
  - Conditions or medications that decrease platelet activity will prolong the closure time.
  - **Normal Range: approx. <120 sec (depending on agonist used)**
  - Patient on anti-platelet meds: >120 seconds (depending on agonist used)

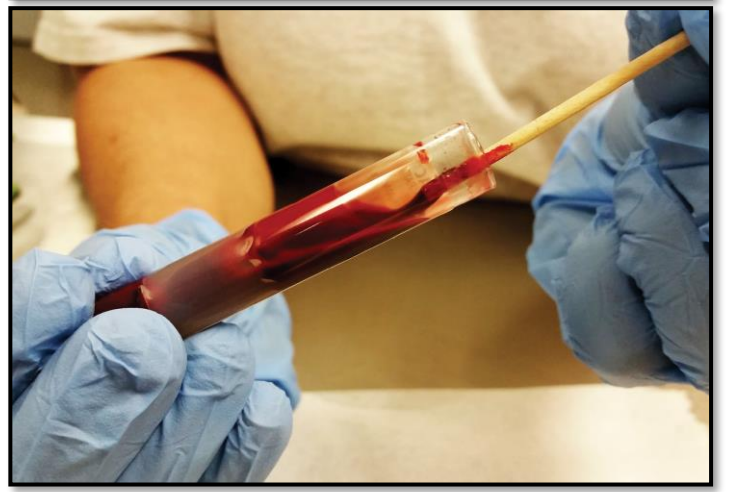


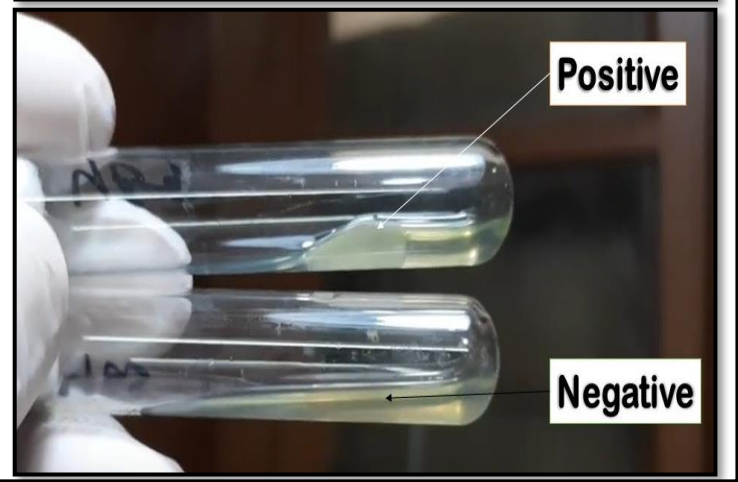
Vessel Injury

Cross section of PFA

CLOTTING TIMES: HOW THEY WORK

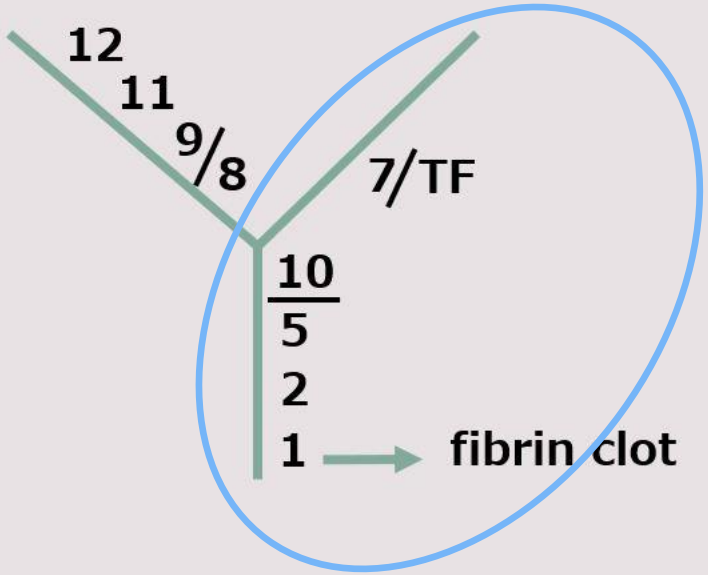
- Assess secondary hemostasis
- Liquid whole blood (or plasma) is combined with reagents to trigger the coagulation cascade of secondary hemostasis
- Timer is simultaneously started
- Analyzer senses when liquid becomes semi-solid/clotted
- Timer stops
- Results typically measured in seconds
- Clotting times greater than the normal range indicate a problem with clot formation OR a patient on anticoagulant therapy





PROTHROMBIN TIME (PT)

- Screening test for inherited and acquired deficiencies in ***extrinsic and common*** pathways of secondary hemostasis
- Also used to monitor long-term Vitamin K antagonist drugs (e.g. warfarin/Coumadin)
  - Warfarin decreases activity of Vitamin K-dependent factors – II, VII, IX, & X
  - Factor VII has the shortest half-life of all factors and is most sensitive to Coumadin
- PT results may vary widely from lab to lab, so INR calculation is done to standardize PT results
- **Normal Range: 12-15 seconds**



INTERNATIONAL NORMALIZED RATIO (INR)

- Calculated using patient's PT results, average normal PT results, and a correction factor

$$\left(\frac{\text{Patient PT}}{\text{Mean normal PT}}\right)^{\text{ISI}}$$ (ISI = International Sensitivity Index)

- **Normal INR: ~1**
- Therapeutic range for hypercoagulable patient on Coumadin: ~2-3
- Example: Patient's PT = 21.5 sec

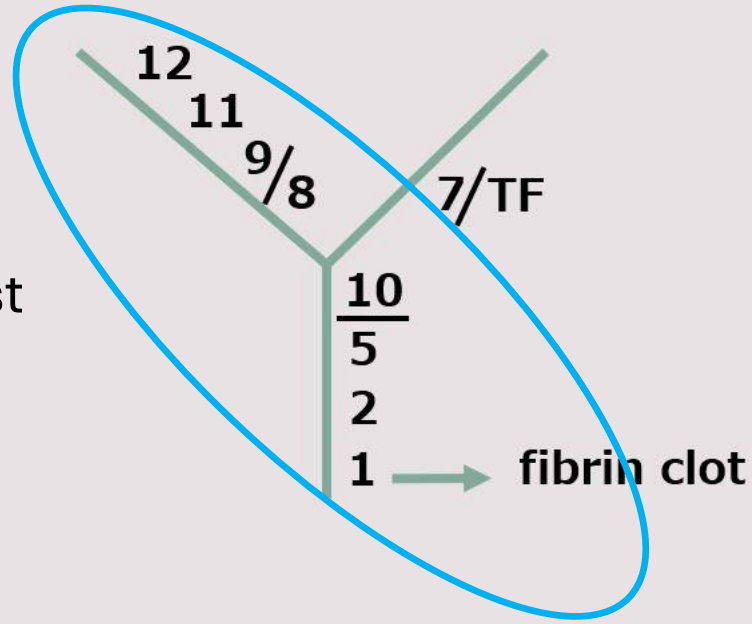
  Mean normal PT = 12.0 sec

  ISI = 1.35

  $INR = (21.5/12.0)^{1.35} = 2.2$

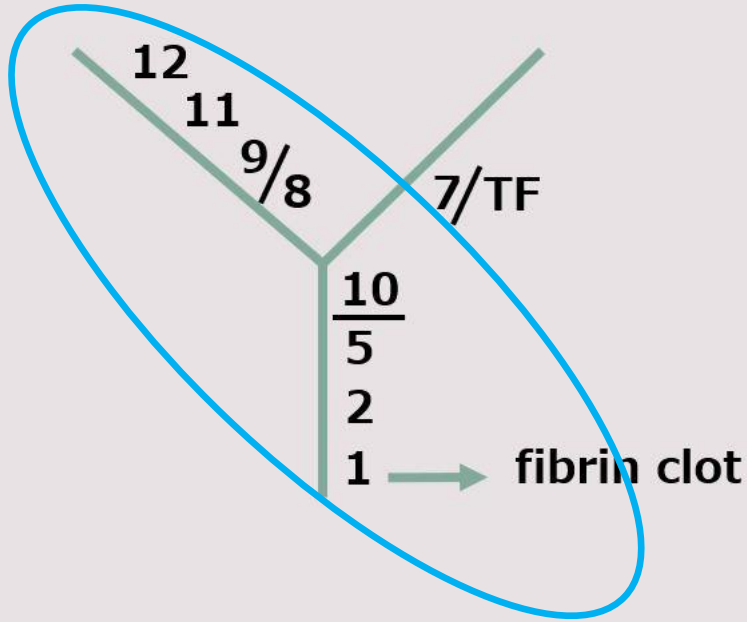
ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

- Screening test for inherited and acquired deficiencies in ***intrinsic and common*** pathways of secondary hemostasis
- Also used to monitor heparin therapy
  - Heparin enhances natural anticoagulant antithrombin (AT), which neutralizes active coagulation enzymes, most of which are part of the intrinsic and common pathways.
- **Normal Range: 25-35 sec**
- Patient on heparin: 60-100 sec



ACTIVATED CLOTTING TIME (ACT)

- Point-of-care test performed before, during, and after medical interventions (at or near bedside) that require high doses of anticoagulants, typically heparin
- Allows for fast turnaround time and dose changes
- Monitors the ***intrinsic and common*** pathways of secondary hemostasis, like the APTT
  - APTT is not designed to test blood that is so highly anticoagulated
  - APTT takes longer to perform than is desirable during such critical interventions
- **Normal Range: 80-130 sec**
- Desired range during cardiopulmonary bypass surgery: >400 sec



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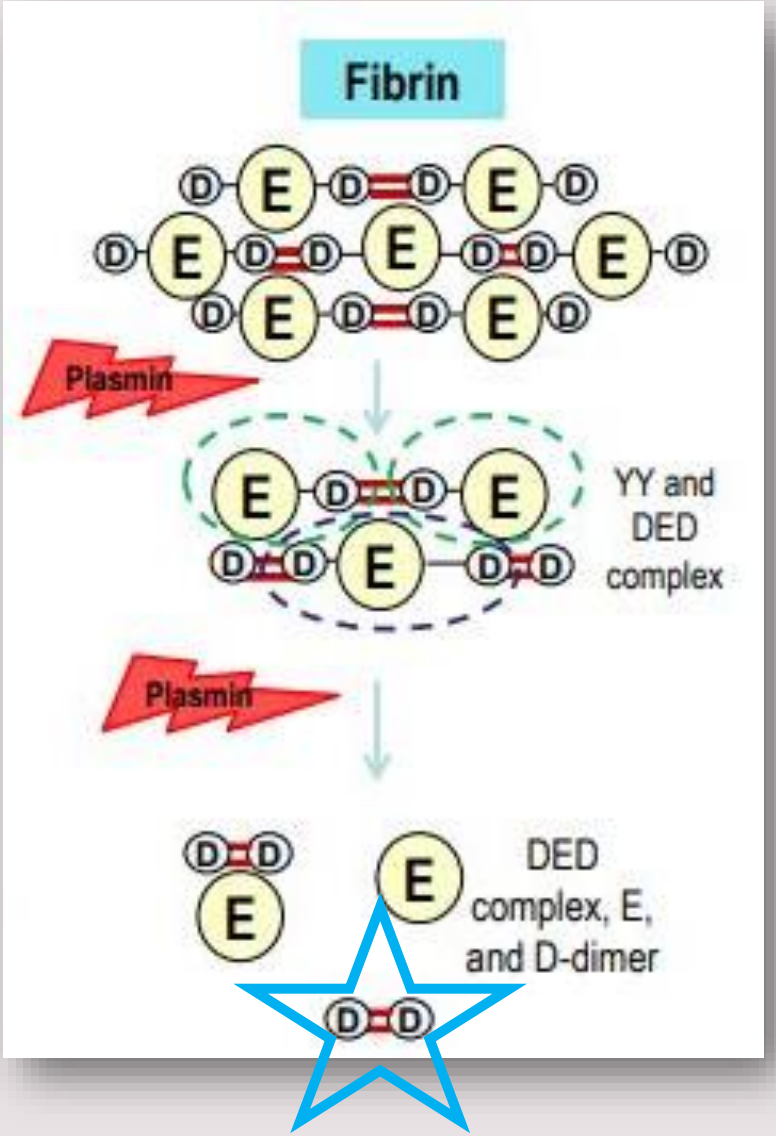
D-DIMER (D=D)

- A specific breakdown product of fibrin that has been digested by plasmin
- Three hemostatic events must occur for D=D to be produced
    1. Clot formation by thrombin
    2. Stabilization by factor XIII
    3. Clot breakdown by plasmin
- Helpful in diagnosis of thrombotic complications like DVT, PE, or DIC (disseminated intravascular coagulation)
- Not as helpful in patients on anticoagulant therapy (false negative) or post-therapeutic procedure (false positive)
- **Normal Range: <0.5µg/mL**
- Elevated in conditions where clotting is occurring /has occurred



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- McRae, H. L., Militello, L., & Refaai, M. A. (2021). Updates in Anticoagulation Therapy Monitoring. *Biomedicines*, *9*(3), 262. https://doi.org/10.3390/biomedicines9030262

QUESTIONS? COMMENTS?

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THANK YOU FOR LISTENING!