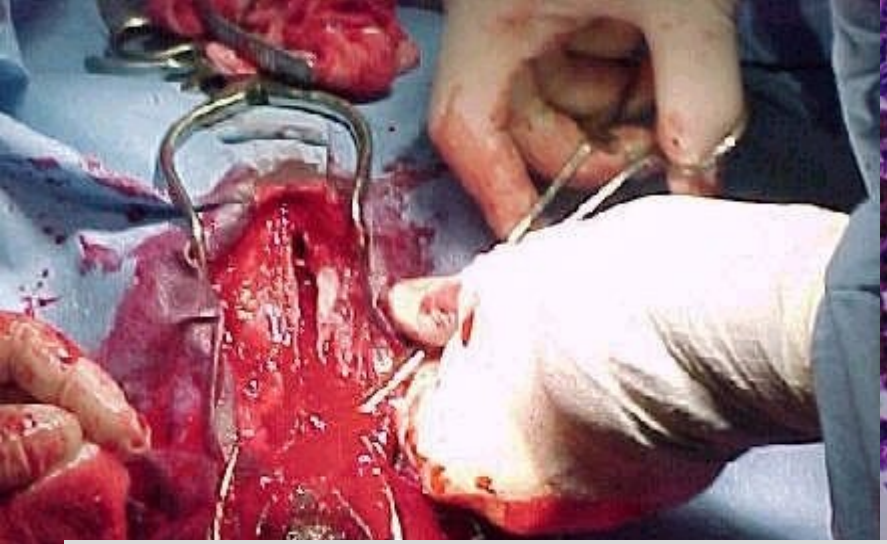
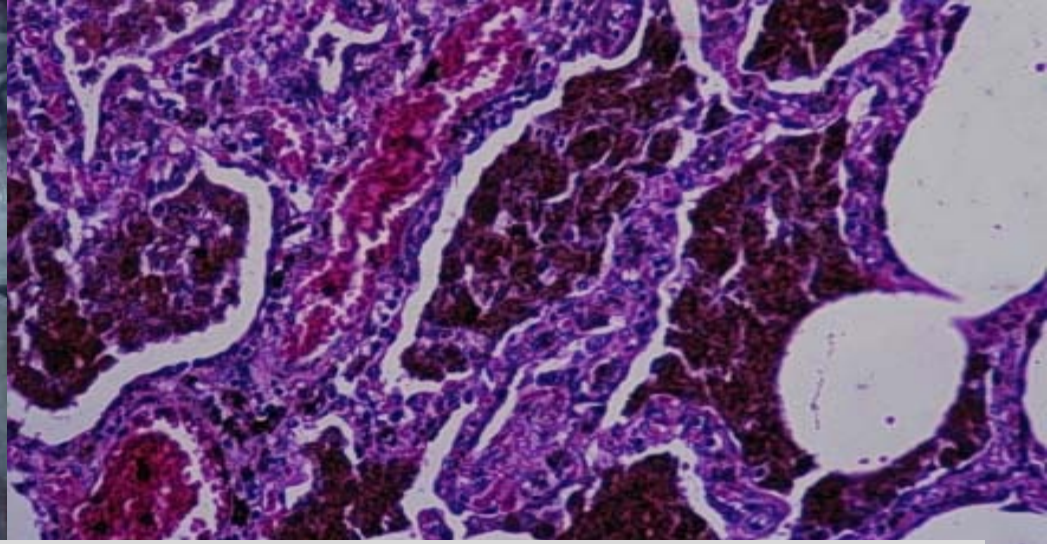
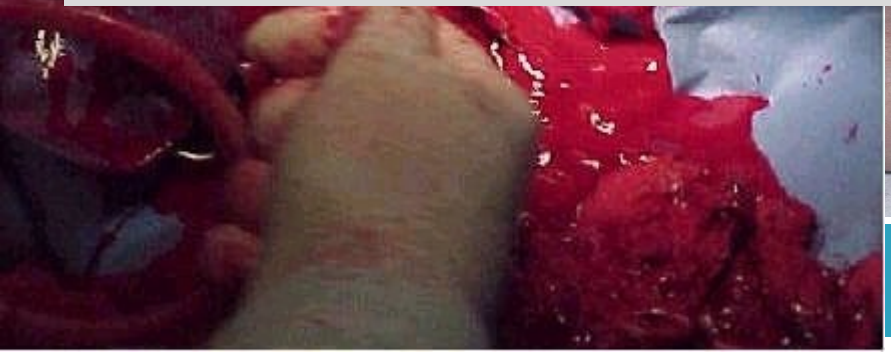
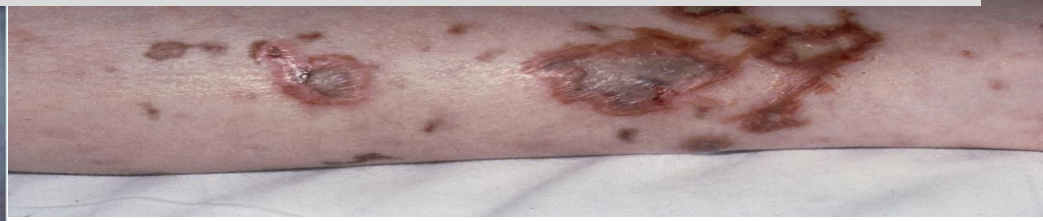




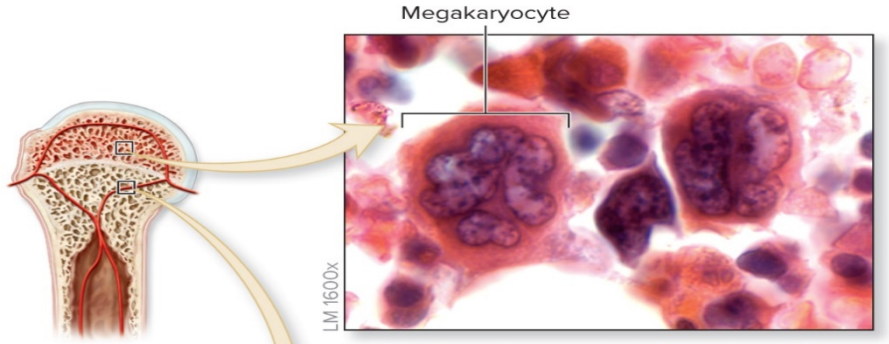
Hemostasis and coagulation



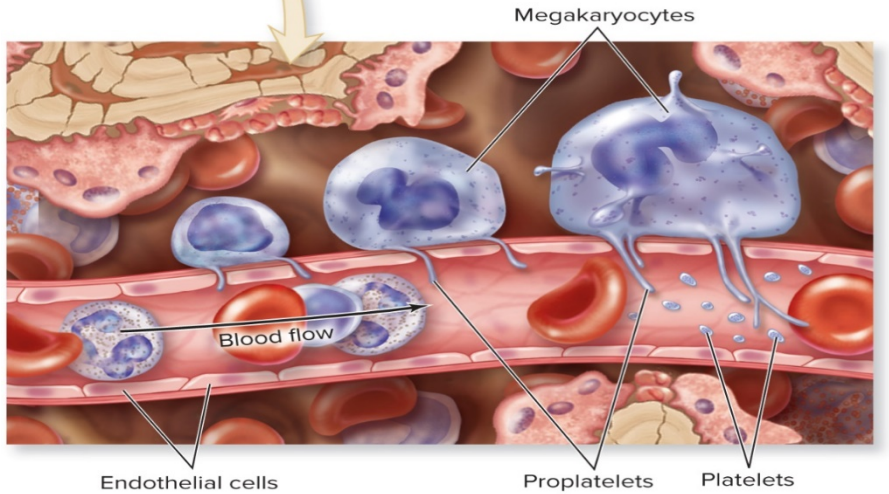


Danil Hammoudi.MD

Figure 18.4



(a) Red bone marrow with megakaryocytes



(b) Platelets forming in blood vessel

(a) ©McGraw-Hill Education/Al Telser

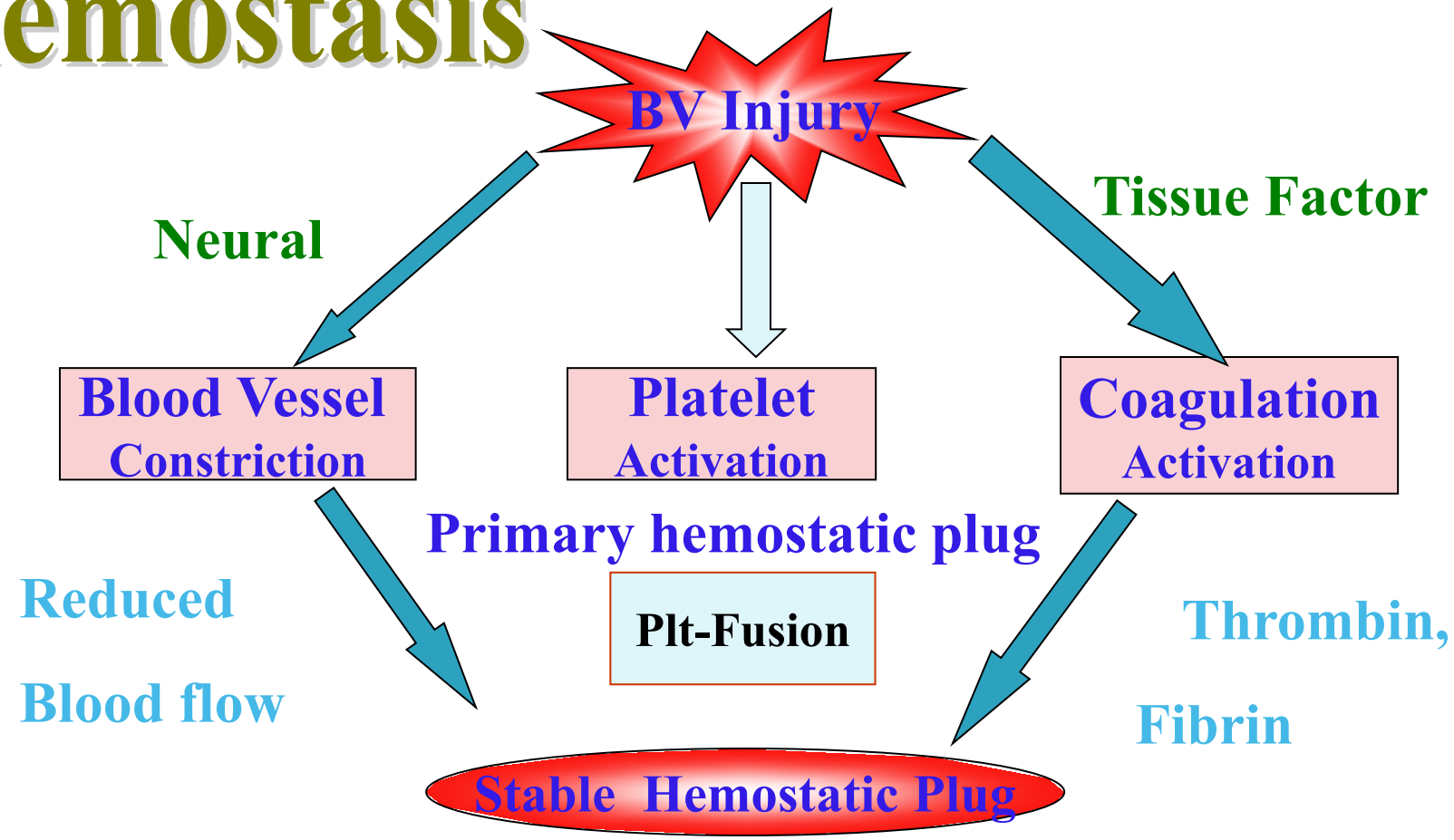
Definition of HEMOSTASIS

- The arrest of bleeding by repair of vessel wall
- Maintaining a balance
  - Coagulation
  - Fibrinolysis
- **Hypocoagulation:** excessive bleeding (inherited or acquired)
- **Hypercoagulation (thrombosis)** inadequate activation of the fibrinolytic system

Systems Involved in Hemostasis

- Vascular system
  - Injured vessel initiates vasoconstriction
- Platelet System
  - Injured vessel exposes collagen that initiates platelet aggregation and help form plug
- Coagulation System
  - protein factors of intrinsic and extrinsic pathways produce a permanent fibrin plug

Hemostasis


BV Injury
Neural
Tissue Factor
Blood Vessel Constriction
Platelet Activation
Coagulation Activation
Primary hemostatic plug
Plt-Fusion
Reduced Blood flow
Thrombin, Fibrin
Stable Hemostatic Plug

HEMOSTASIS & THROMBOSIS

- Platelets
- Coagulation Cascade
- Regulation of Coagulation
- Disseminated Intravascular Coagulation

HEMOSTATIC DISORDERS

Suspicions

- Spontaneous bleeding
- Prolonged or excessive bleeding after procedures or trauma
- Simultaneous bleeding from multiple sites

HEMOSTASIS

Primary vs. Secondary vs. Tertiary

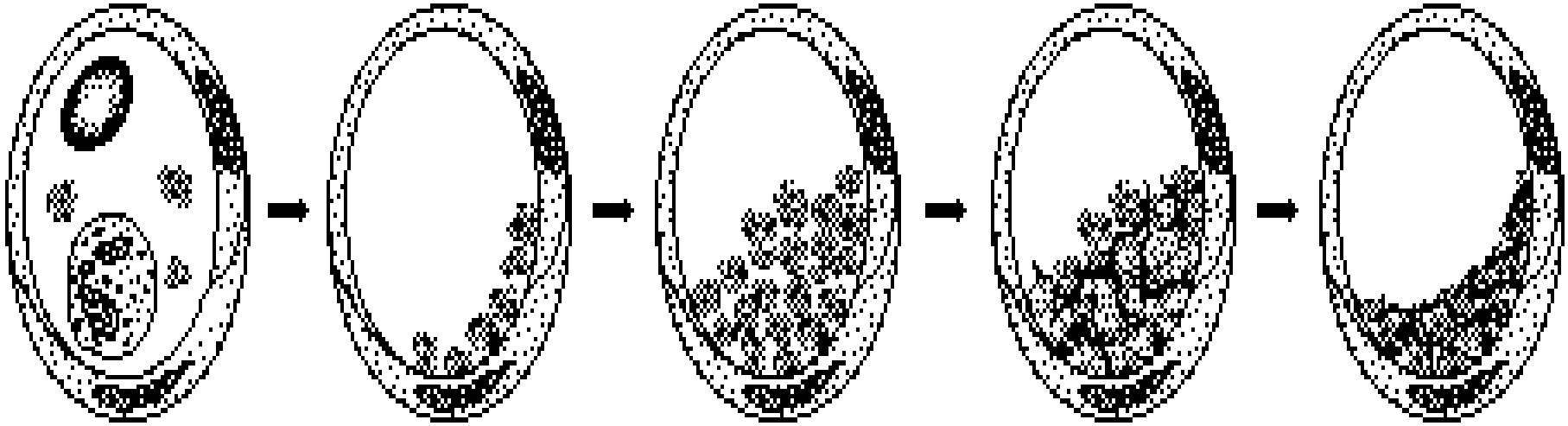
- Primary Hemostasis
  - Platelet Plug Formation
  - Dependent on normal platelet number & function
  - Initial Manifestation of Clot Formation

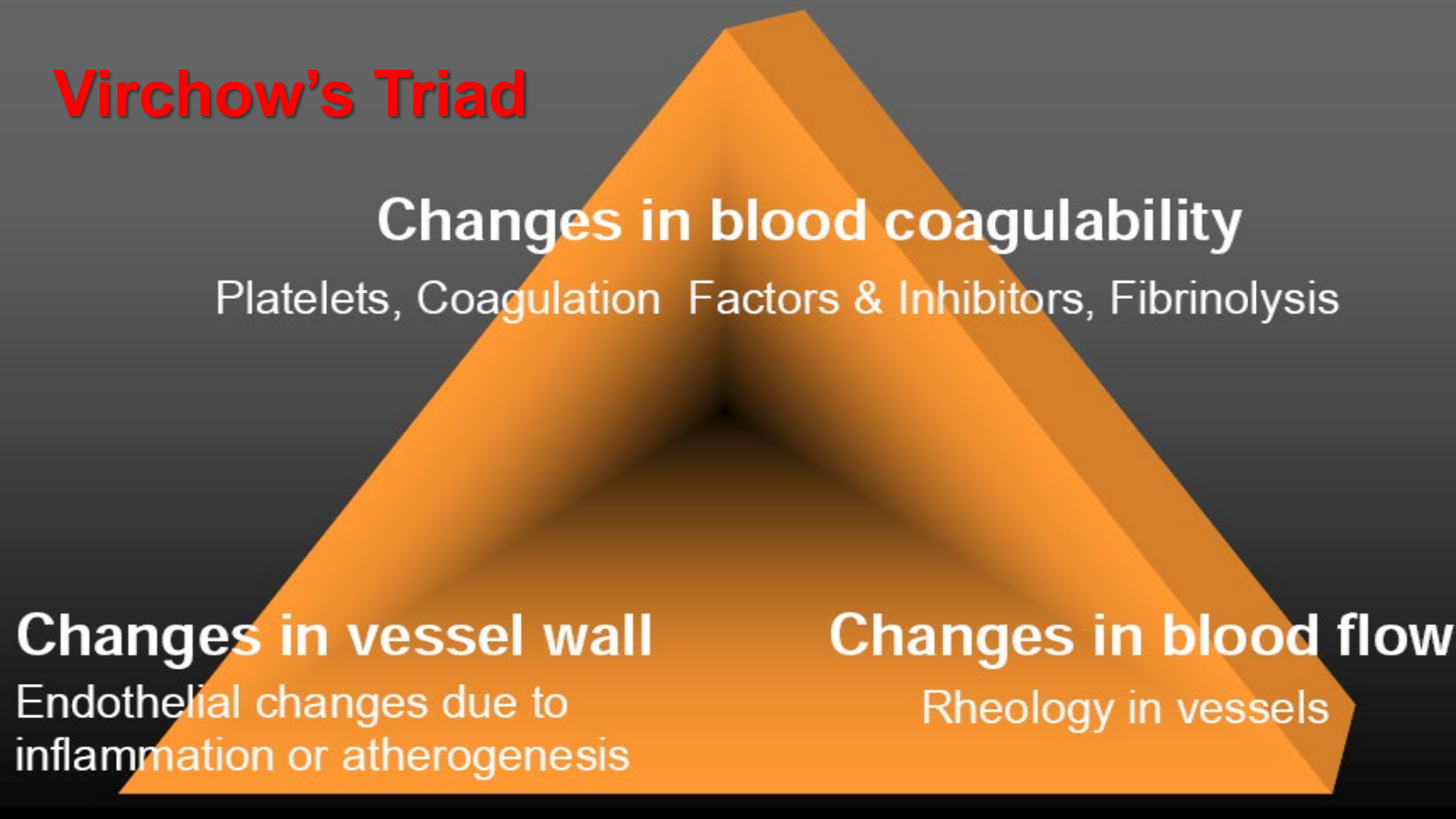
Secondary Hemostasis

- Activation of Clotting Cascade ☒ Deposition & Stabilization of Fibrin

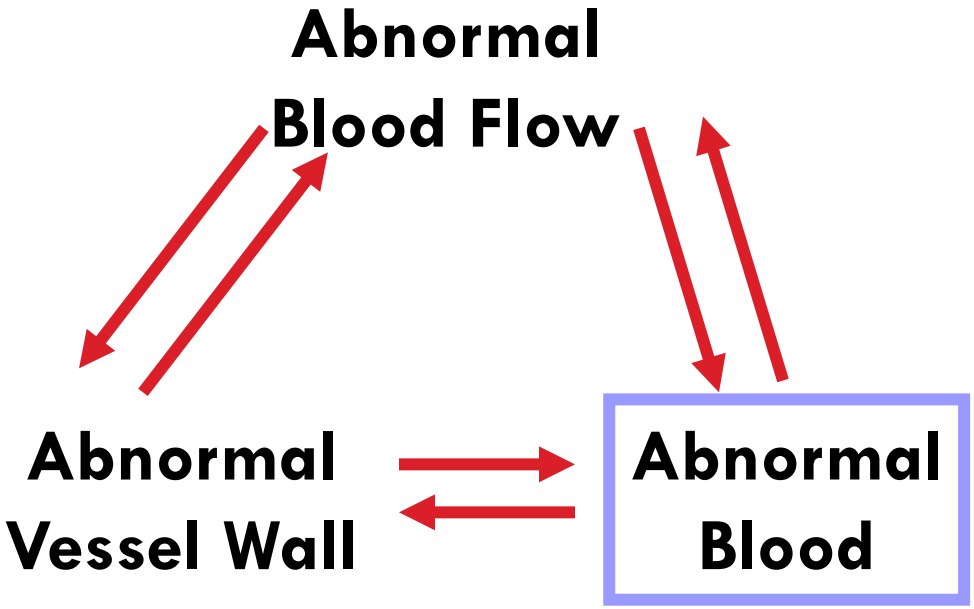
Tertiary Hemostasis

- Dissolution of Fibrin Clot
- Dependent on Plasminogen Activation


Circulating platelets; endothelial damage
Platelet adhesion
Platelet aggregation
Platelet-Fibrin plug
Clot retraction


Virchow's Triad
Changes in blood coagulability
Platelets, Coagulation Factors & Inhibitors, Fibrinolysis
Changes in vessel wall
Endothelial changes due to inflammation or atherogenesis
Changes in blood flow
Rheology in vessels

Dr. Rudolph Virchow
1821-1902



The Hypercoagulable State

Cause – Bleeding Diathesis

Acquired

- Anticoagulation with warfarin / heparin
- Liver failure / Vitamin K deficiency / DIC
- Snake venom e.g Rattle snake, viper
- Viral hemorrhagic fever
- Leukemia

Autoimmune

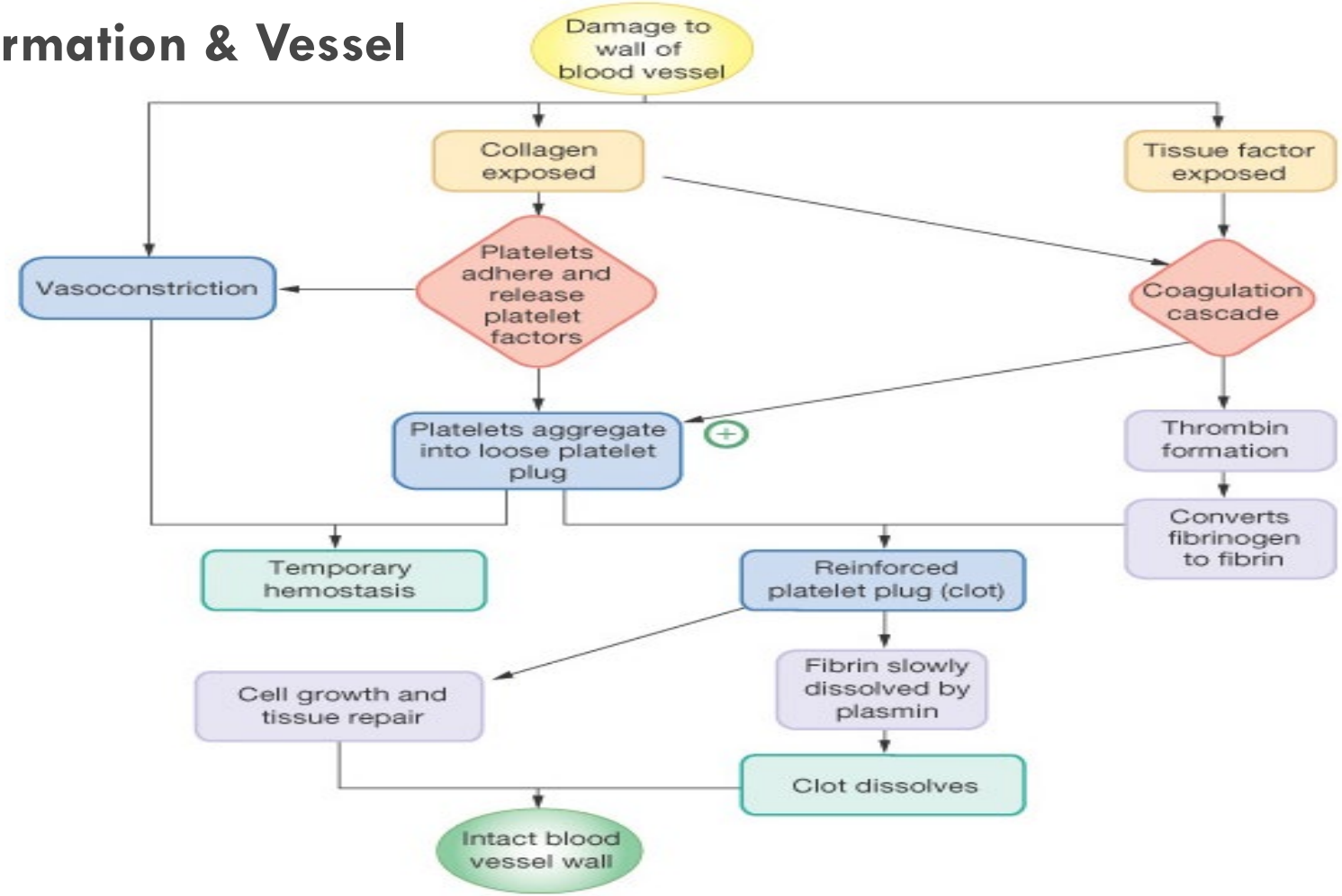
- Acquired antibodies to coagulation factors
- Inhibitor directed
  - Against Factor VIII
  - Antiphospholipid

Genetic

Lack of coagulation factor protien producing genes

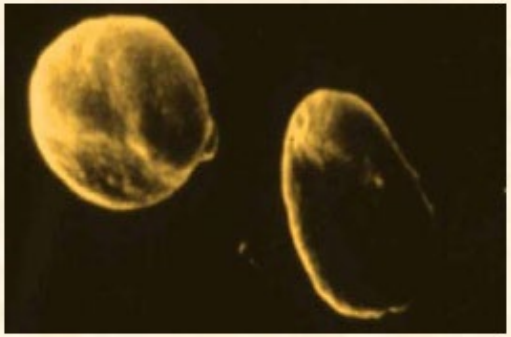
- Hemoplilia (VIIIA, IXB deficiency)
- Von willebrand (protein regured for platlet adhesion)
- Bernard souller (GpIb), the receptor for vWF)
- Wiskott Aldrich (autoimmune haemolytic anaemia-defects in homeostasis)
- Glenzmann thromasthenia (platelets lack GP IIb/IIIa. Hence, no fibrinogen bridging

Overview of Hemostasis: Clot Formation & Vessel Repair

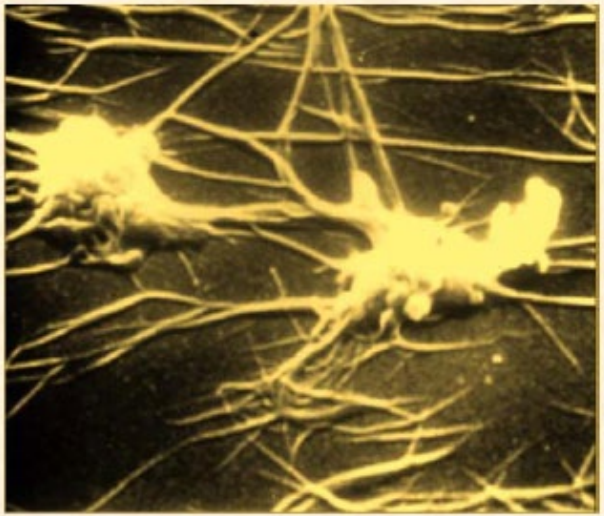


Hemostasis

- A series of reactions for stoppage of bleeding
- During hemostasis, three phases occur in rapid sequence
  - **Vascular spasms – immediate vasoconstriction in response to injury**
  - **Platelet plug formation**
  - **Coagulation (blood clotting)**



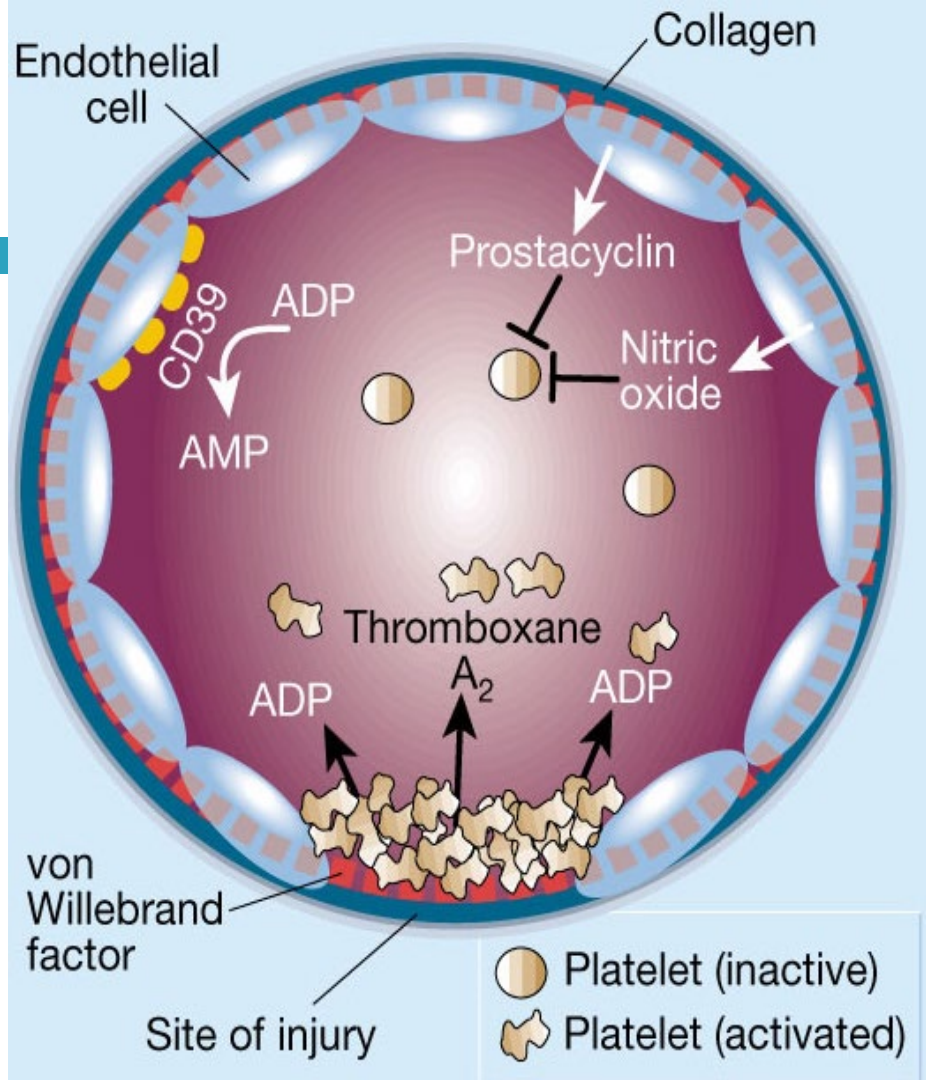
Resting platelets



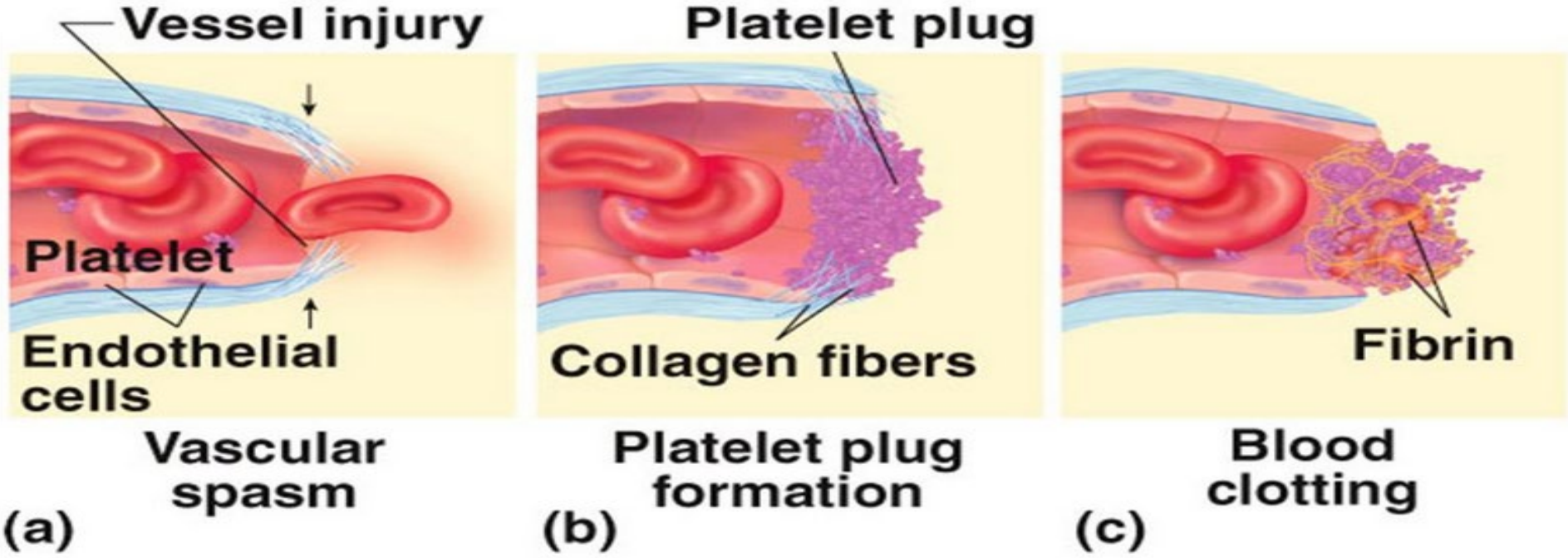
Activated platelets

Platelet Plug Formation

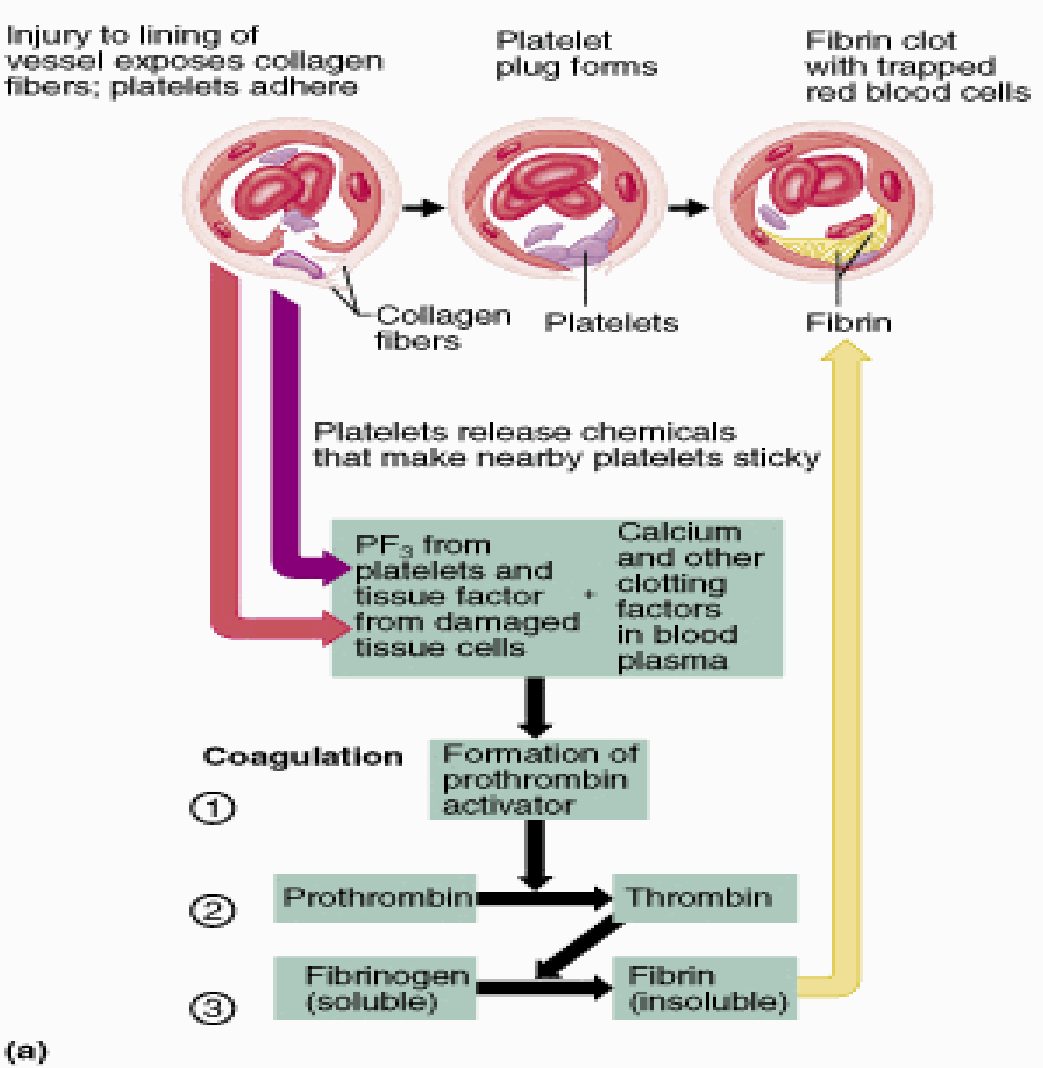
- Platelets do not stick to each other or to blood vessels
- Upon damage to blood vessel endothelium platelets:
  - **With the help of von Willebrand factor (VWF) adhere to collagen**
  - **Are stimulated by thromboxane A2**
  - **Stick to exposed collagen fibers and form a platelet plug**
  - **Release serotonin and ADP, which attract still more platelets**
- ***The platelet plug is limited to the immediate area of injury*** by **prostacyclin**



Steps of Hemostasis



https://www.youtube.com/watch?v=HFNWGCx_Eu4



Platelets release

↓

PF3

tissue factor other clotting factors

↓

Prothrombin activator is formed,

Activator transforms prothrombin

↓

Prothrombin becomes thrombin

↓

catalyzes fibrinogen activates factor XIII

↓

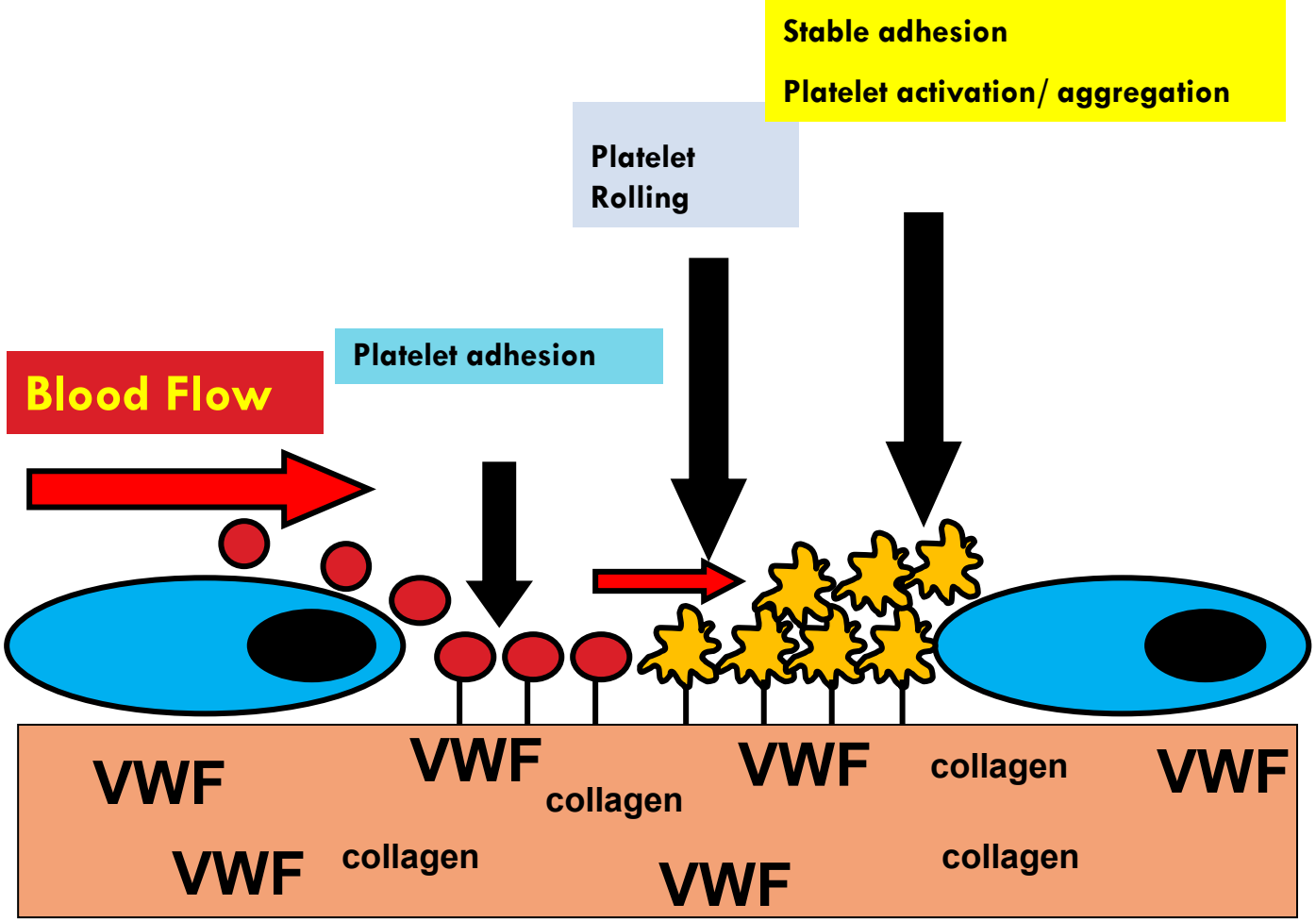
fibrinogen becomes fibrin

↓

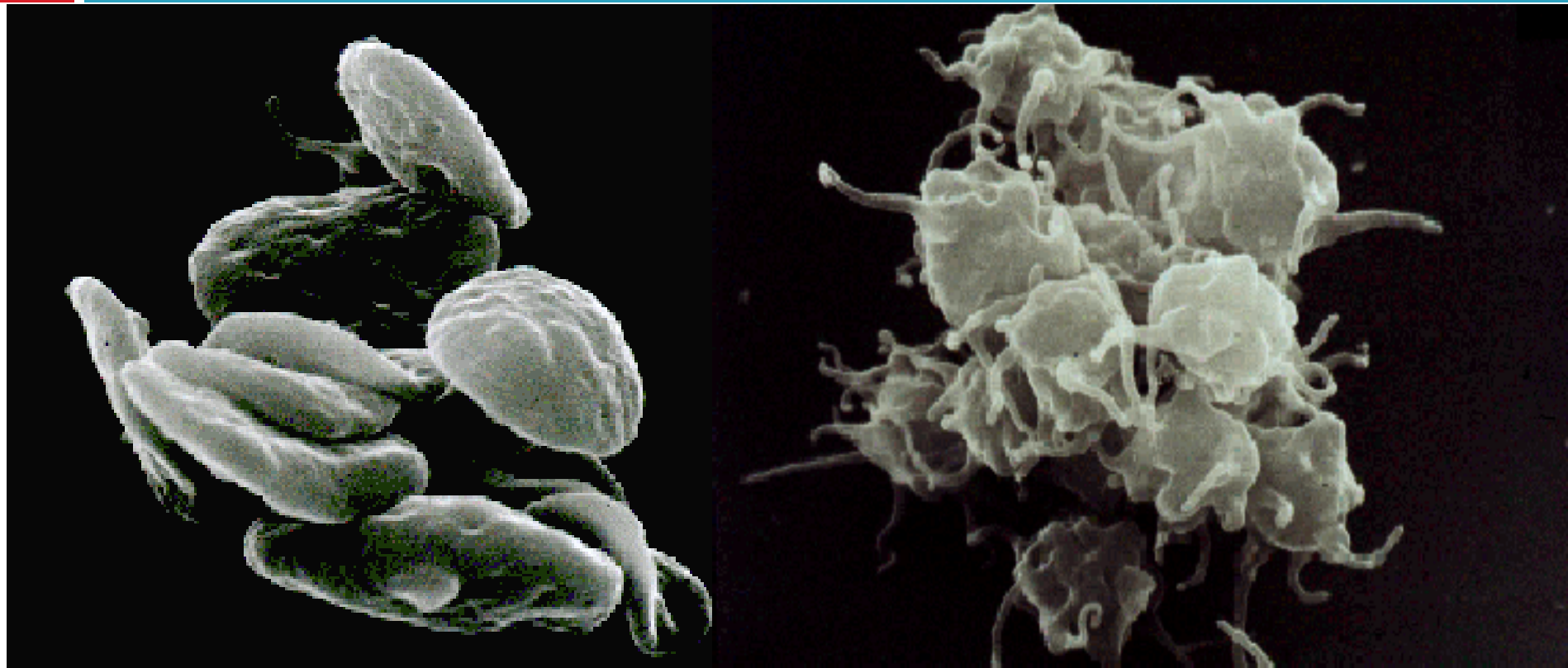
fibrin stabilizing factor

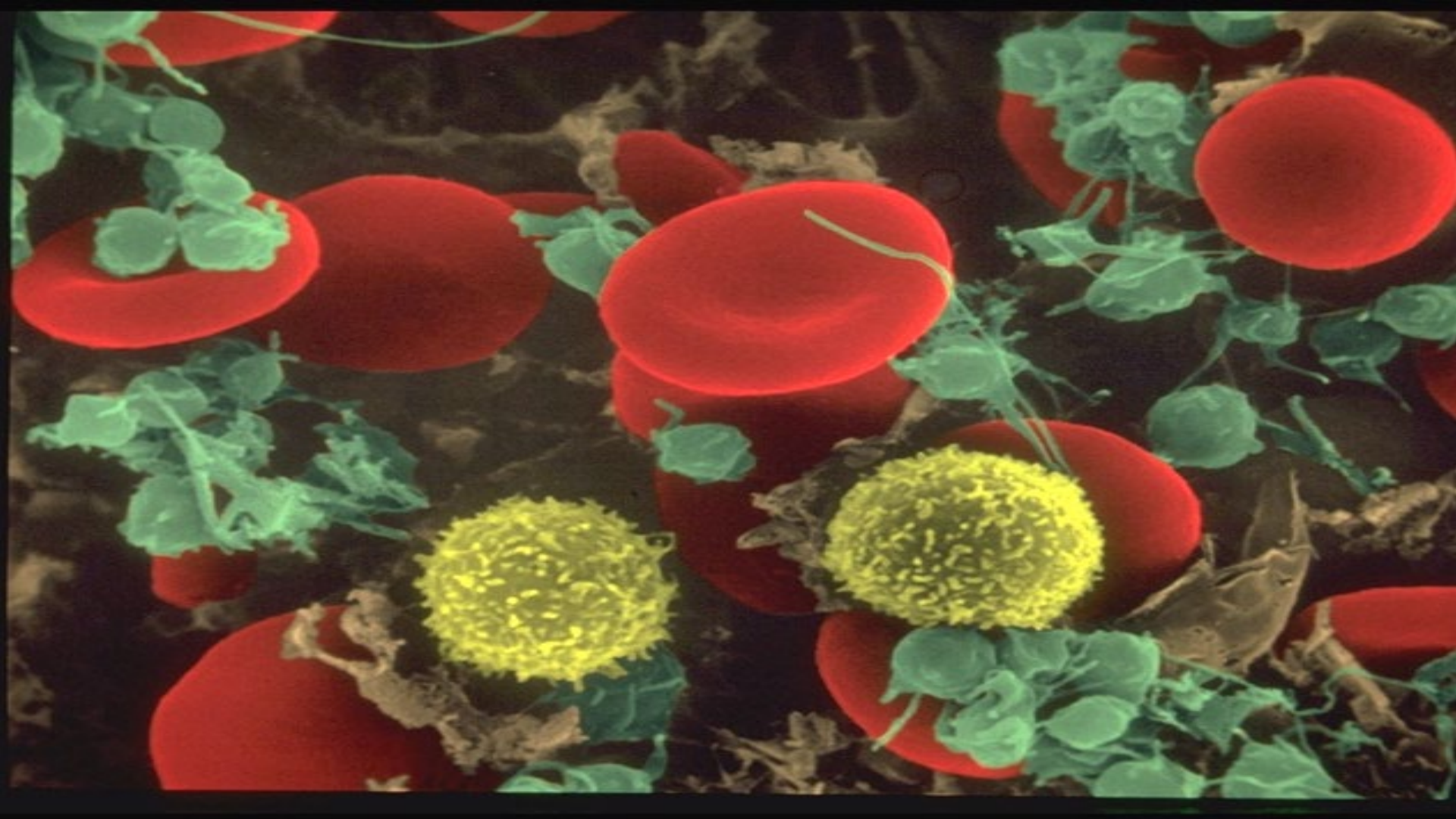
Fibrin Mesh Forms

Clot Forms


Stable adhesion
Platelet activation/ aggregation
Platelet
Rolling
Platelet adhesion
Blood Flow
VWF
VWF
collagen
VWF
collagen
VWF
VWF
collagen
VWF
collagen

Inactive *Active*





Antithrombotic Properties of the Endothelium

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

Antithrombotic Properties of the Endothelium

Anticoagulant properties

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

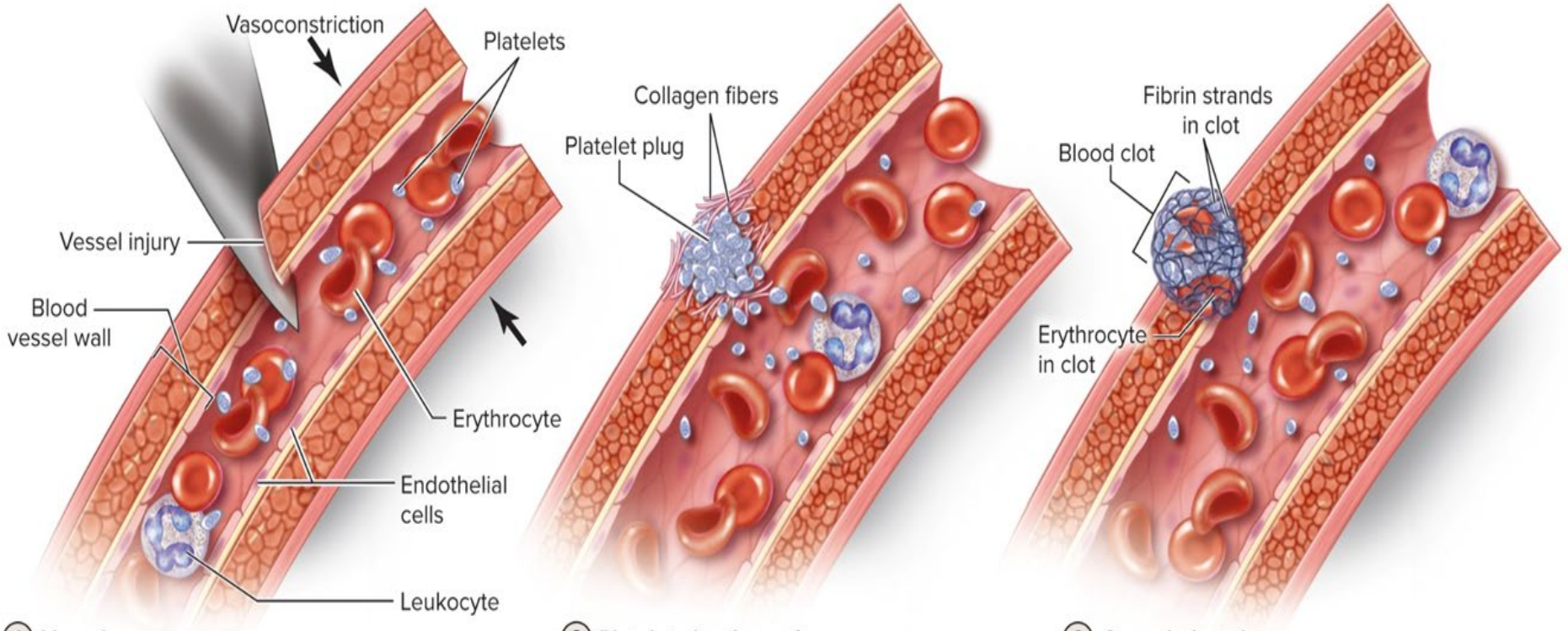
*__THROMBOMODULIN__: changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa

*Endothelial cells produce **tPA** which activates fibrinolysis via plasminogen to plasmin

Prothrombotic Properties of the Endothelium

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

Hemostasis



1 Vascular spasm
Blood vessel constricts to limit blood escape.

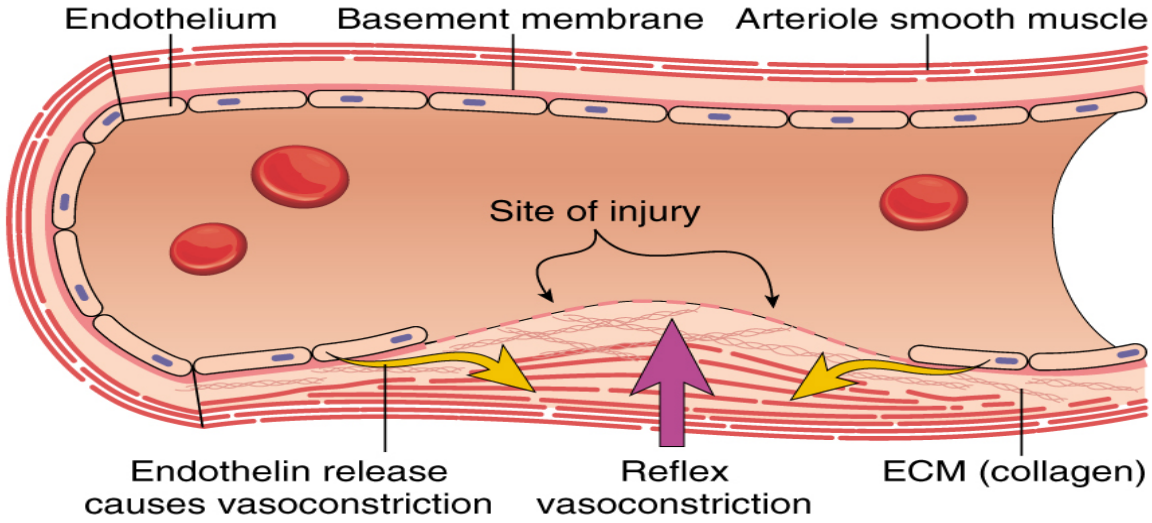
2 Platelet plug formation
Platelets arrive at site of injury and stick to exposed collagen fibers.

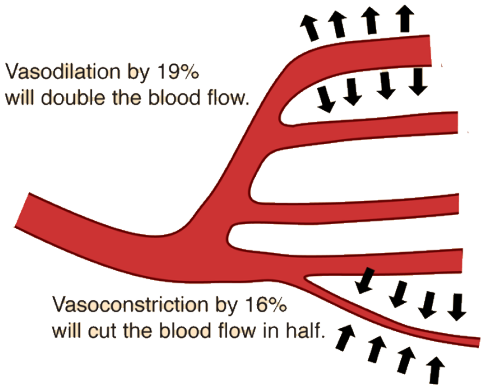
3 Coagulation phase
Coagulation cascade converts inactive proteins to active forms, which ultimately forms fibrin strands of a blood clot.

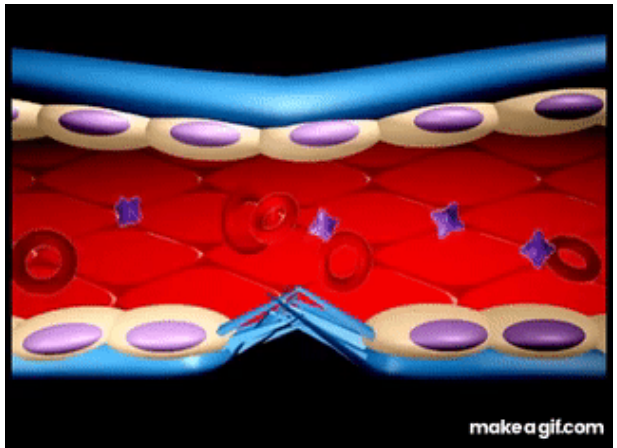
Glycocalyx

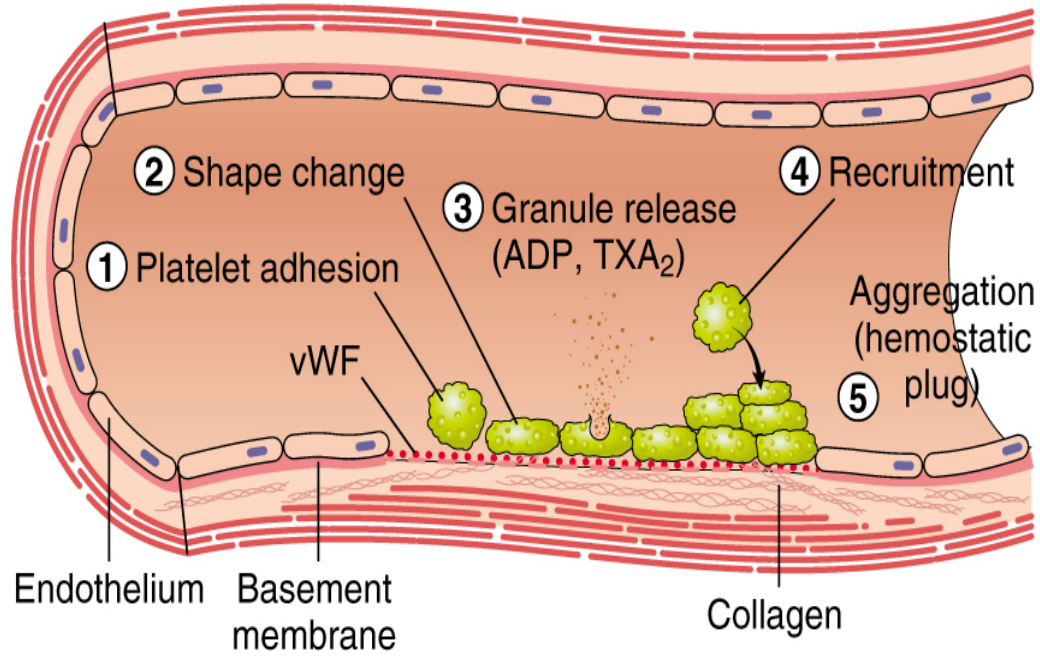
- Glycoproteins
  - Ib (GPIb)
    - Receptor site for vWF
  - IIb, IIIa (GPIIb/IIIa)
    - Complex becomes receptor site for fibrinogen

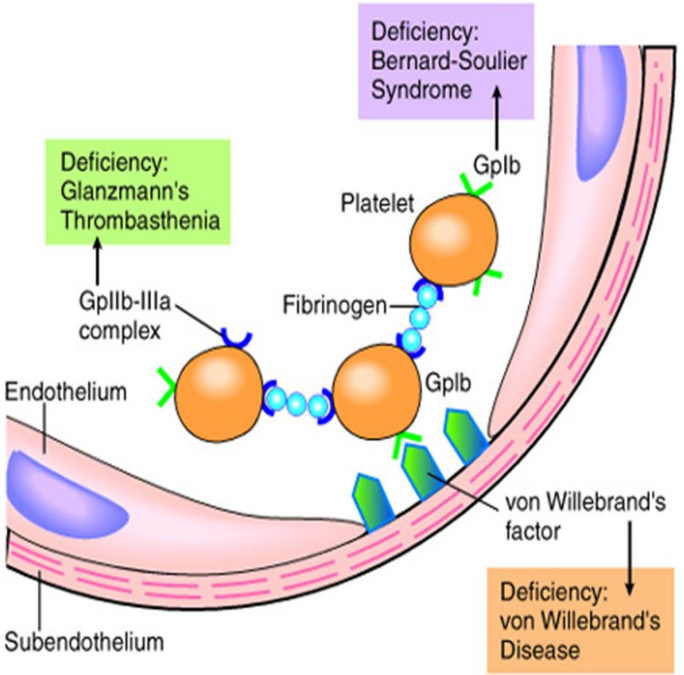
A. VASOCONSTRICTION





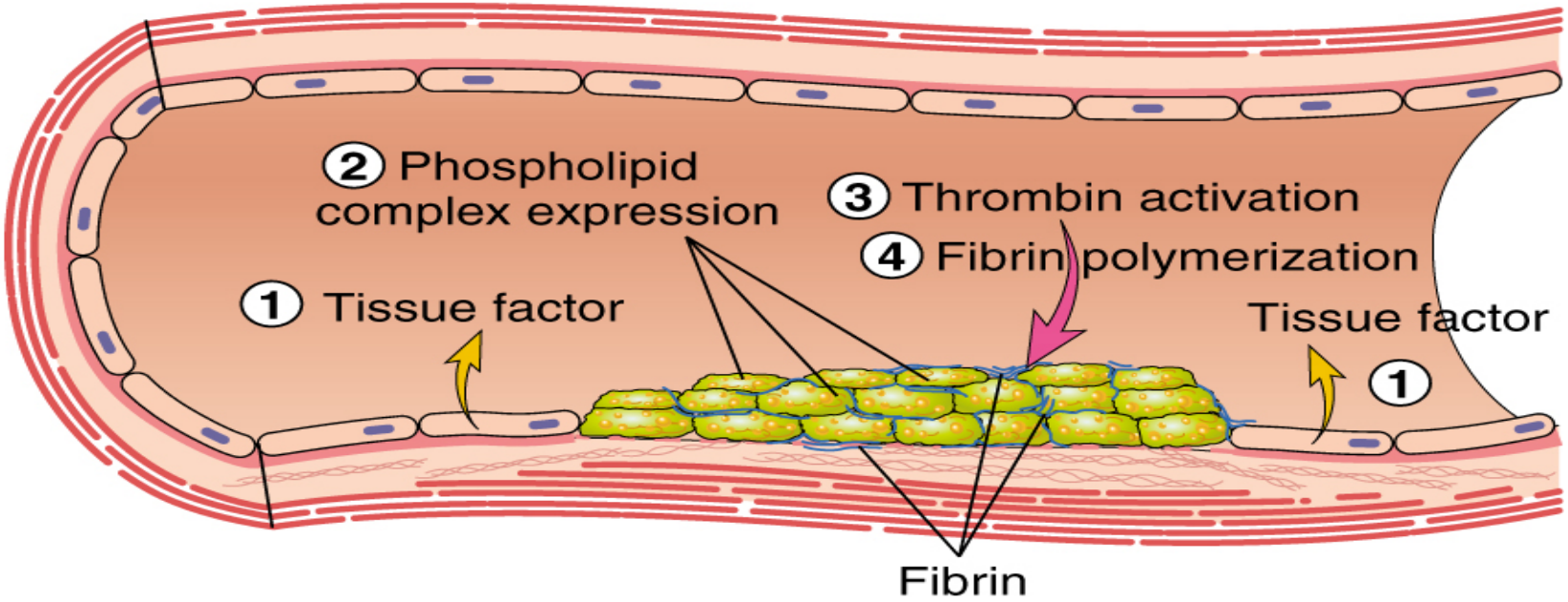






https://www.youtube.com/watch?v=R8JMfbYW2p4

C. SECONDARY HEMOSTASIS

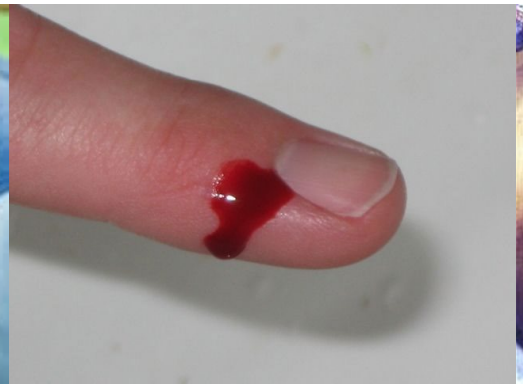
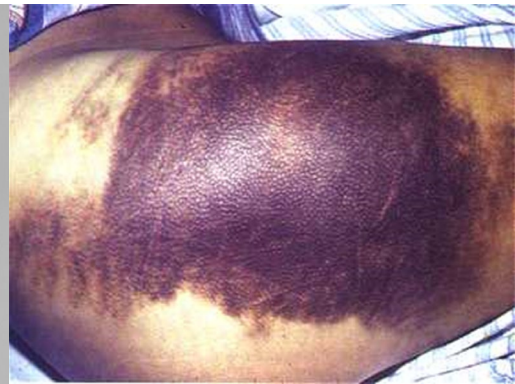


https://www.cast-pharma.com/portfolio/hemophilia/

Coagulation

- A set of reactions in which blood is transformed from a liquid to a gel
- **Coagulation follows intrinsic and extrinsic pathways**
- The final three steps of this series of reactions are:
  - **Prothrombin activator is formed**
  - **Prothrombin is converted into thrombin**
  - **Thrombin catalyzes the joining of fibrinogen into a fibrin mesh**



Secondary hemostasis

- **Intrinsic Pathway**
  - All components required for initiating this pathway are circulating in the blood
  - **triggered by contact with collagen or glass**
- **Extrinsic Pathway**
  - Initiated by the **release of tissue thromboplastin and calcium from damaged tissue**
- **Common Pathway**
  - Leads to clot formation including the platelet plug and fibrin produced

Coagulation Proteins

- Zymogens
  - enzyme precursors II, VII, IX, X, XI, XII, Prekallkrein
  - When activated become serine proteases
- Cofactors
  - Nonenzymatic V, VIII, HMWK, Tissue factor(thromboplastin)
- Kinin factors prekallikrein, kallikrein, HMWK
  - Roles include coag activation as well as fibrinolytic activation

Coag factors (by group)

- Fibrinogen group: I,V,VIII,XIII
  - most labile, are consumed in coagulation, found on platelets
- Prothrombin group: II,VII,IX,X
  - Vitamin K dependent, may be affected by coumarin,diet, antibiotics
- Contact group: XI,XII,HMWK, Prekallikrein
  - initiate intrinsic path and fibrinolysis

Table 45.2 Proteins of blood coagulation

Factor	descriptive name	function/active form
Coagulation factors		
I	Fibrinogen	Fibrin
II	Prothrombin	**Serine protease**
III	Tissue factor	Receptor, cofactor
IV	Ca^{2+}	Cofactor
V	Proaccelerin, labile factor	Cofactor
VII	Proconvertin	**Serine protease**
VIII	**Antihemophilia factor A**	Cofactor
IX	Antihemophilia factor B	**Serine protease**
X	Stuart-Prower factor	**Serine protease**
XI	Plasma thromboplastin antecedent	**Serine protease**
XIII	Fibrin-stabilizing factor	Ca^{2+}-dep transglutaminase
Regulatory factors		
Thrombomodulin	endothelial cell receptor, binds thrombin	
Protein C	activated by thrombomodulin-bound thrombin; serine protease	
Protein S	cofactor; binds activated protein C	

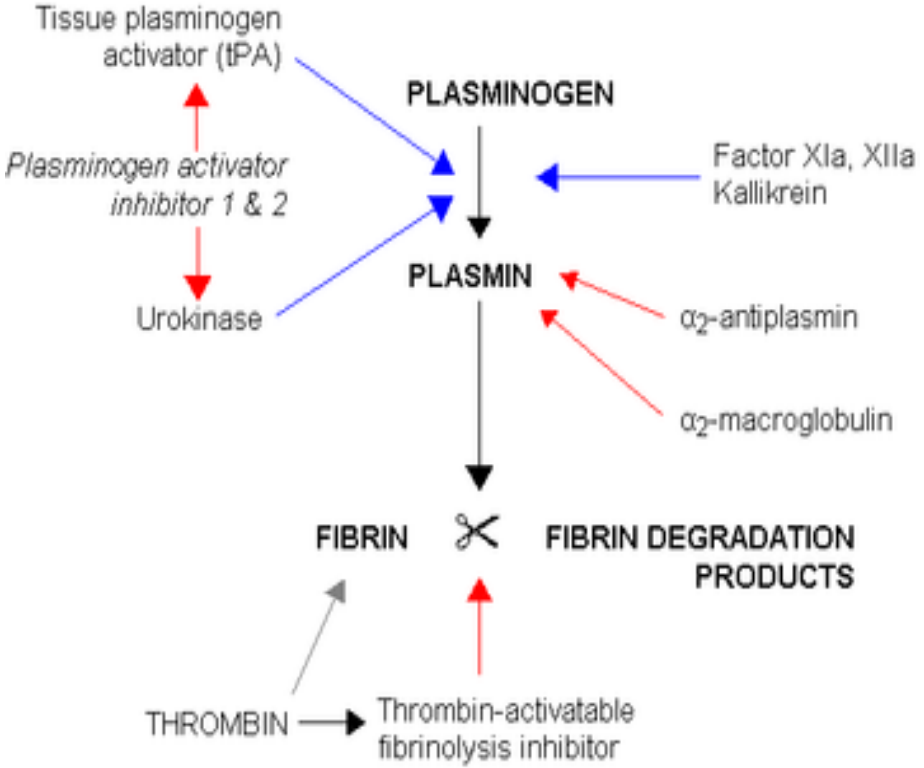
Table 18.8 Clotting Factors

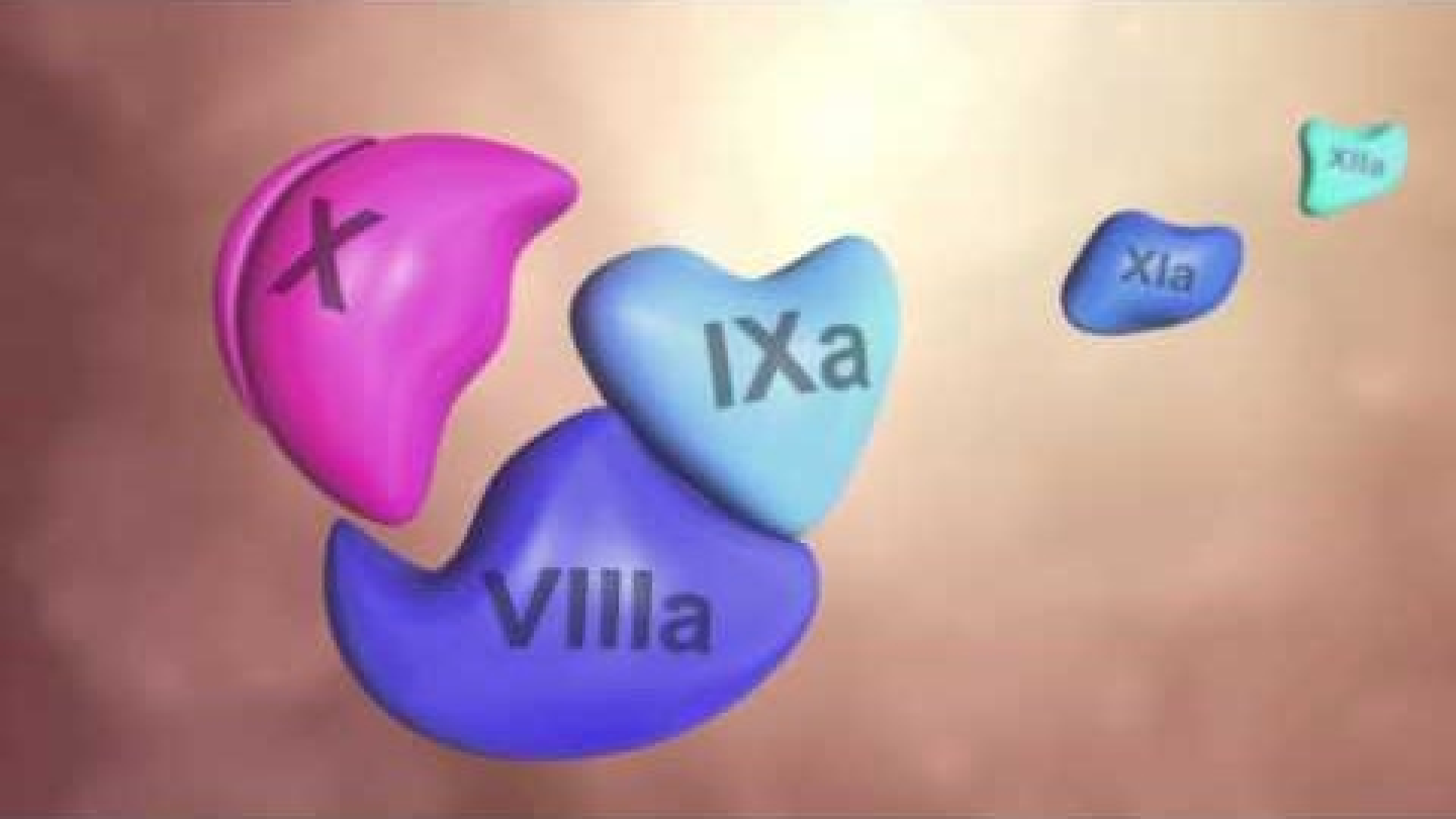
Factor Designator[1]	Name	Function	Extrinsic or Intrinsic Pathway	Clinical Syndrome (If Clotting Factor Is Deficient)
I	Fibrinogen	Activated to fibrin	Both	Afibrinogemia (autosomal recessive disorder); during pregnancy can cause premature separation of placenta
II	Prothrombin	Protease; activated to thrombin	Both	Hypoprothrombinemia (autosomal recessive disorder); decreased synthesis in liver generally due to insufficient vitamin K (Note that mutation in the prothrombin gene causes hypercoagulation problems.[2])
III	Tissue factor (thromboplastin)	Cofactor; activates factor VII	Extrinsic	None known
IV	Calcium	Ion essential to both pathways	Both	None known
V	Proaccelerin	Cofactor; activates factor VII; combines with factor X to form prothrombin activator	Both	Parahemophilia (autosomal recessive) (Note that Leiden mutation causes hypercoagulation problems.[2])
VI	Accelerin	Redundant to activated factor V	Both	None known
VII	Proconvertin	Protease; activates factor X	Extrinsic	Hypoconvertinemia (autosomal recessive)
VIII	Antihemophilic factor A	Cofactor; activates factor X	Intrinsic	Hemophilia A (classical hemophilia); congenital X-linked trait
IX	Antihemophilic factor B (Christmas factor)	Protease; activates factor VIII	Intrinsic	Hemophilia B (Christmas disease[3]); congenital X-linked trait
X	Thrombokinase	Protease; combines with factor V to form prothrombin activator	Both	Stuart-Prower factor deficiency (autosomal recessive)
XI	Antihemophilic factor C	Protease; activates factor IX	Intrinsic	Hemophilia C, also known as plasma thromboplastin antecedent (PTA) deficiency (autosomal dominant)
XII	Hageman factor	Protease; activates factor XI and plasmin; converts prekallikrein to kallikrein	Intrinsic	Hageman trait (autosomal recessive disorder)
XIII	Fibrin-stabilizing factor	Cross-links fibrin	Both	The rarest of all of the clotting deficiencies; bleeding disorders apparent at birth (autosomal recessive disorder)

1. All proteins produced by the liver except tissue factor (thromboplastin; factor III) formed by perivascular tissue; fibrin-stabilizing factor (factor XIII) produced by platelets and plasma; and factor IV, which is simply Ca^{2+} (not a protein). Hageman factor is produced by both the liver and platelets. Additional factors released from platelets: platelet factors 1, 2, 3, and 4.
2. This item is a hypercoagulation problem and not due to deficient clotting factor.
3. Named for the first person diagnosed with the disease.

FIBRINOLYTIC SYSTEM

- Definition: **temporary fibrin clot systematically and gradually dissolved as the vessel heals**
- Key components
  - Plasminogen (inactive form)
  - Plasminogen activators
  - Plasmin
  - Fibrin
  - Fibrin Degradation Products (FDP)
  - Inhibitors of plasminogen activators and plasmin




X
IXa
VIIIa
XIa
XIIa

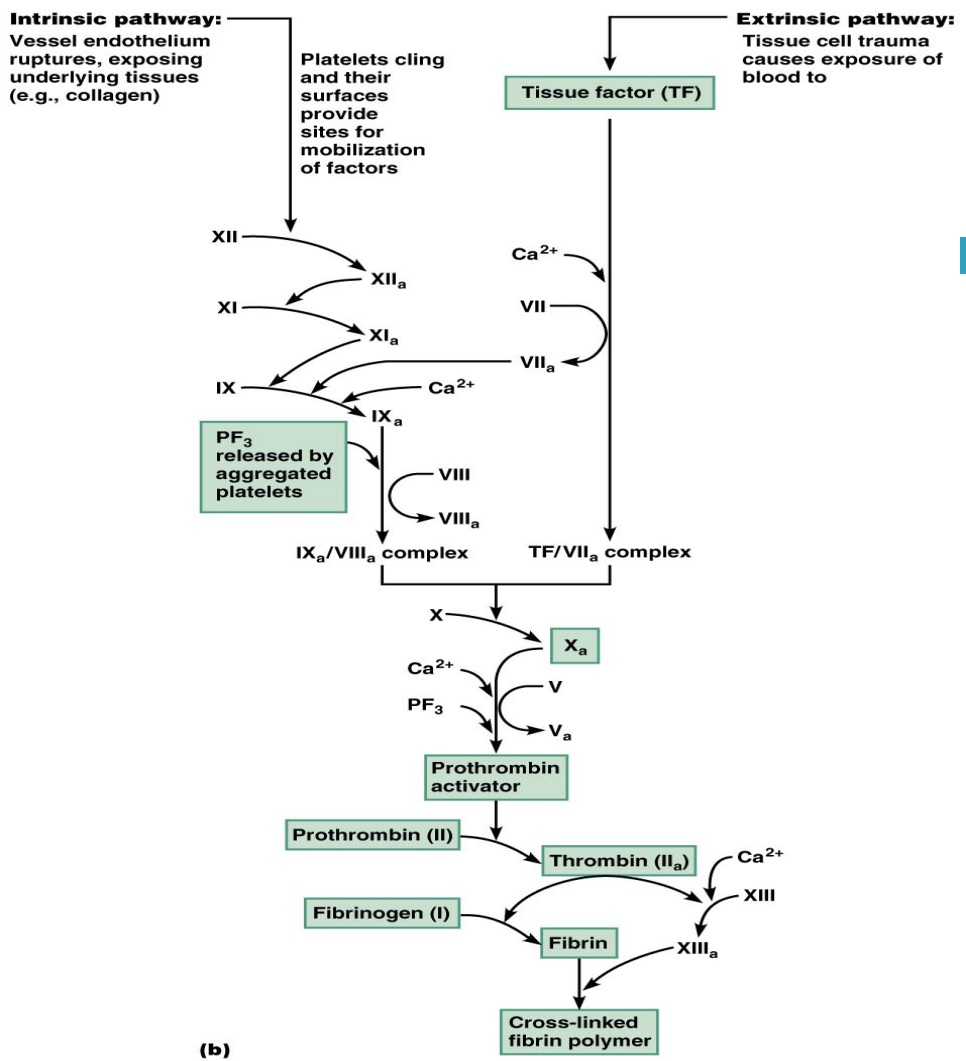
Activators of Fibrinolysis

- Intrinsic activators
  - Factor XIIa, XIa, kallikrein
- Extrinsic activators
  - Tissue type plasminogen activator (t-PA)
  - Urokinase type plasminogen acitvator (u-PA)
- Exogenous activators
  - Streptokinase (derived from beta strep)

Inhibitors of Fibrinolysis

- Plasminogen Activator Inhibitors (PAI)
- α_2 –antiplasmin
- α_2 -macroglobulin

Coagulation



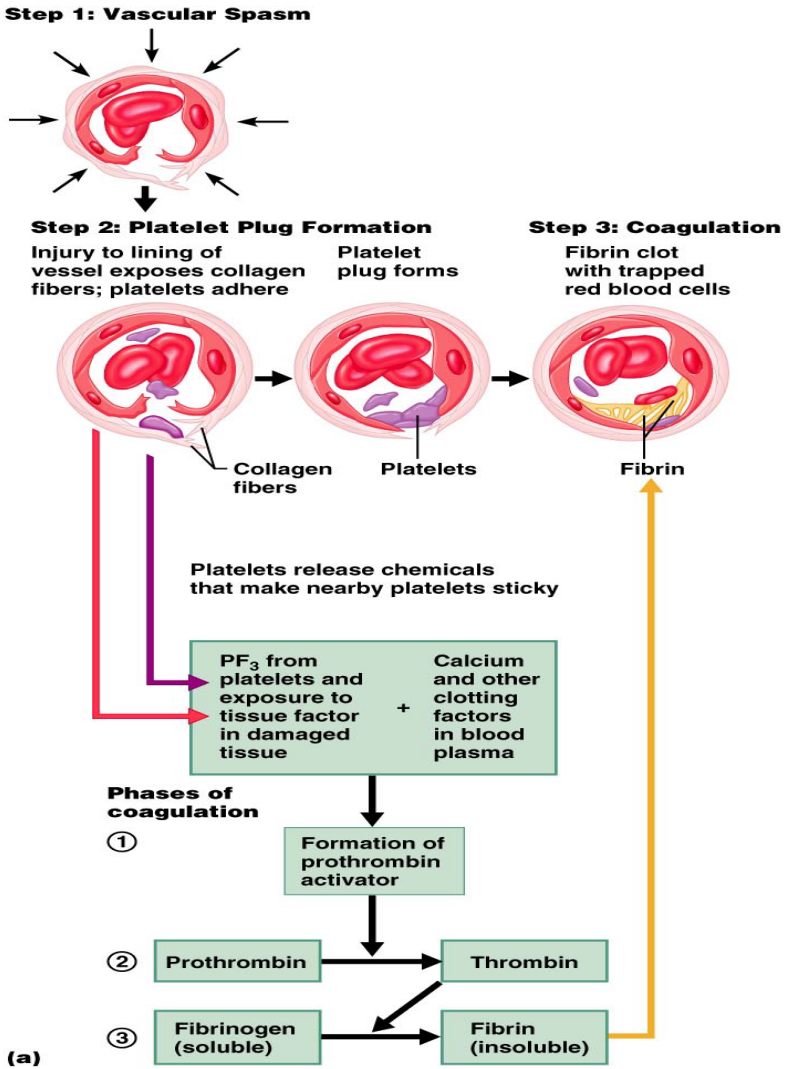
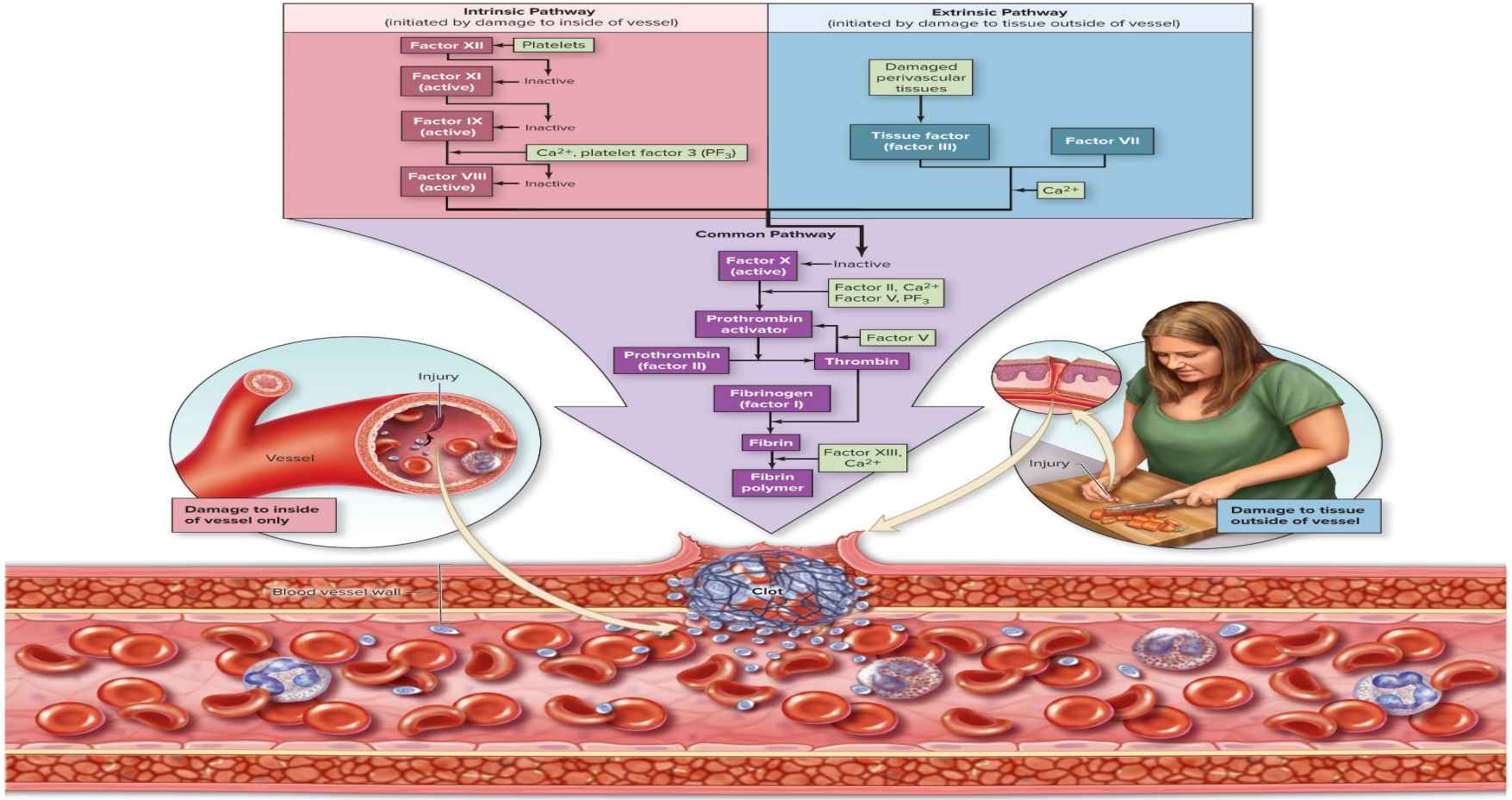


Figure 18.13

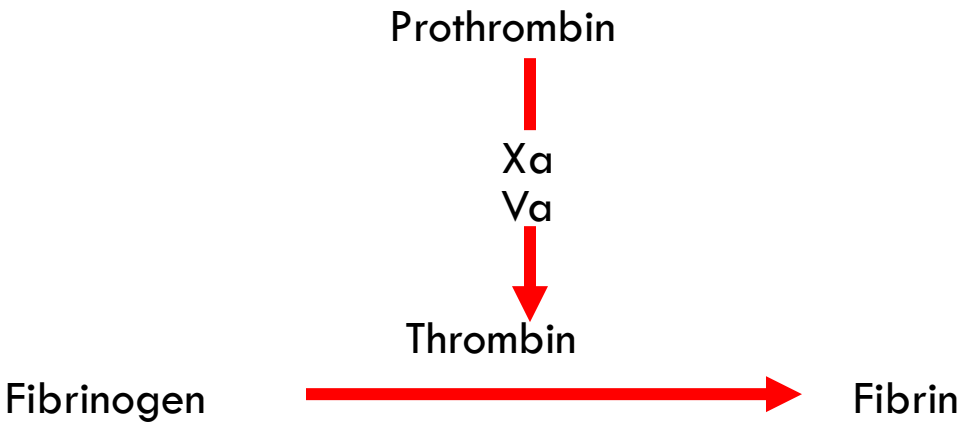


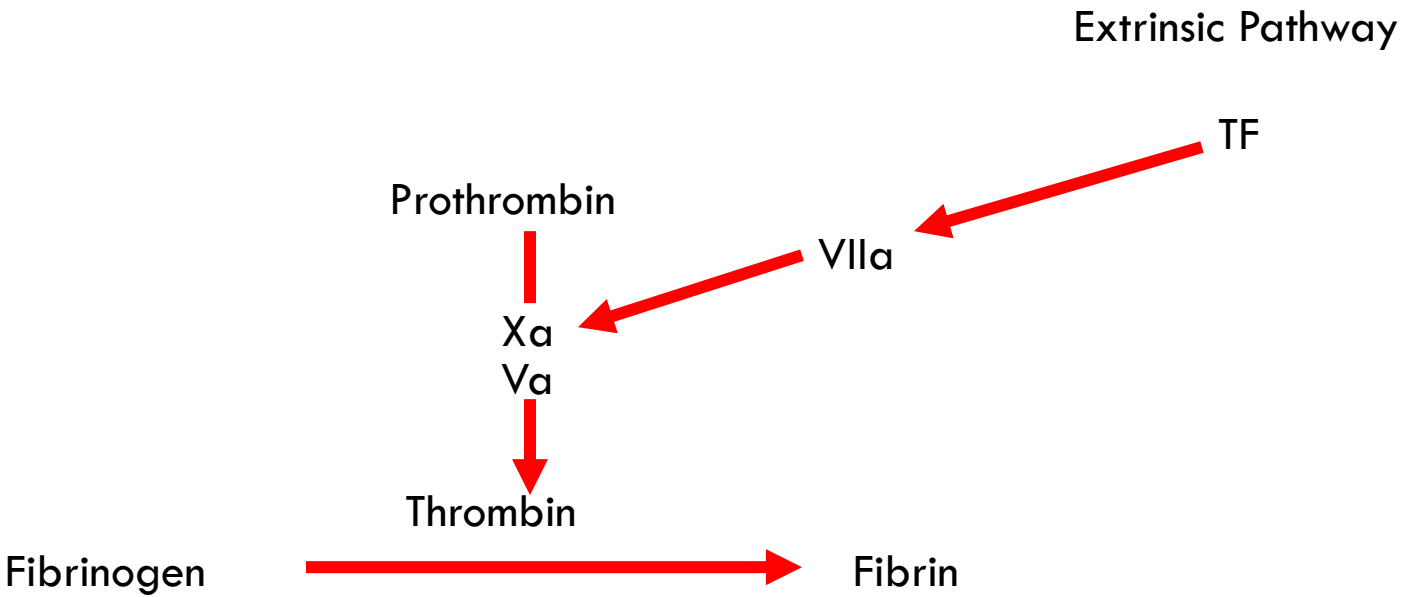
https://youtu.be/cy3a__OOa2M

https://youtu.be/SGzp9wqeu84

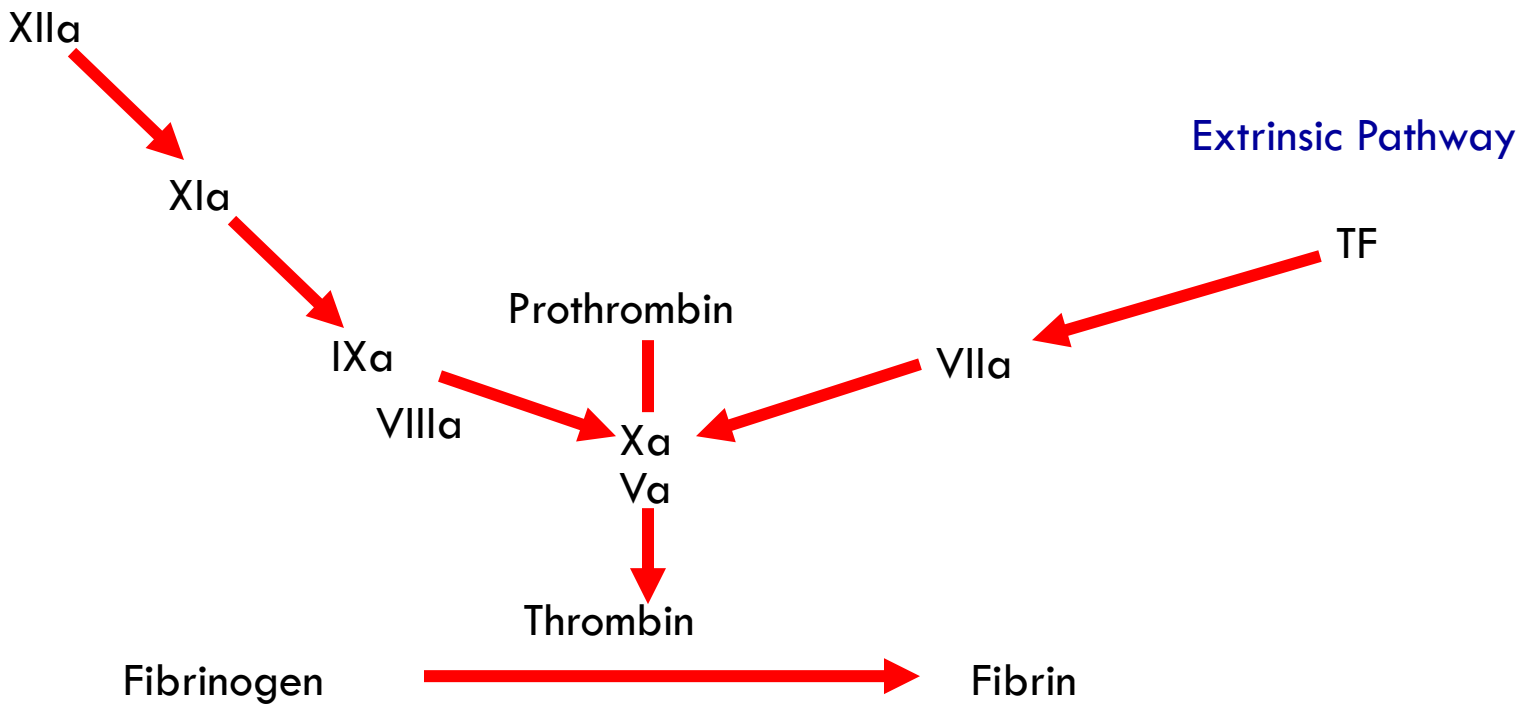
Fibrinogen  Fibrin

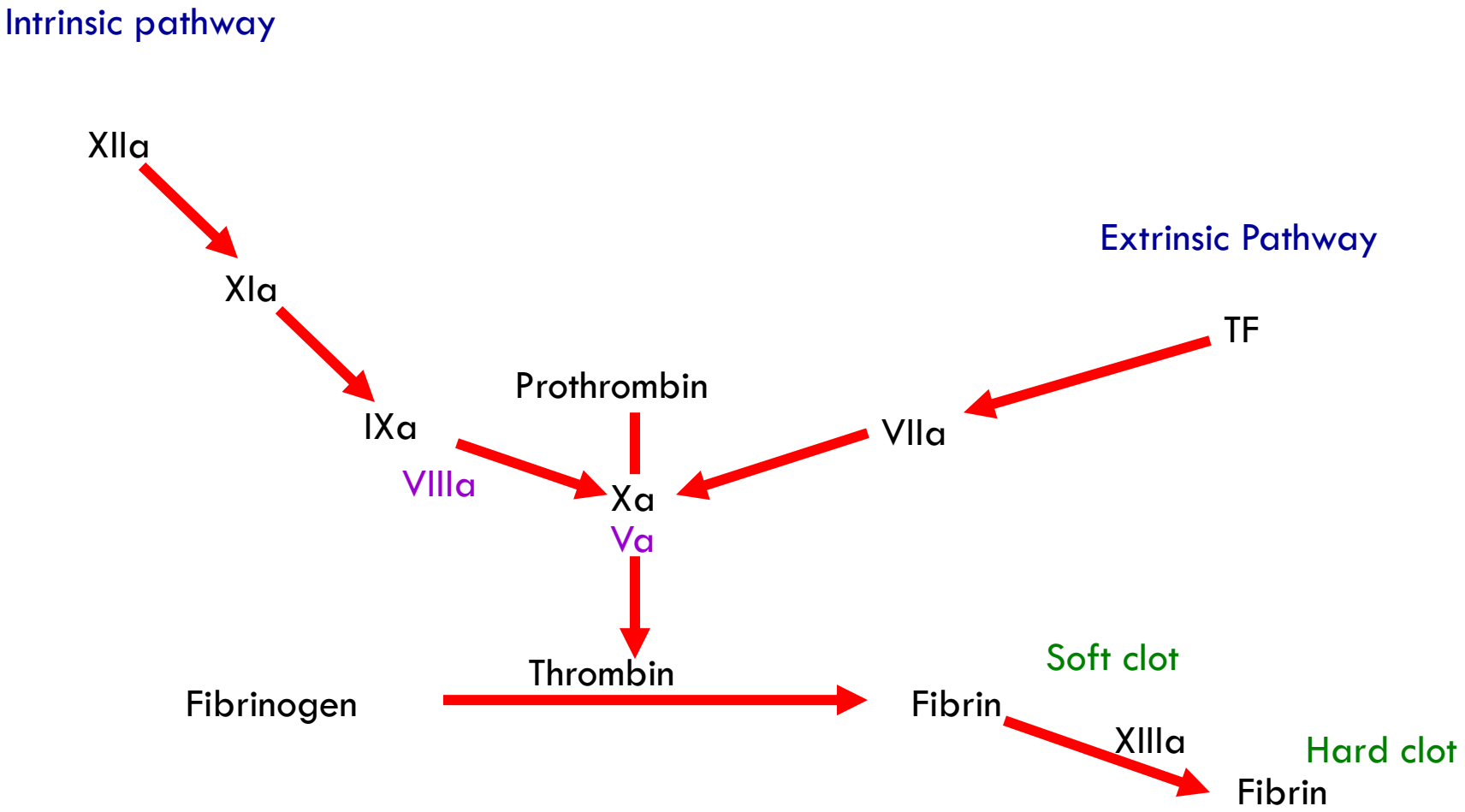

Thrombin
Fibrinogen
Fibrin

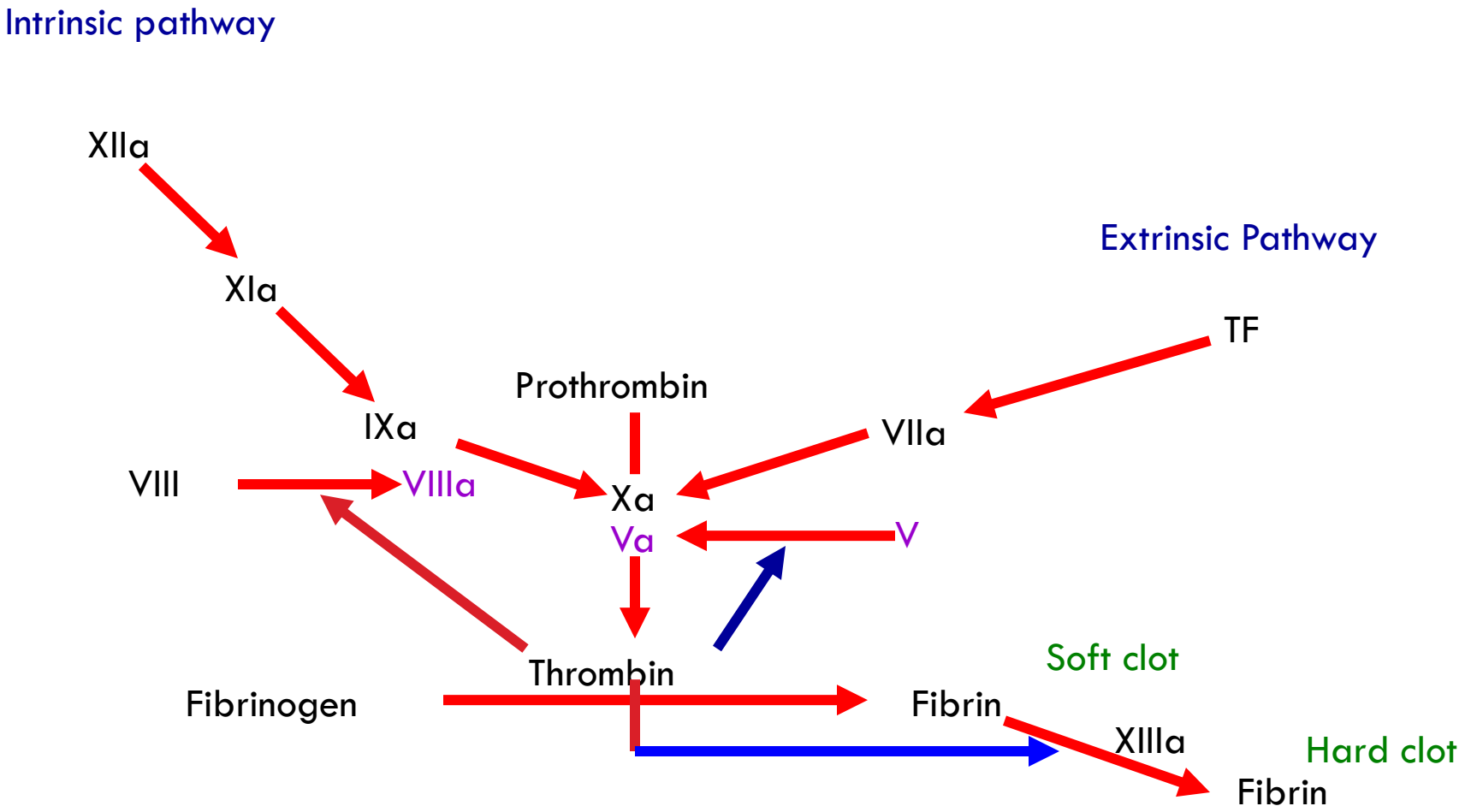

Prothrombin
Xa
Va
Thrombin
Fibrinogen
Fibrin


Extrinsic Pathway
TF
Prothrombin
VIIa
Xa
Va
Thrombin
Fibrinogen
Fibrin

Intrinsic pathway




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


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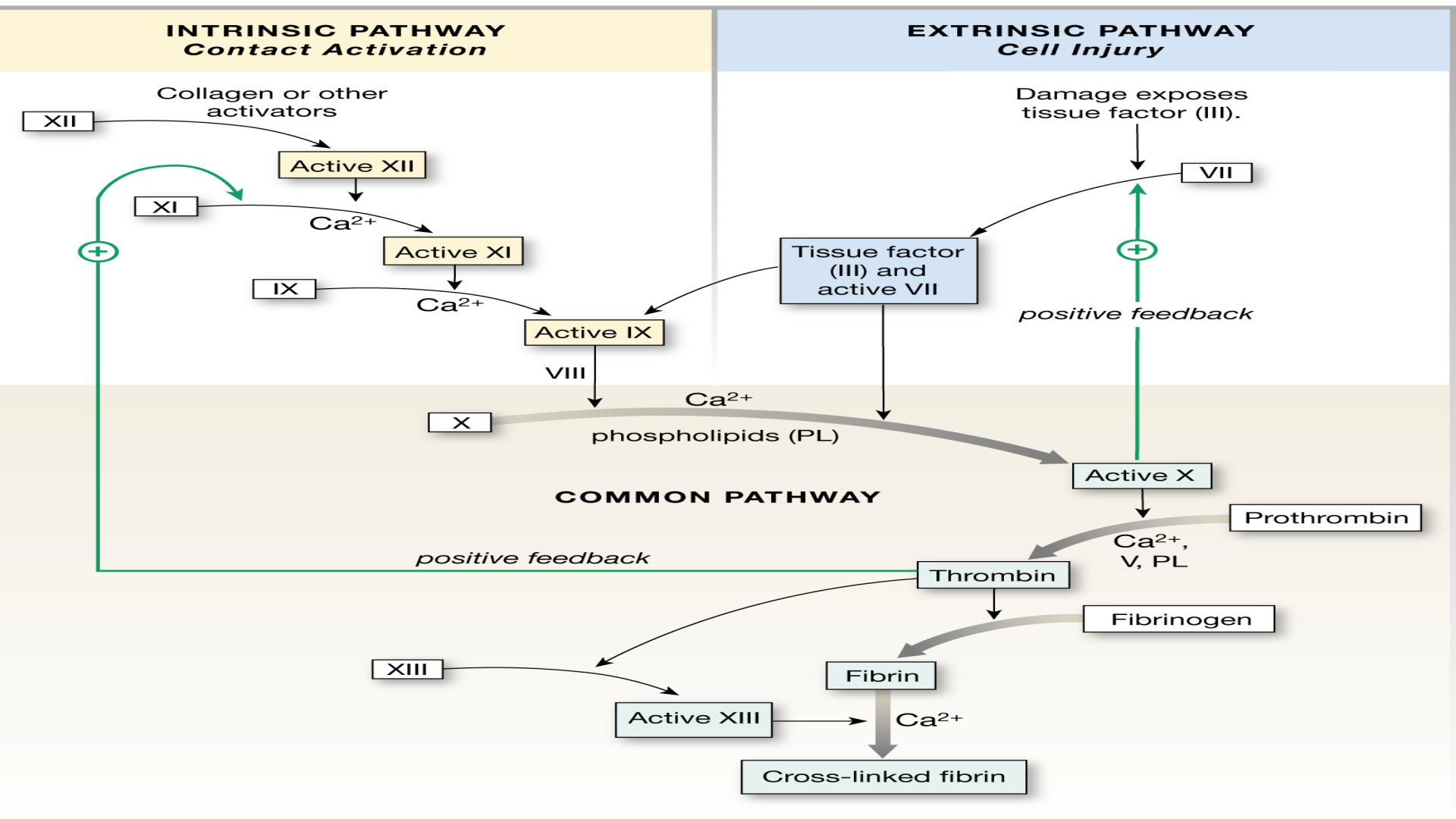
Prothrombin
Xa
Va
V
Extrinsic Pathway
TF
VIIa
Thrombin
Fibrinogen
Fibrin
Soft clot
XIIIa
Hard clot
Fibrin


INTRINSIC PATHWAY
Contact Activation
EXTRINSIC PATHWAY
Cell Injury
Collagen or other activators
XII
Active XII
XI
Ca2+
Active XI
IX
Ca2+
Active IX
VIII
X
Damage exposes tissue factor (III).
VII
Tissue factor (III) and active VII
positive feedback
Ca2+
phospholipids (PL)
Active X
COMMON PATHWAY
Prothrombin
Ca2+, V, PL
positive feedback
Thrombin
Fibrinogen
XIII
Fibrin
Active XIII
Ca2+
Cross-linked fibrin

Secondary Hemostasis: *Coagulation Cascade*

Authors:
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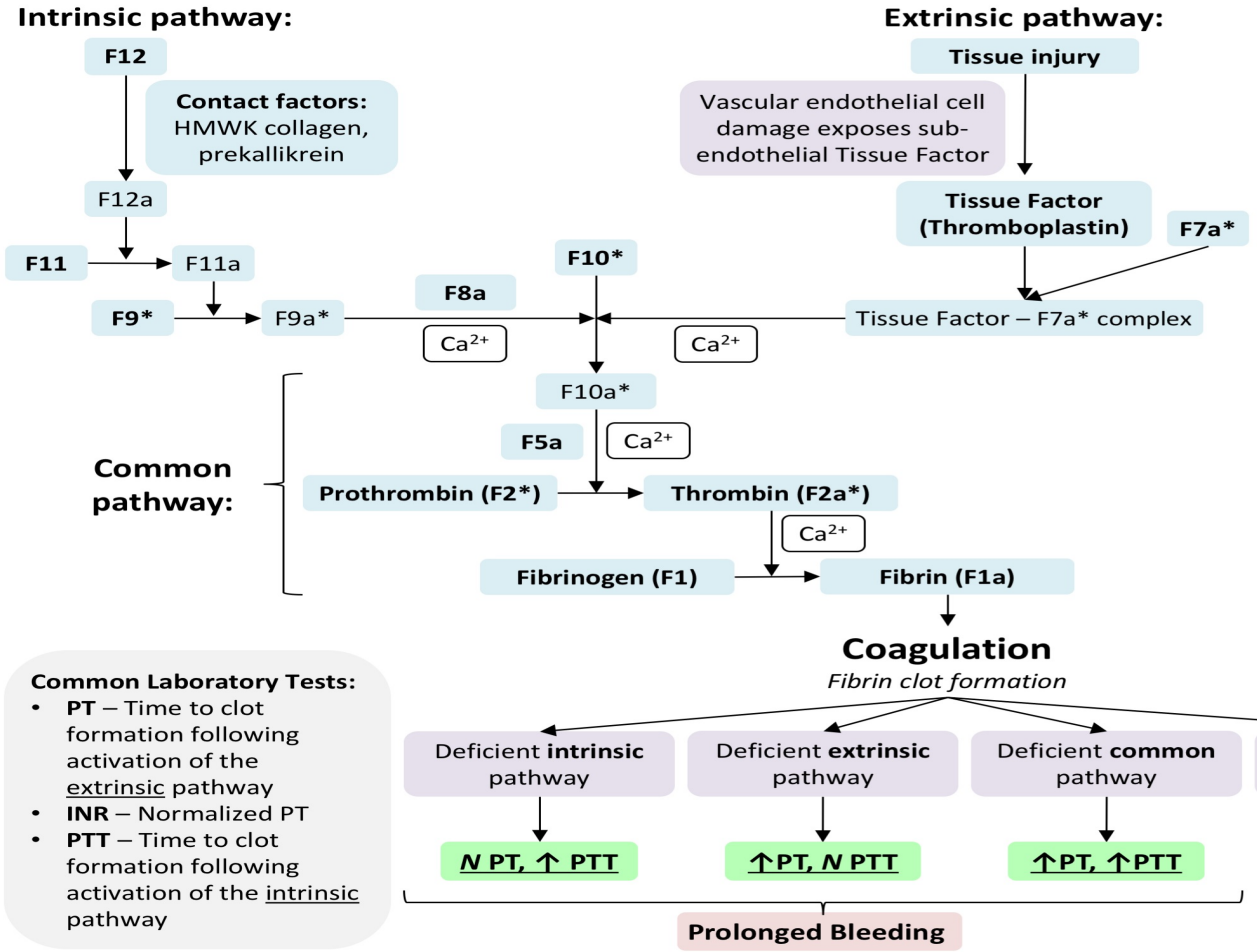
David Lincoln
Yan Yu*
Lynn Savoie*
** MD at time of publication*



Abbreviations:

- **PT** – Prothrombin Time
- **INR** - International Normalized Ratio
- **PTT** – Partial Thromboplastin Time
- **F** – Coagulation Factor
- **a** – Activated coagulation factor
- ***N*** – Normal
- **HMWK** – High molecular weight kininogen
- * – Vitamin K dependent (for more information, see *Vitamin K Deficiency* slide)

Memory Aids:

- PT = Extrinsic: Play Tennis outside
- PTT = Intrinsic: Play Table Tennis Inside
- Intrinsic factors – TENET: Twelve, Eleven, Nine, Eight, Ten
- Common pathway factors – 10/5=2, 2/2=1 (F10, 5, 2, 1)
- Vitamin K dependent factors – 1972 (the year Canada won the Summit Series in hockey vs. the USSR): F10, 9, 7, 2

Common Laboratory Tests:

- **PT** – Time to clot formation following activation of the extrinsic pathway
- **INR** – Normalized PT
- **PTT** – Time to clot formation following activation of the intrinsic pathway

Legend: Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

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Coagulation Factors

- Factor XII (FXII) → activated FXII (FXIIa)
- Factor XI (FXI) → activated FXI (FXIa)
- Factor X (FX) → activated FX (FXa)
- Factor IX (FIX) → activated FIX (FIXa)
- Factor VIII (FVIII) → activated FVIII (FVIIIa)
- Factor VII (FVII) → activated FVII (FVIIa)
- Factor V (FV) → activated FV (FVa)

Factor II (prothrombin) is converted to thrombin (FIIa)

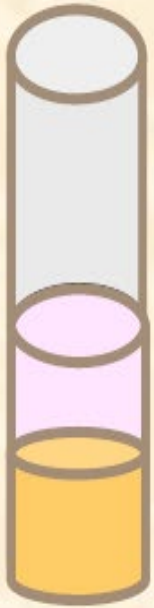
Factor I (fibrinogen) is converted to fibrin

Coagulation

- The prothrombin time (PT) and its derived measures of **prothrombin ratio** (**PR**) and **international normalized ratio** (**INR**) are measures of the ***extrinsic pathway of coagulation***.
- They are used to determine the **clotting tendency of blood**, in the measure of
  - **warfarin dosage,**
  - **liver damage,**
  - **vitamin K status.**
- The reference range for prothrombin time is usually around 12–15 seconds;
- the normal range for the INR is 0.8–1.2. PT **measures factors I, II, V, VII, and X.**
- It is used in conjunction with the activated partial thromboplastin time (aPTT) which measures the *intrinsic pathway*.

CLOT-ED

Prothrombin Time (PT)



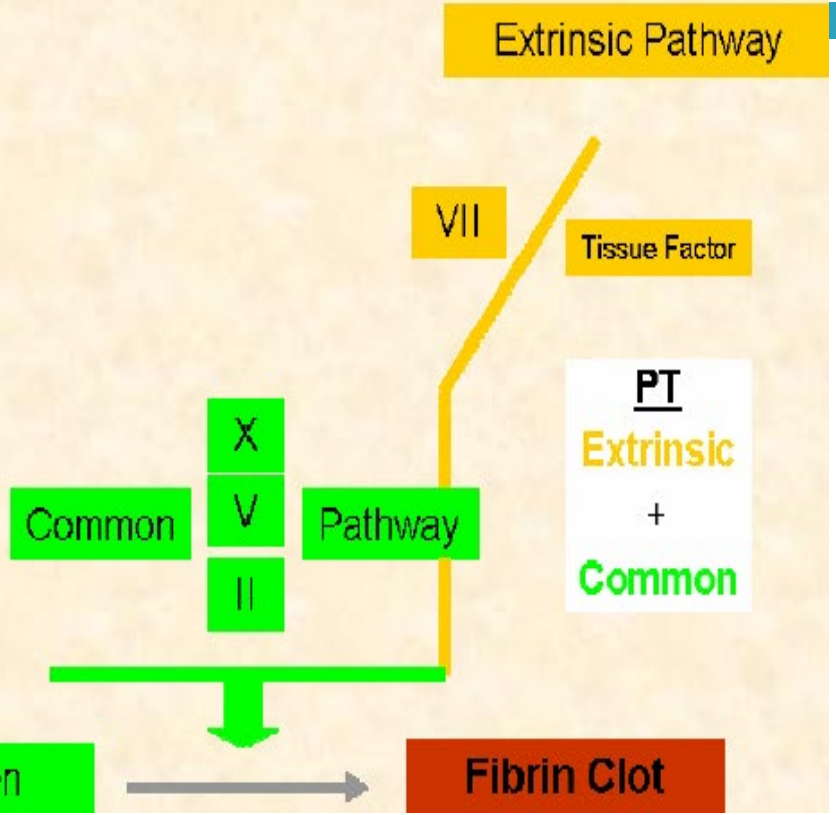
Time for clot formation

~ 12 seconds

Incubate at 37 °C for ~3 minutes

0.1 ml Thromboplastin + Ca^{++}

0.1 ml Plasma

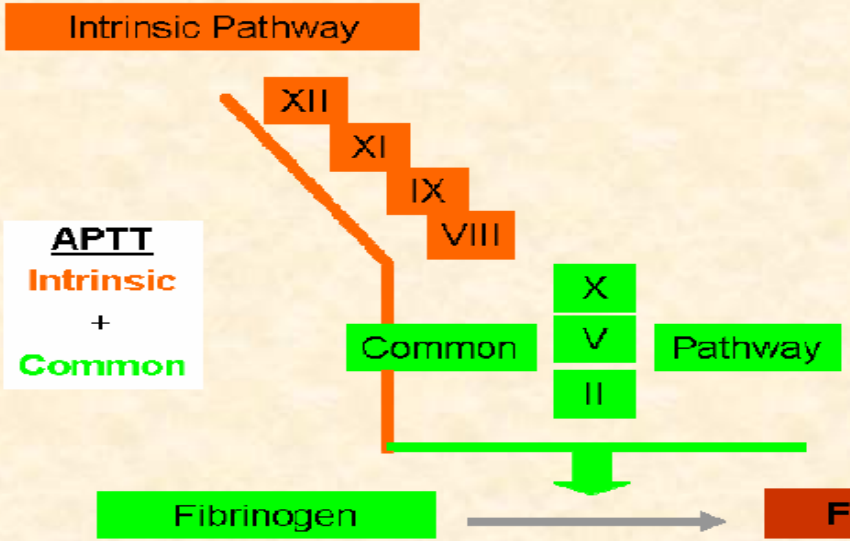


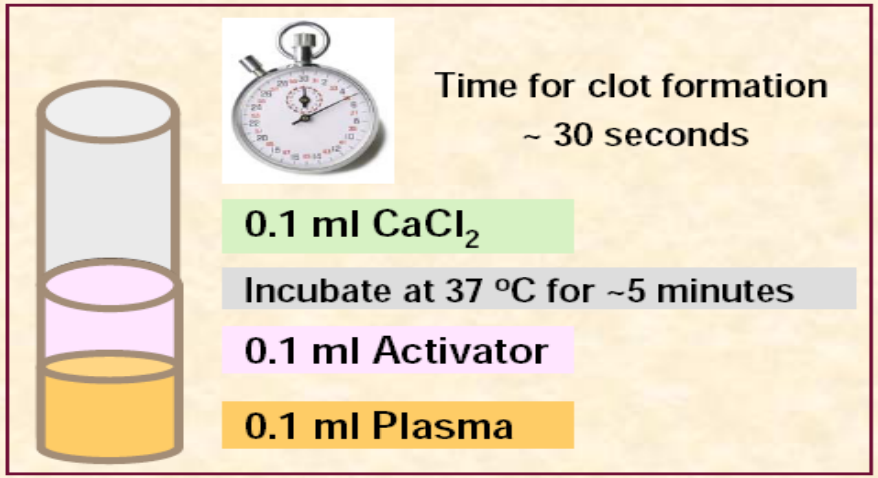
- **The partial thromboplastin time (PTT) or activated partial thromboplastin time (aPTT or APTT)**
  - is a performance indicator measuring the efficacy of both the **"intrinsic"** (now referred to as the contact activation pathway) and the common coagulation pathways.

monitor the treatment effects with heparin, a major anticoagulant

Kaolin cephalin clotting time (KccT) is a historic name for the activated partial thromboplastin time

Activated Partial Thromboplastin Time





APTT Reagent Composition

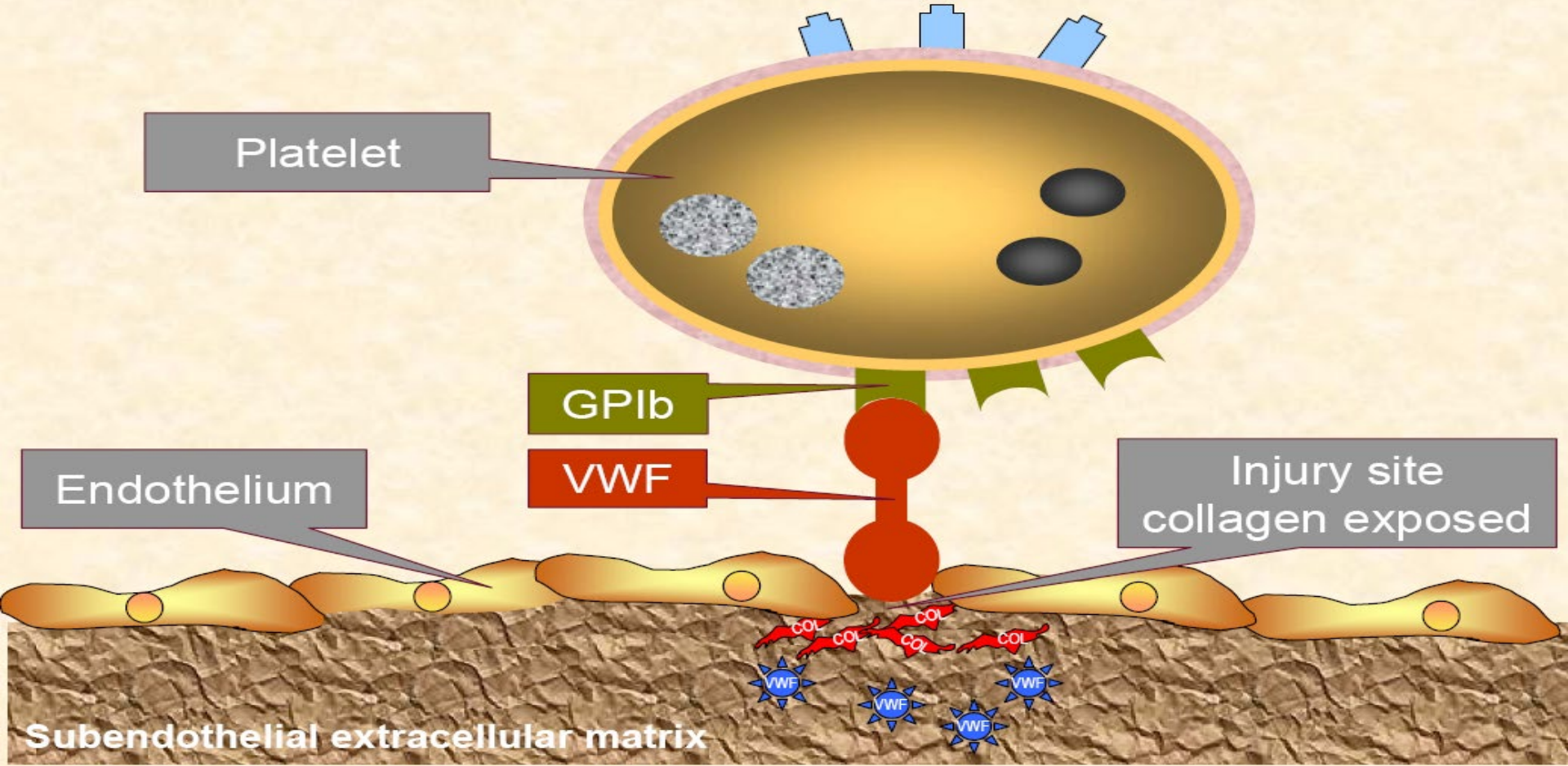
- Activator to convert FXII to FXIIa
- Phospholipid (replaces "in vivo" platelet surface on which coagulation reactions occur)
- $CaCl_2$ – used to reintroduce calcium ions that were chelated by sodium citrate
- Referred to as "partial thromboplastin" since no Tissue Factor is used
  - Two-stage assay (activation and re-calcification)

- Prolonged APTT may indicate:

1. use of heparin (or contamination of the sample)
2. antiphospholipid antibody (especially lupus anticoagulant, which paradoxically increases propensity to thrombosis)
3. coagulation factor deficiency (e.g. hemophilia)

CLOT-ED

Platelet Adhesion



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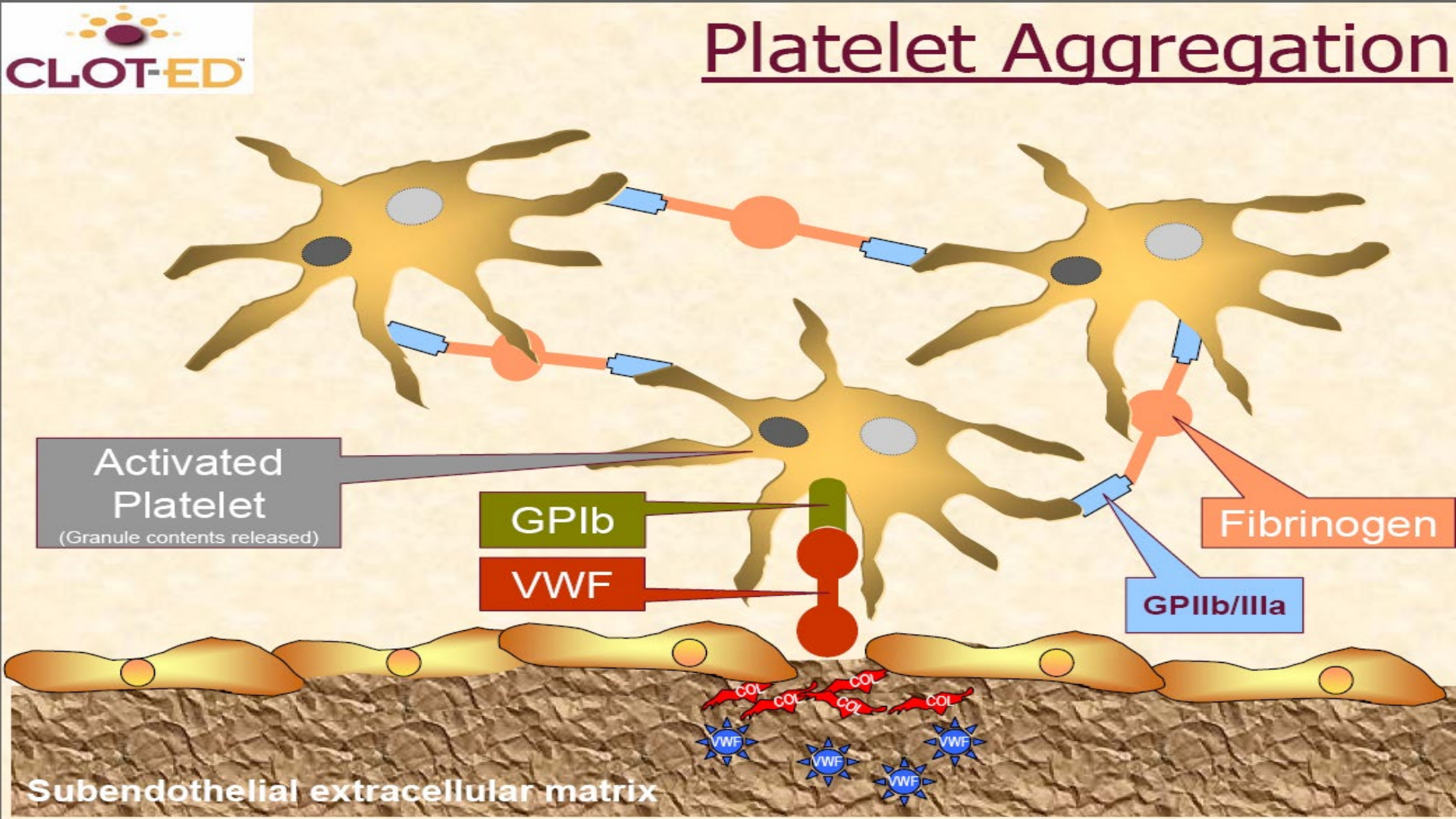
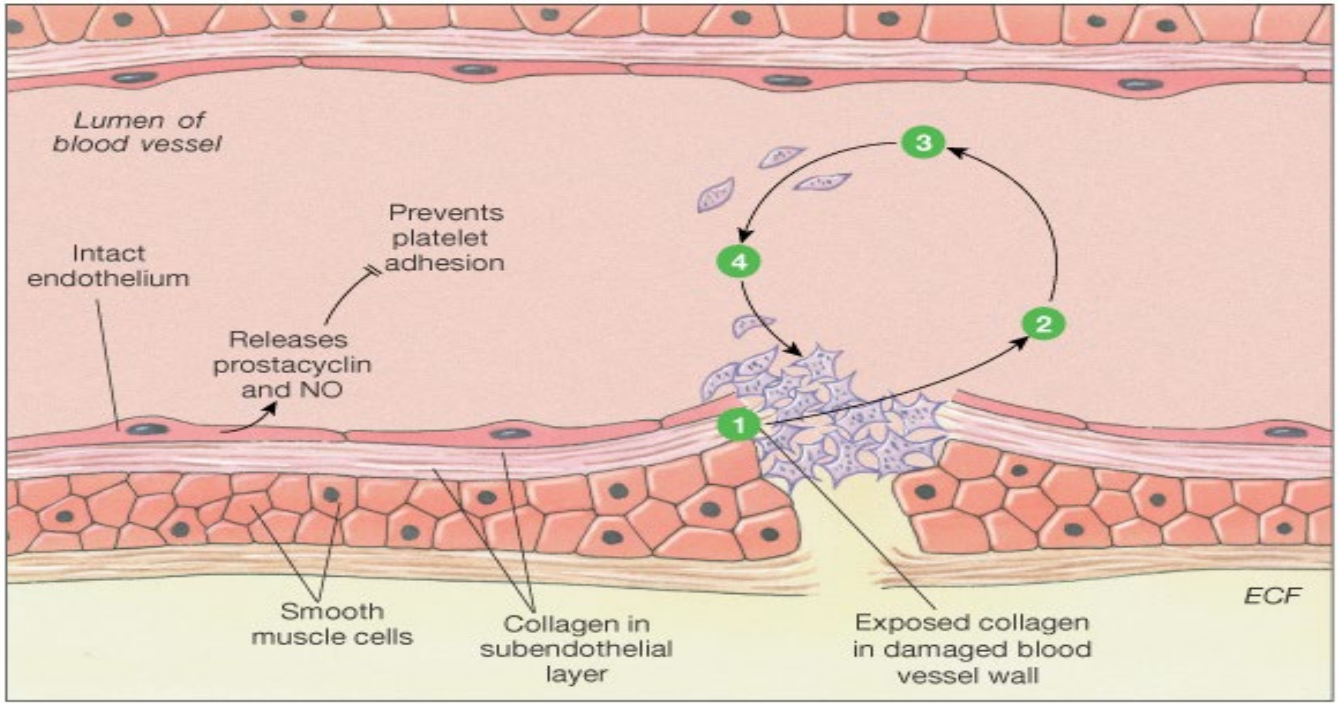
Platelet Aggregation



Hemostasis: Vasoconstriction & Plug Formation



1. Exposed collagen binds and activates platelets.
2. Release of platelet factors
3. Attracts more platelets
4. Aggregate into platelet plug

Figure 16-12: Platelet plug formation

Coagulation Phase 1: Two Pathways to Prothrombin Activator

- May be initiated by either the intrinsic or extrinsic pathway
  - Triggered by tissue-damaging events
  - Involves a series of procoagulants
  - Each pathway cascades toward factor X
- Once **factor X has been activated, it complexes with calcium ions, PF_3, and factor V to form prothrombin activator**

Coagulation Phase 2: Pathway to Thrombin

- Prothrombin activator catalyzes the transformation of prothrombin to the active enzyme thrombin

Coagulation Phase 3: Common Pathways to the Fibrin Mesh

- Thrombin catalyzes the polymerization of fibrinogen into fibrin
- Insoluble fibrin strands form the structural basis of a clot
- Fibrin causes plasma to become a gel-like trap
- Fibrin in the presence of calcium ions activates factor XIII that:
  - Cross-links fibrin
  - Strengthens and stabilizes the clot

Clot Retraction and Repair

- **Clot retraction** – stabilization of the clot by squeezing serum from the fibrin strands
- **Repair**
  - Platelet-derived growth factor (PDGF) stimulates rebuilding of blood vessel wall
  - Fibroblasts form a connective tissue patch
  - **Stimulated by vascular endothelial growth factor (VEGF), endothelial cells multiply and restore the endothelial lining**

Factors Limiting Clot Growth or Formation

- Two homeostatic mechanisms prevent clots from becoming large
  - Swift removal of clotting factors
  - Inhibition of activated clotting factors

Inhibition of Clotting Factors

- Fibrin acts as an anticoagulant by binding thrombin and preventing its:
  - Positive feedback effects of coagulation
  - Ability to speed up the production of prothrombin activator via factor V
  - Acceleration of the intrinsic pathway by activating platelets

Inhibition of Clotting Factors

- Thrombin not absorbed to fibrin is inactivated by antithrombin III
- Heparin, another anticoagulant, also inhibits thrombin activity

Factors Preventing Undesirable Clotting

- Unnecessary clotting is prevented by endothelial lining the blood vessels
- **Platelet adhesion is prevented by:**
  - **The smooth endothelial lining of blood vessels**
  - **Heparin and PGI_2 secreted by endothelial cells**
  - **Vitamin E quinone, a potent anticoagulant**

Hemostasis: Coagulation & Clot Stabilization

- Prothrombin
- Ca++
- Fibrinogen
- Fibrin
- Polymerization

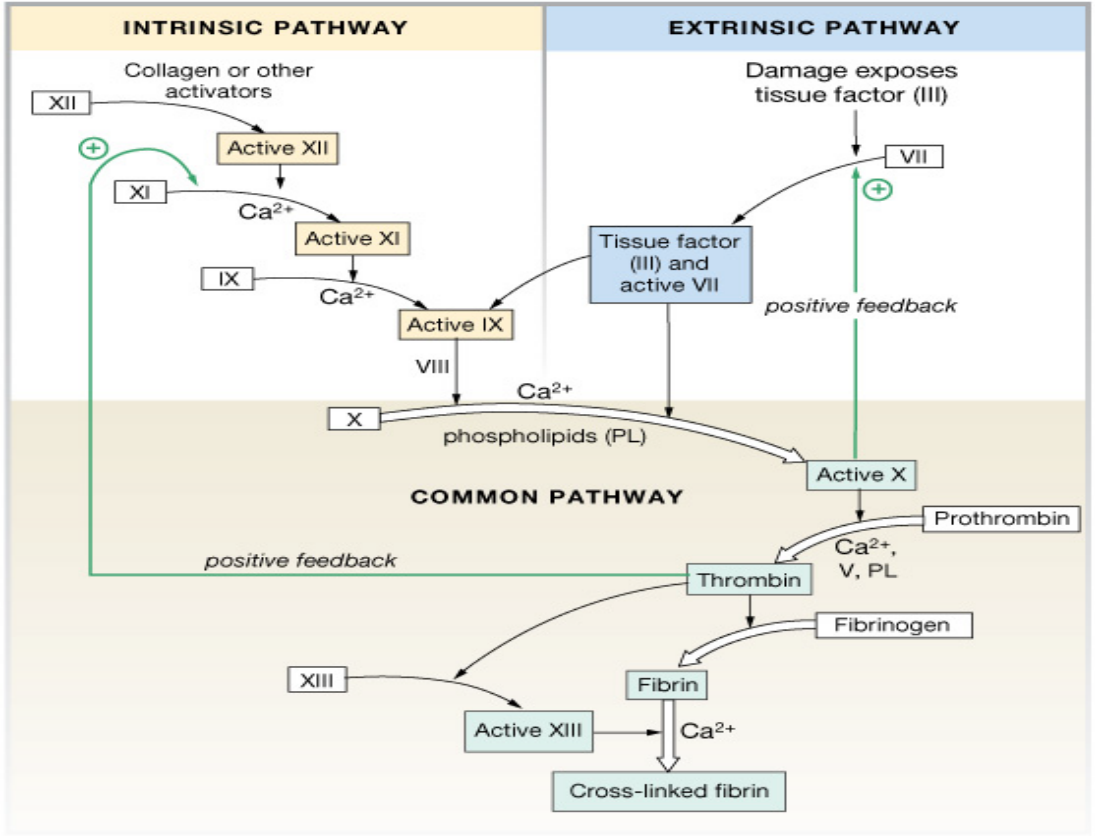


Figure 16-13: The coagulation cascade

Dissolving the Clot and **Anticoagulants**

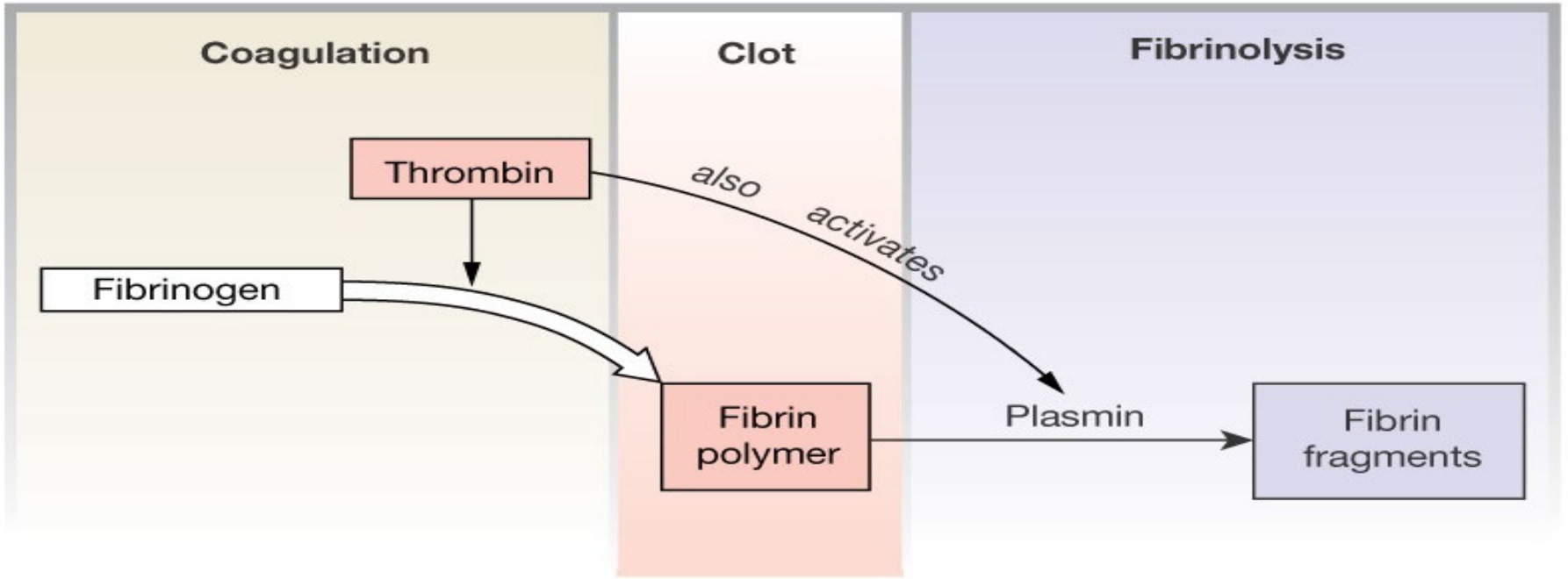


Figure 16-14: Coagulation and fibrinolysis

Tests for Primary Hemostasis

- **Bleeding Time**

– Assesses all components of Virchow's triad

– in vivo test – performed directly on patient

– Has fallen into disrepute and replaced by instruments that perform "in vitro" bleeding times

- **Platelet Aggregation studies**

– Measure ability of platelets to aggregate, in vitro, when subjected to various stimulators (agonists)

– Predominantly assesses function of platelet glycoprotein IIb/IIIa receptor

- **Von Willebrand Factor (VWF) assays**

– Measure amount and function of VWF, a protein that works with platelets so that they adhere to site of injury

– Assesses function of VWF ligand in its interaction with platelet glycoprotein Ib receptor

CLOT-ED

Time Frame for Hemostasis

Platelets	Coagulation Factors	Fibrinolytic Proteins
Primary Hemostasis	**Secondary Hemostasis**	**Fibrinolysis**
• Vessel constriction occurs immediately • Platelet adhesion occurs in seconds • Platelet aggregation takes minutes	• Activation of coagulation factors occurs in seconds • Fibrin forms in minutes	• Activation of fibrinolytic proteins happens immediately • Dissolving the thrombus requires hours

- Examination

Platelet Disease

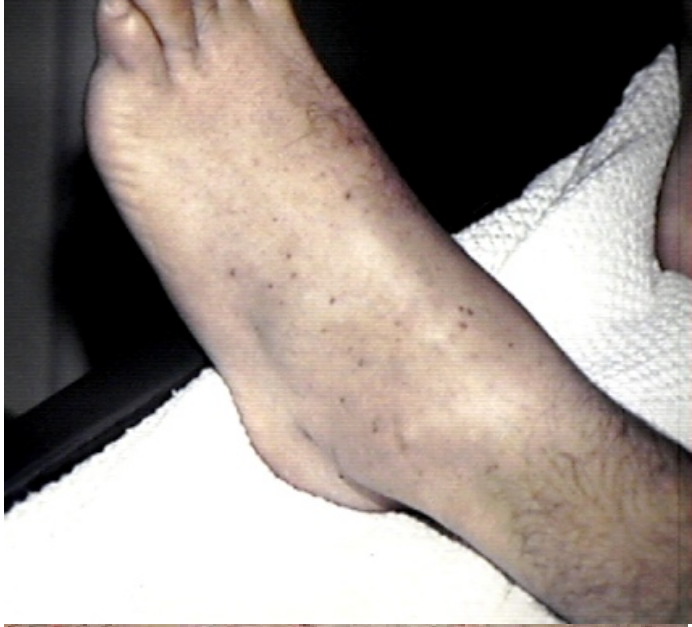
Mucosal/cutaneous bleeding

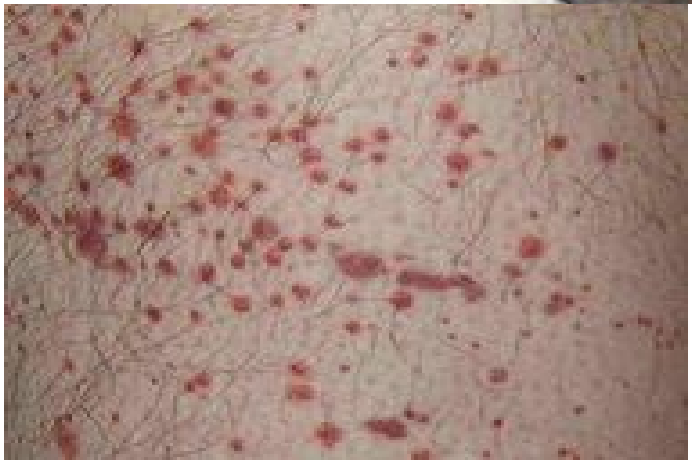
Lack vessel protection by submucosal tissue

Bleed immediately after vascular trauma

- **Petechiae**
  - From small capillary
  - In areas of increased venous pressure (dependent parts of the body)
  - Asymptomatic and not palpable
  - D/D small telangiectasias

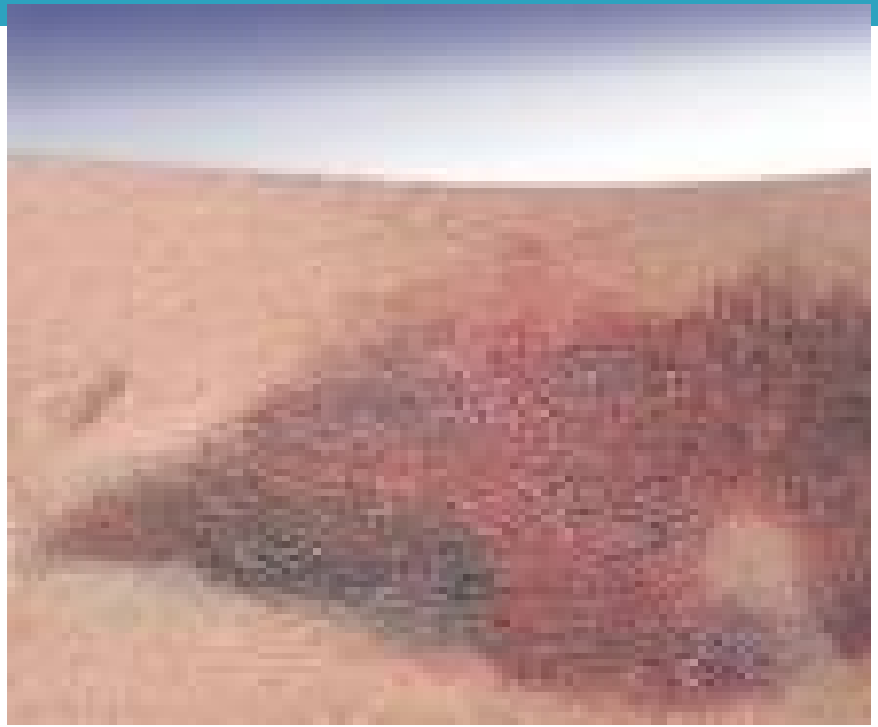
    (Angiomas, Vasculitic purpura, Wiskott-Aldrich Syndrome, Leukaemia, Vit K deficiency
- **Purpura**
  - Characteristically purple in colour
  - Small, multiple, and superficial in location
  - Develop without noticeable trauma / not spread into deeper tissues
  - Seen in – (Acute / Chronic leukaemia, Vitamin K deficiency)





Examination
Coagulation Disorders

- **Ecchymoses**
  - Large palpable ecchymoses
  - Spreading into deep tissue - haematomas – Hemarthrosis- severe coagulation disorder- haemophilia
  - Coagulation disorder bleeding onset may be delayed after surgery

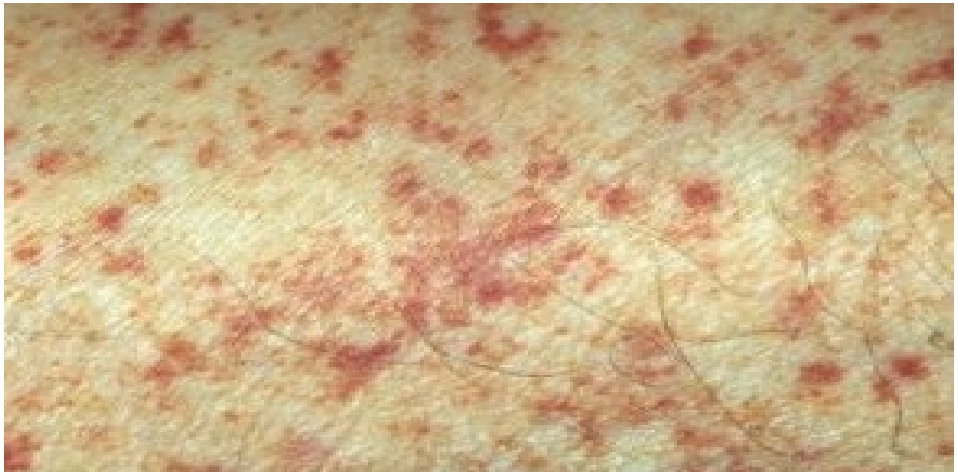


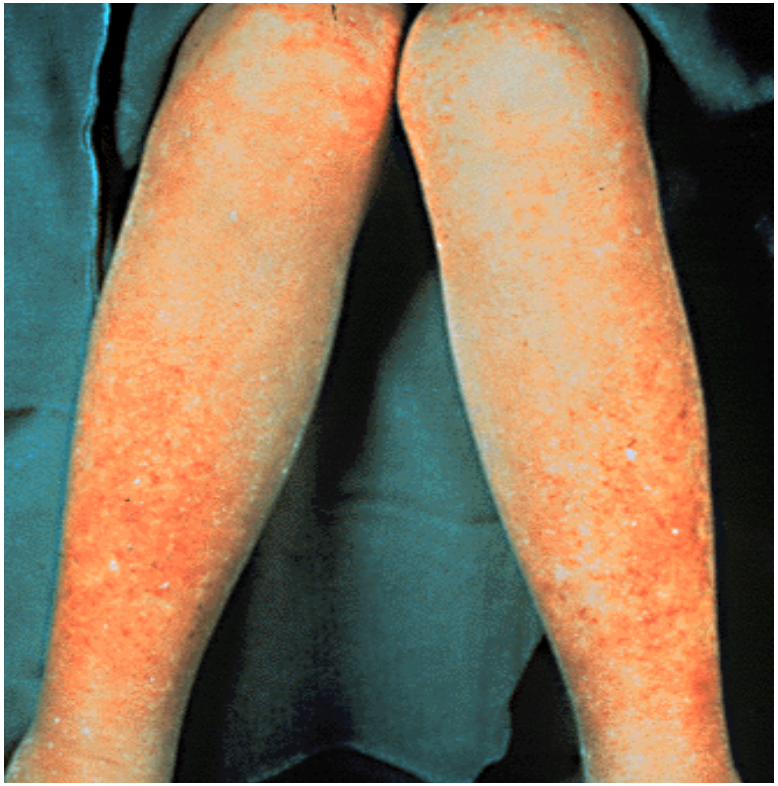
Role of PT, PTT: Warfarin, Heparin Monitoring

Anticoagulant	PT	PTT
Low dose Heparin	Normal	Prolonged
High dose heparin	Prolonged	Prolonged
Low dose warfarin	Prolonged	Normal
High dose warfarin	Prolonged	Prolonged

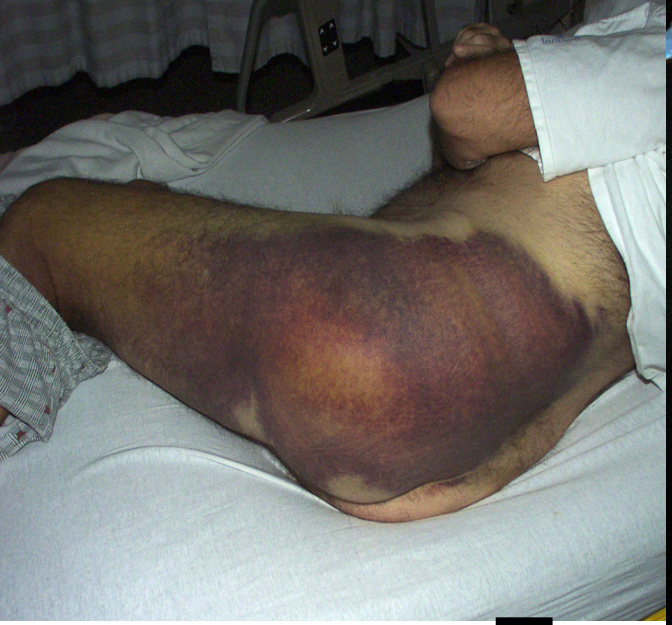
Physical Examination

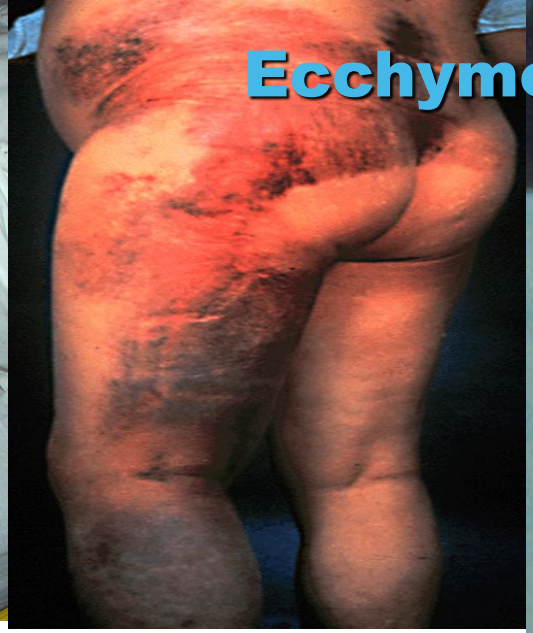
- Petechiae

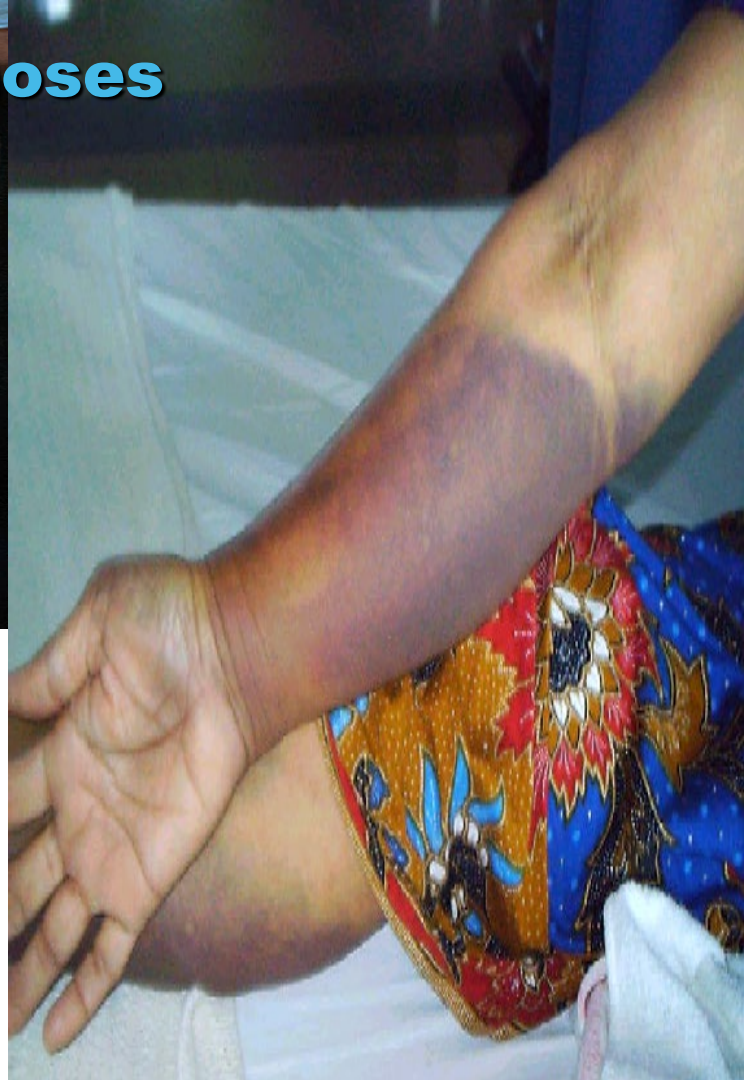




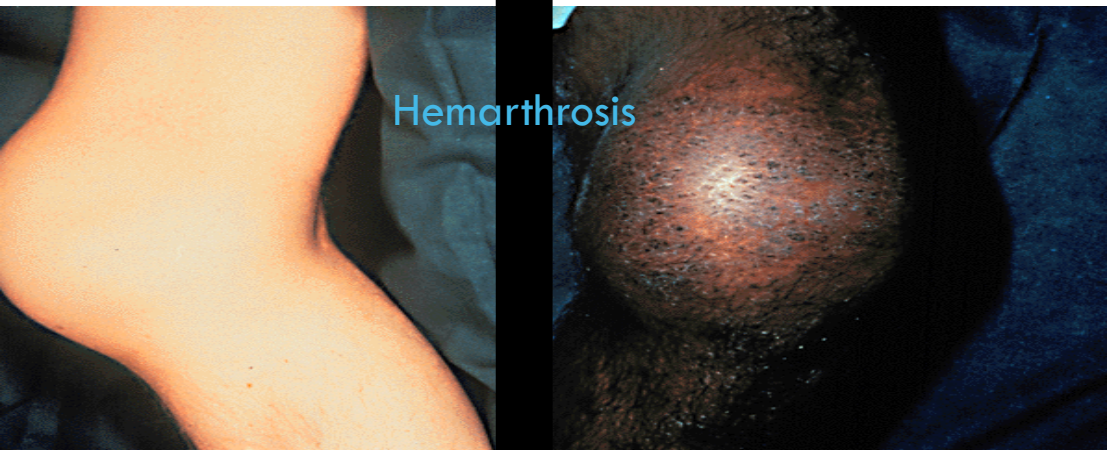
Ecchymoses







Hemarthrosis



52 year old male

Severe Hemophilia

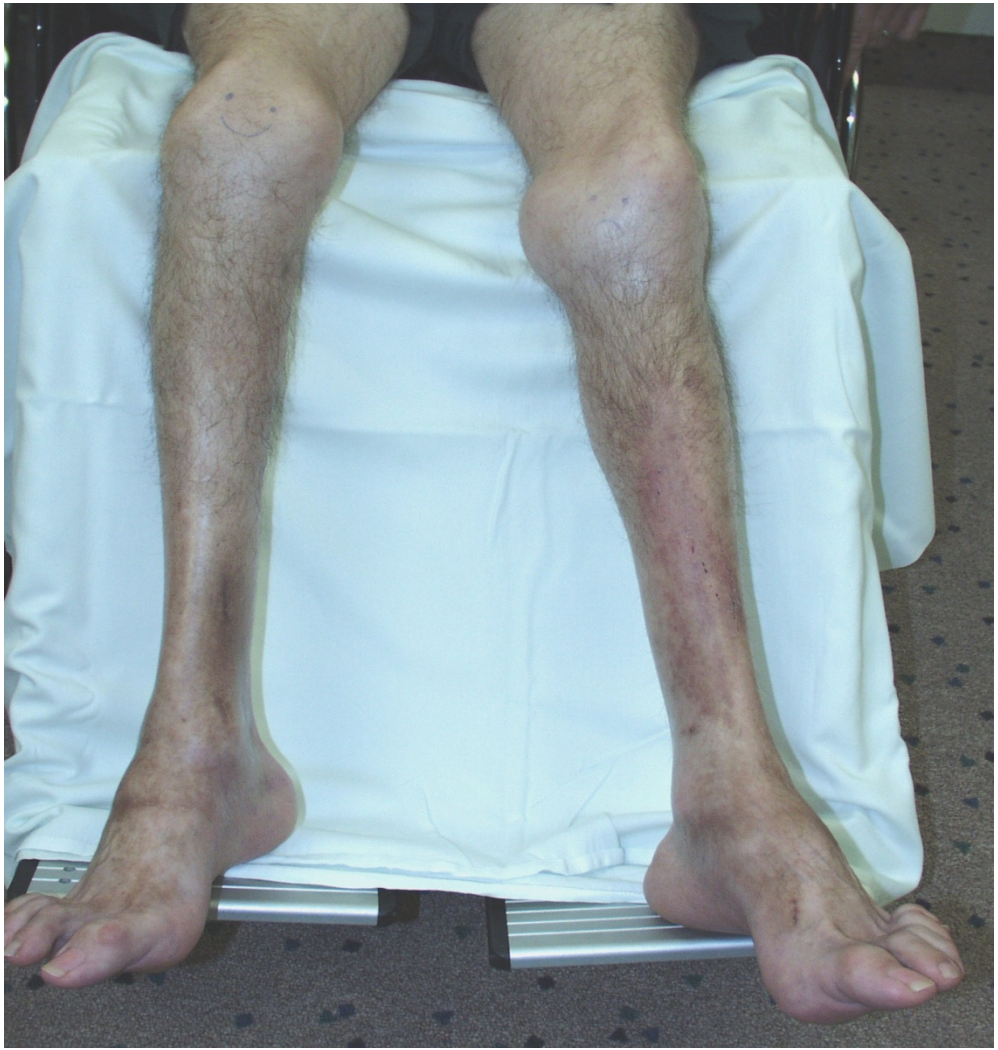
Now bleeds 3x month

Severe muscle wasting

Joint immobility

Atrophic skin changes

HIV and HCV +ve



Role of vitamin K

Play key roles in the regulation of three physiological processes:

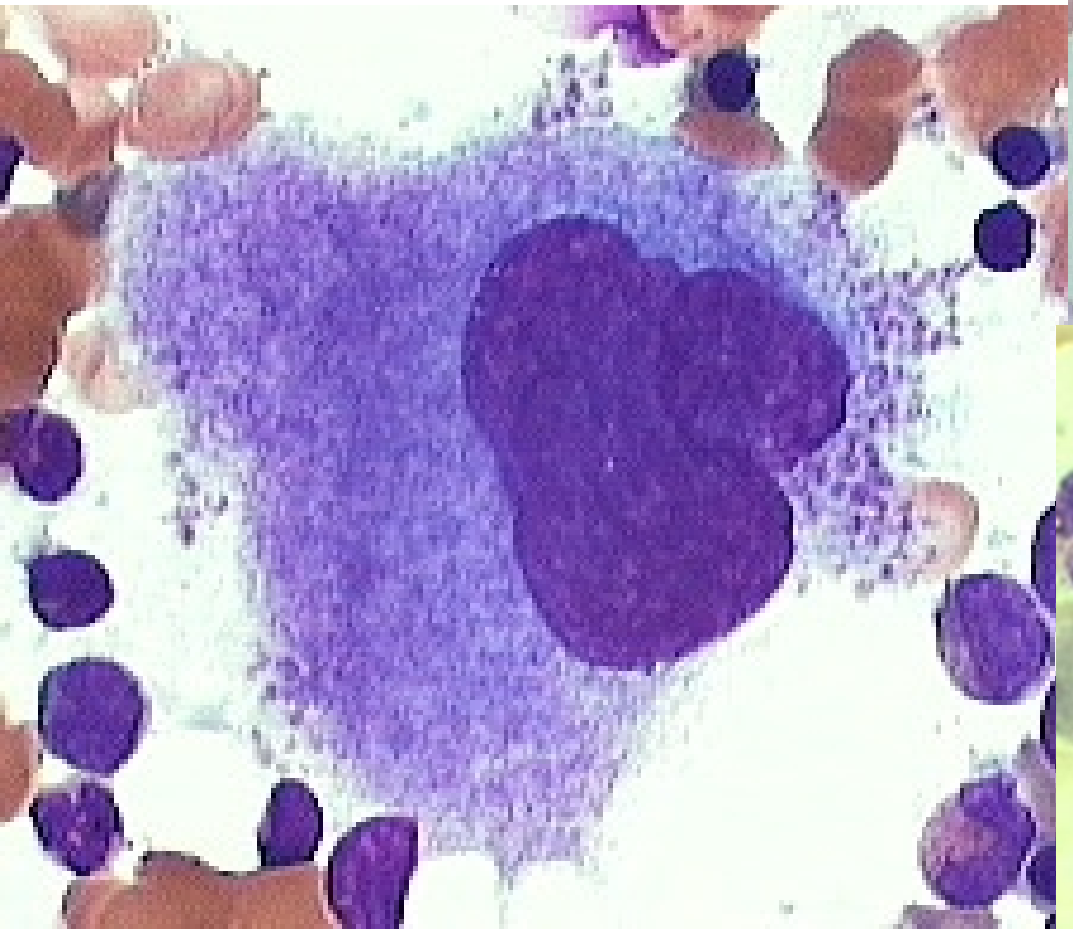
•Blood coagulation: (prothrombin (factor II), factors VII, IX, X, protein C, protein S, and protein Z).

•Bone metabolism: osteocalcin, also called bone Gla-protein (BGP), and matrix gla protein (MGP).

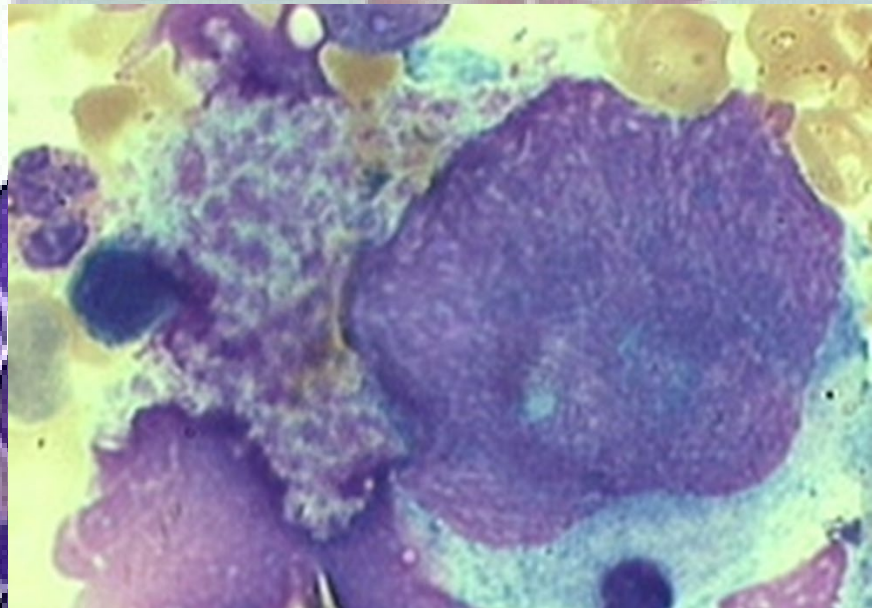
•Vascular biology.

Like other liposoluble vitamins (A, D, E), vitamin K is stored in the fat tissue of the human body.

Megakaryocyte



Normal platelets and one giant platelet



Clinical View: Bleeding and Blood Clotting Disorders 1

Hemophilia: bleeding disorders

- **Hemophilia A** and **hemophilia B** most common
  - Occur in X-linked recessive pattern
  - Males exhibit full-blown disease; females typically carriers
  - Result from deficiency of factor VIII, factor IX, or factor XI (more rare)

Thrombocytopenia: platelet deficiency

- Increased breakdown or decreased production
- May occur in bone marrow infections or cancer

Certain drugs interfere with clotting (can cause bleeding)

- E.g., aspirin, ibuprofen, warfarin, ginkgo

Clinical View: Bleeding and Blood Clotting Disorders 2

Hypercoagulation

- Increased tendency to clot blood
- Can lead to **thrombus,** blood vessel clot
- When dislodged within blood, **embolus**
- If lodges in lungs, **pulmonary embolism**
  - Can cause breathing problems and death
- Can have drug-related, environmental, and genetic causes
  - E.g., birth control pills, prolonged inactivity

Blood group (refer to lab)

Population Distribution of Major Blood Groups

O	Rh pos	38%
O	Rh neg	7%
A	Rh pos	34%
A	Rh neg	6%
B	Rh pos	9%
B	Rh neg	2%
AB	Rh pos	3%
AB	**Rh neg**	**1%**

Human Blood Groups

- RBC membranes have glycoprotein antigens on their external surfaces
- These antigens are:
  - Unique to the individual
  - Recognized as foreign if transfused into another individual
  - Promoters of agglutination and are referred to as agglutinogens
- Presence or absence of these antigens is used to classify blood groups
- Humans have 30 varieties of naturally occurring RBC antigens
- The antigens of the **ABO and Rh** blood groups cause vigorous transfusion reactions when they are improperly transfused
- Other blood groups (**M, N, Dufy, Kell, and Lewis**) are mainly used for legalities
- The ABO blood groups consists of:
  - **Two antigens (A and B) on the surface of the RBCs**
  - **Two antibodies in the plasma (anti-A and anti-B)**
- ABO blood groups may have various types of antigens and preformed antibodies
- Agglutinogens and their corresponding antibodies cannot be mixed without serious hemolytic reactions

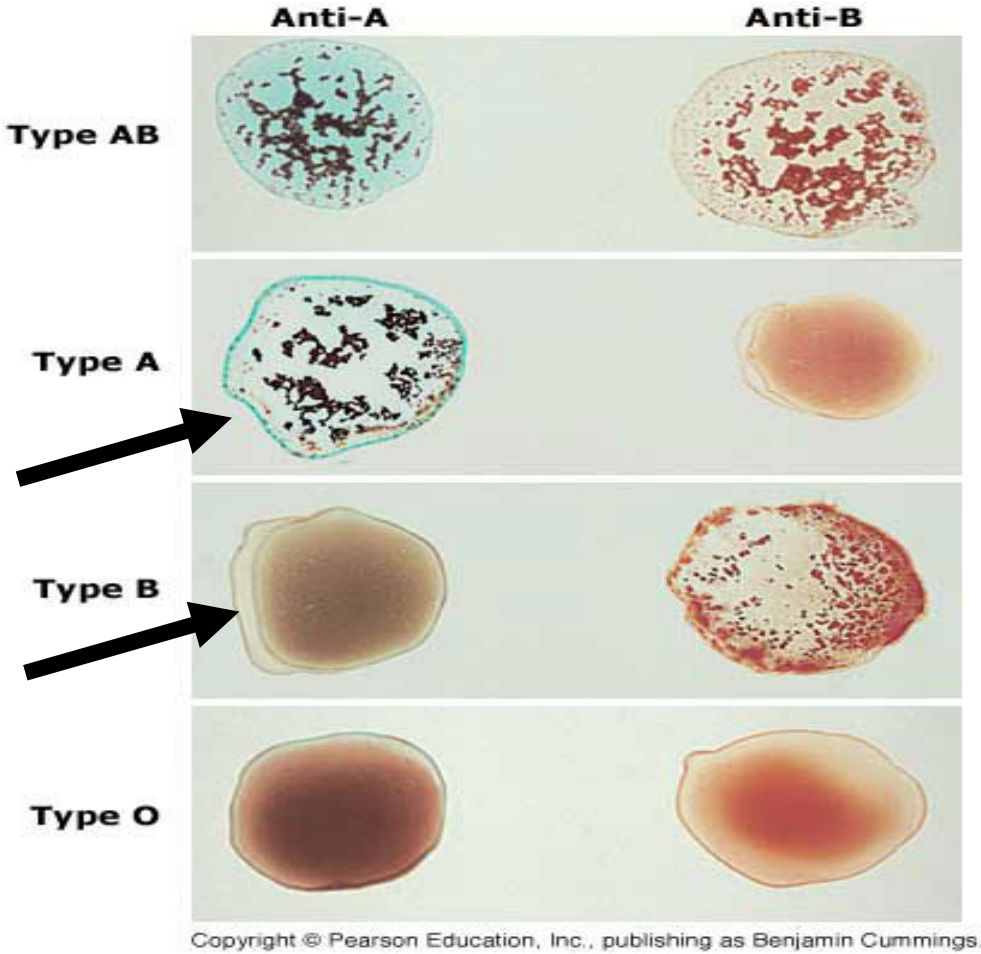
AGGLUTINOGENS

- Also called antigens.
- These agglutinogens are present on the outer surface of the Erythrocyte membranes.
- They are antigenic and have epitopes or antigenic determinants, which are glycoproteins.
- In ABO groups, three types of agglutinogens can be present.

- Some individuals will have Erythrocytes with an agglutinogen called as “A”.
- Others have one called “B”
- The third type of agglutinogen is non antigenic and it is called “H”
- H doesn’t cause production of antibodies.
- So those having H antigen are called O group individuals.

Multiple alleles

ABO blood group s



Type A blood transfused into Type B person

Type B blood transfused into Type B person - OK

A medical problem - some blood transfusions produce lethal clumping of cells.

Don't worry about details yet...

ABO BLOOD GROUPS

Blood Group	Antigens on RBCs	Antibodies in Serum	Genotypes
A	**A**	**Anti-B**	***AA* or *AO***
B	**B**	**Anti-A**	***BB* or *BO***
AB	**A** and **B**	**Neither**	***AB***
O	Neither	**Anti-A and Anti-B**	***OO***

A AND B, INDIVIDUALS

- Those having the A agglutinogen on their erythrocytes are called A blood group people.
- Those having the B agglutinogen are called the B blood group people.

•Some have both the A and B agglutinogens on their erythrocytes and they are called AB type.

•Others have neither A nor B agglutinogens. They have the non antigenic H on their RBCs and are called O group people.

AGGLUTININS

- The antibodies to the agglutinogens are called Agglutinins.
- These are present naturally in ABO groups.
- They are always present in the plasma of the individual.
- There are two types of agglutinins in the ABO blood system:
  - Anti A or α: Alpha
  - Anti B or β: Beta

•The A group people have the Beta or anti B agglutinin in their plasma.

•Similarly the B group people have the Alpha or Anti-A agglutinin in their plasma.

•The AB group of people have no agglutinins in their plasma.

•The O group people have both Alpha and Beta types of agglutinins in their plasma

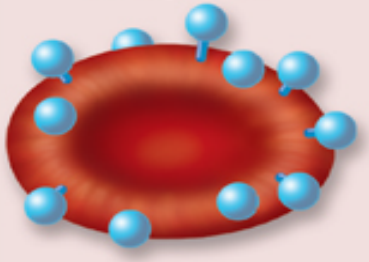
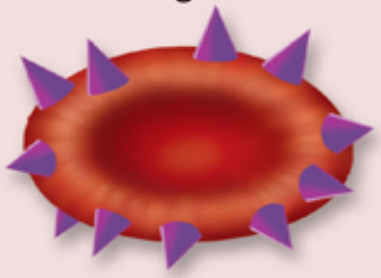
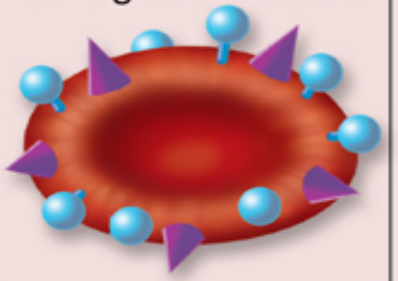
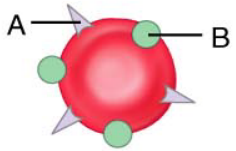
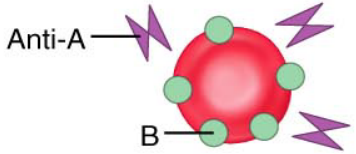
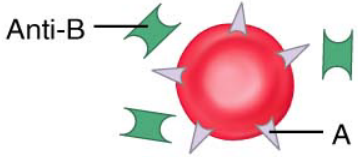
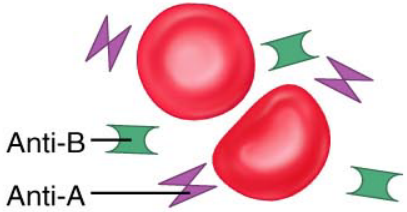
Erythrocytes	Antigen A 	Antigen B 	Antigens A and B 	Neither antigen A nor B 
Plasma	Anti-B antibodies 	Anti-A antibodies 	Neither anti-A nor anti-B antibodies	Both anti-A and anti-B antibodies 
Blood type	**Type A** Erythrocytes with type A surface antigens and plasma with anti-B antibodies	**Type B** Erythrocytes with type B surface antigens and plasma with anti-A antibodies	**Type AB** Erythrocytes with both type A and type B surface antigens, and plasma with neither anti-A nor anti-B antibodies	**Type O** Erythrocytes with neither type A nor type B surface antigens, but plasma with both anti-A and anti-B antibodies

TABLE 17.4 ABO Blood Groups

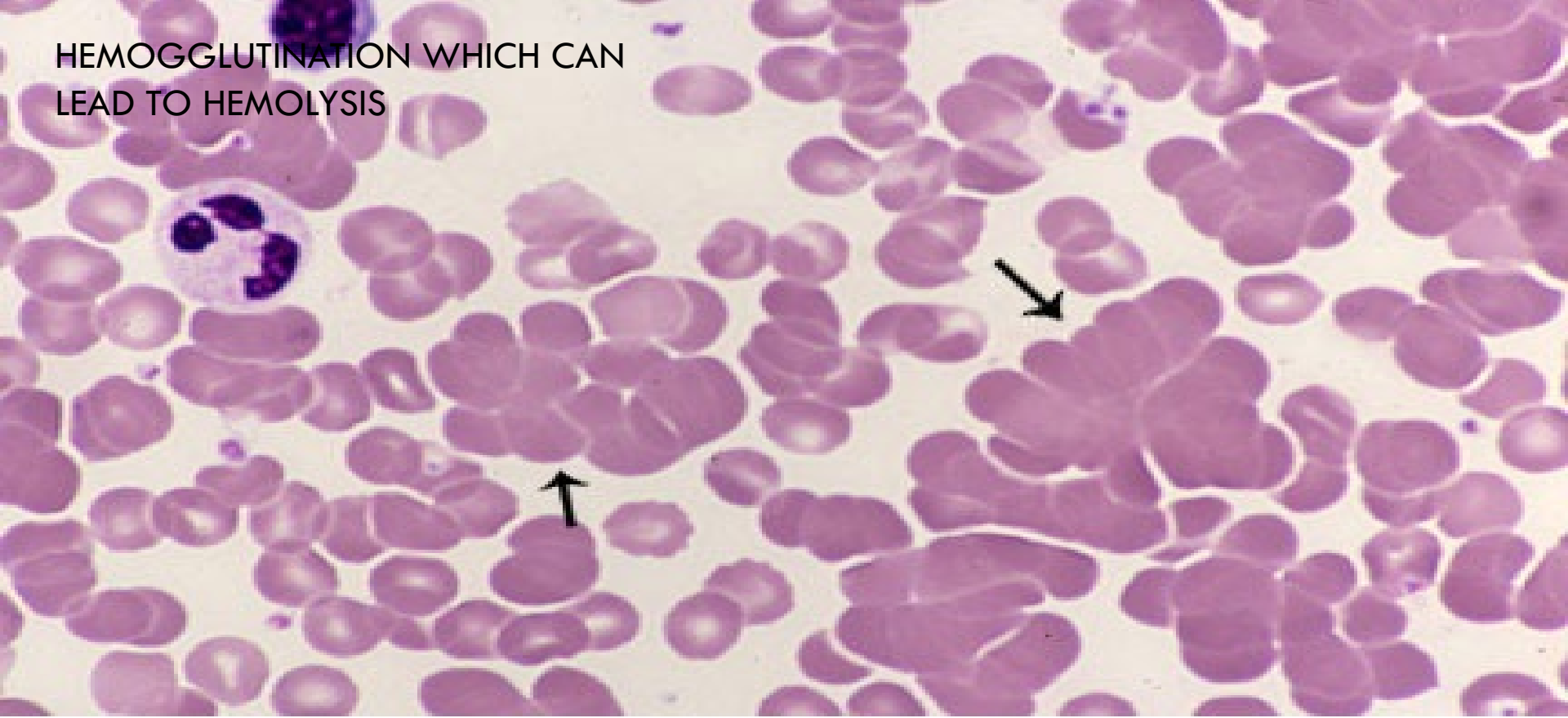
BLOOD GROUP	FREQUENCY (% U.S. POPULATION) WHITE	BLACK	ASIAN	NATIVE AMERICAN	RBC ANTIGENS (AGGLUTINOGENS)	ILLUSTRATION	PLASMA ANTIBODIES (AGGLUTININS)	BLOOD THAT CAN BE RECEIVED
AB	4	4	5	<1	A B		None	A, B, AB, O (Universal recipient)
B	11	20	27	4	B		Anti-A (a)	B, O
A	40	27	28	16	A		Anti-B (b)	A, O
O	45	49	40	79	None		Anti-A (a) Anti-B (b)	O (Universal donor)

HEMAGGLUTINATION

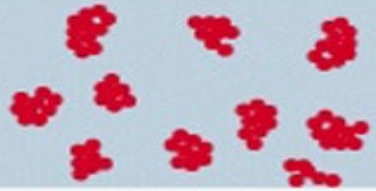
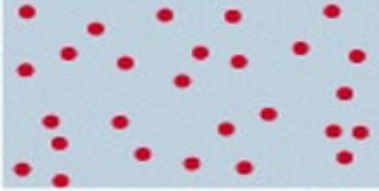
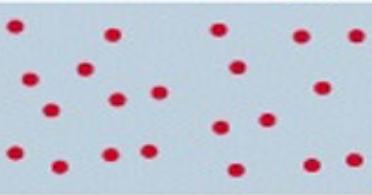
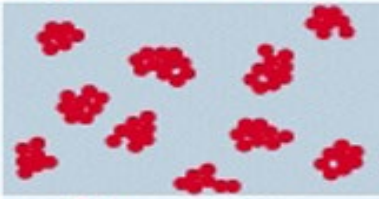
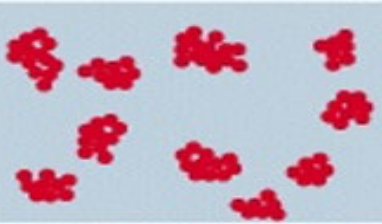
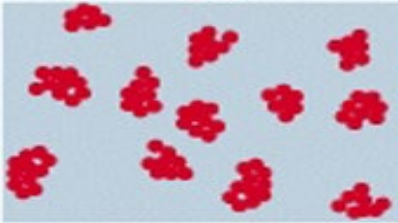
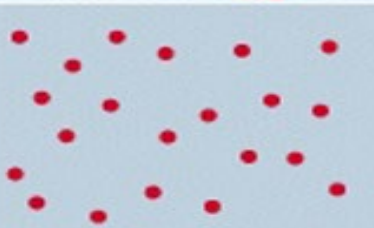
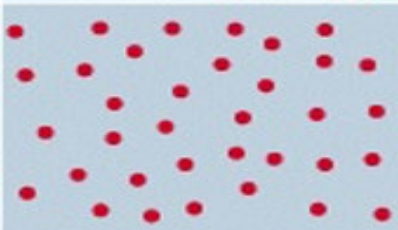
Agglutination or clumping is seen whenever the respective agglutinogens and agglutinins are mixed.

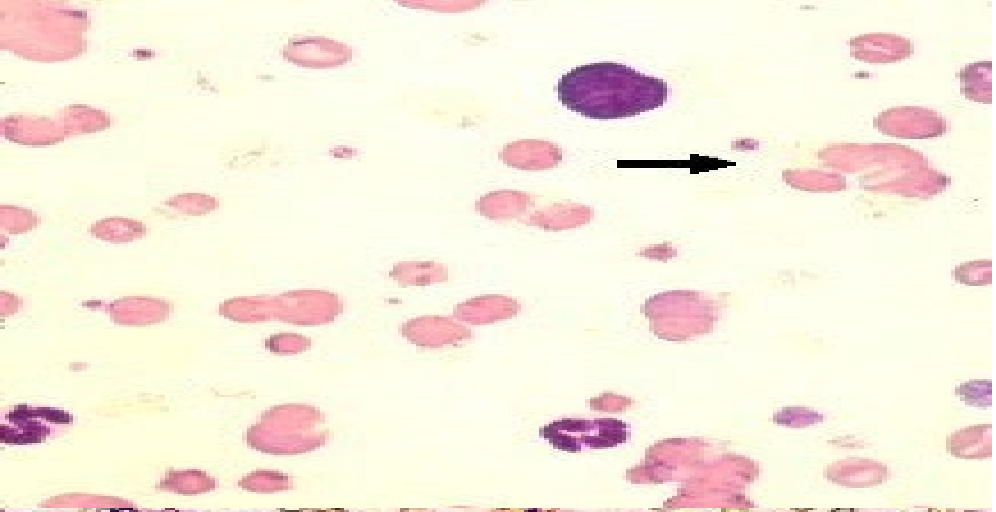
- **Agglutinogen A + Agglutinin Alpha = Agglutination.**
- **Agglutinogen B + Agglutinin Beta = Agglutination.**
- **Both agglutinogens + Both antisera = Agglutination.**
- **No agglutinogens = No agglutination.**

HEMOGGLUTINATION WHICH CAN LEAD TO HEMOLYSIS



Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:
Lichtman's Atlas of Hematology: http://www.accessmedicine.com

Blood type of cells	Genotype	Antibodies made by body	Reaction to added antibodies	
			Anti-A	Anti-B
A	I^AI^A or I^AI^O	Anti-B		
B	I^BI^B or I^BI^O	Anti-A		
AB	I^AI^B	Neither anti-A nor anti-B		
O	I^OI^O	Both anti-A and anti-B		

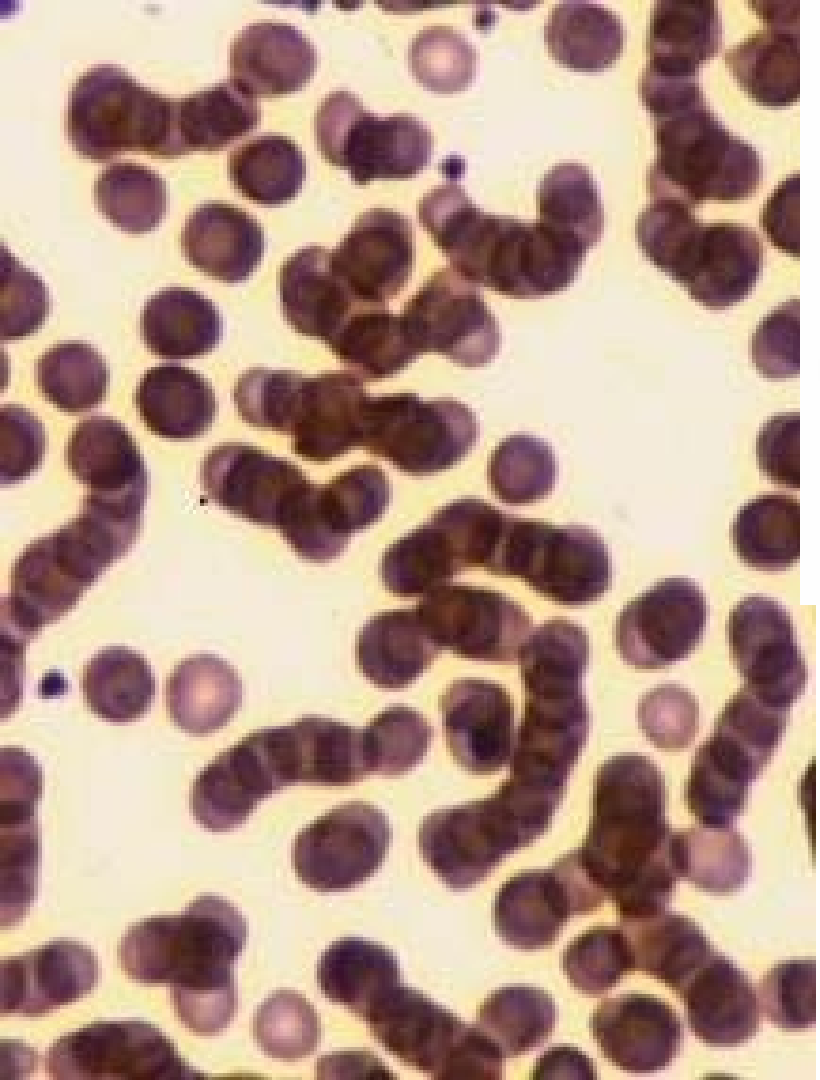


The observation of red blood cell agglutination (also referred to as autoagglutination) must be distinguished from rouleaux formation which is a physiological phenomenon. The presence of antibodies (usually IgM) on the surface of red blood cells is responsible for the phenomenon of autoagglutination.

Agglutination can be observed during immune-mediated hemolytic anemia, but also during 'cryoglobulinemia' (a far more rare condition).

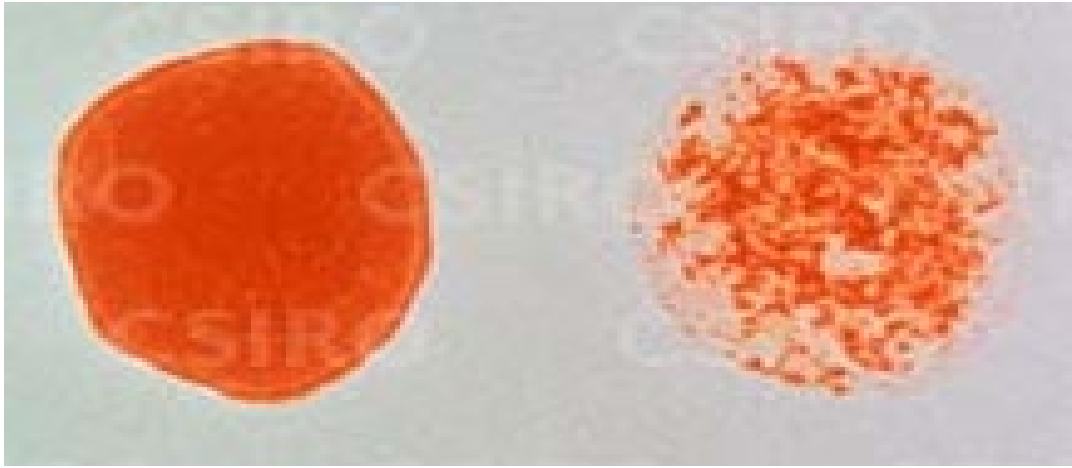
Agglutinating red blood cells resemble grapelike clusters whereas red blood cells in rouleaux formation resemble a stack of coins.

In order to clearly distinguish erythrocyte agglutination from rouleaux formation, a simple saline test can be performed.

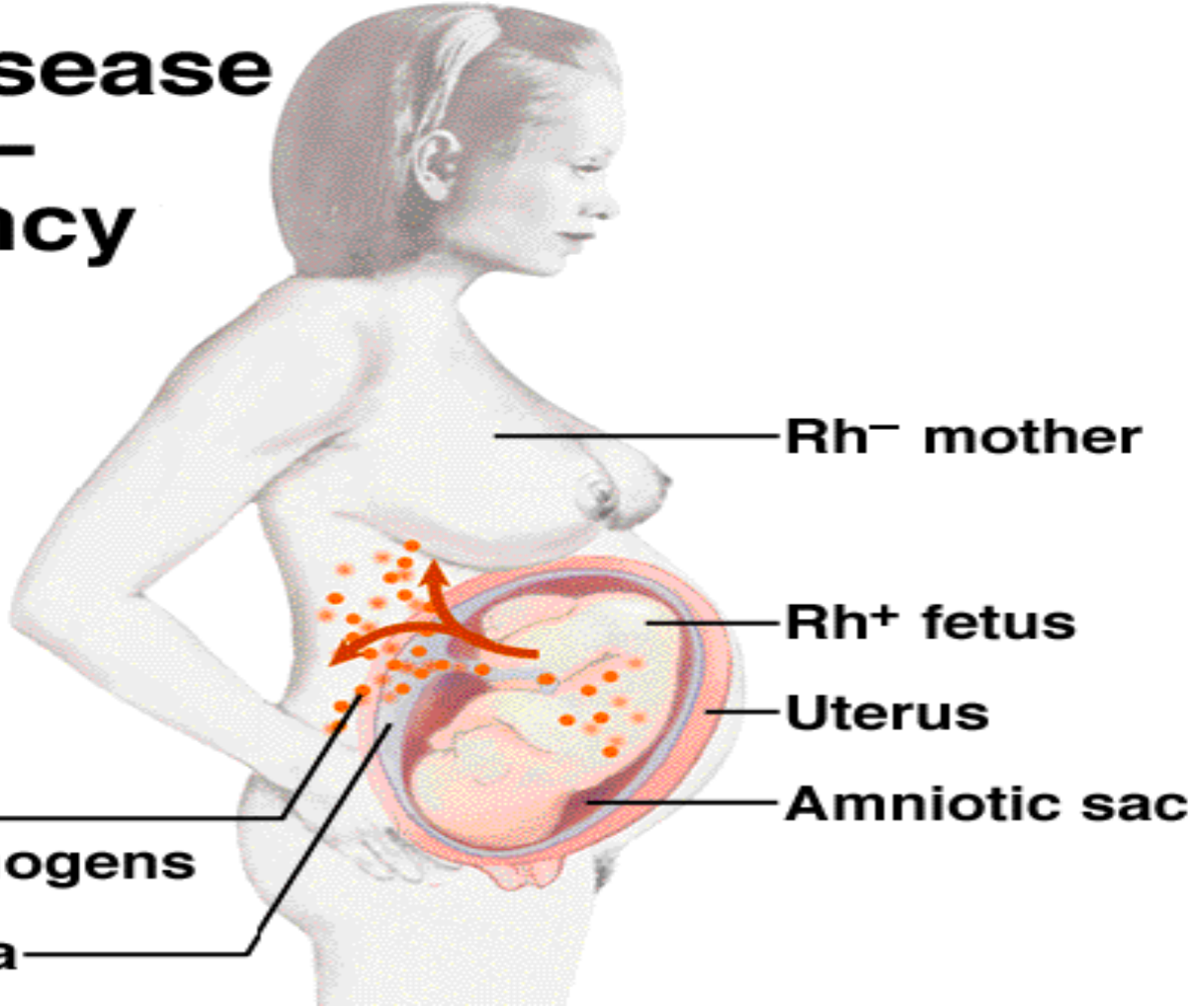


Actual blood sample taken during a demonstration showing red blood cells glued together lacking oxygen.

The same blood cells 30 minutes after the person drank x20 treated water showing blood cells floating free & full of oxygen



Hemolytic Disease of Newborn — First Pregnancy



Rh TYPING: INTRODUCTION

- **It is the second most important typing of blood.**
- **These blood groups were originally discovered in Rhesus monkeys**
- **Rh is another type of agglutinogen.**
- **It is also present on the outer surface of the erythrocytes.**

- **There are eight different Rh agglutinogens, three of which (C, D, and E) are common**
- **Presence of the Rh agglutinogens on RBCs is indicated as Rh^+**
- Anti-Rh antibodies are not spontaneously formed in Rh^- individuals
- However, if an Rh^- individual receives Rh^+ blood, anti-Rh antibodies form
- A second exposure to Rh^+ blood will result in a typical transfusion reaction

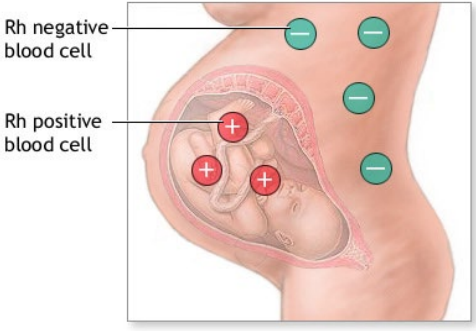
ABO System & Pregnancy

- Majorities of hemolytic diseases are due to ABO incompatibility
- **Foetus inherits one gene from each parent.**
  - **O + O = O,**
  - **O + A= O or A,**
  - **O + B= O or B,**
  - **O + AB= A or B.**
- There is a 20% chance of ABO incompatibility of mother & foetus
- Only 5% chance of developing hemolytic disease only in type A & B infants of type O mothers, that too only of milder forms

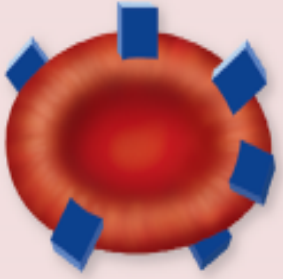
Rhesus

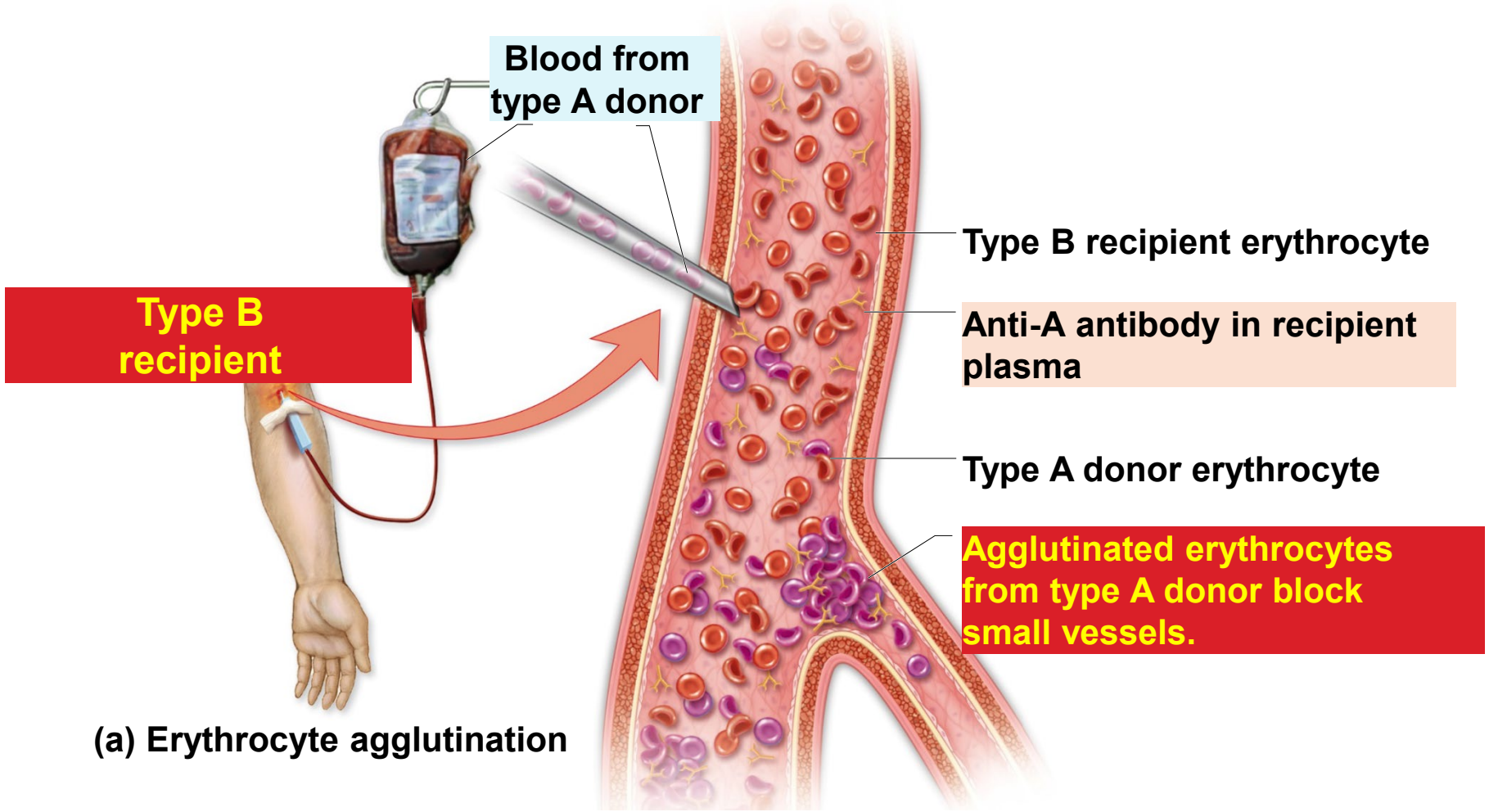
47 Antigens make up the Rhesus Blood Group
The most significant is the D antigen

Rh or D Agglutinins



- Anti-D agglutinins or antibodies do not occur naturally.
- They are produced by the Immune systems as and when it is exposed to the D antigens.
- So these Anti D agglutinins are found only in some of the Rh Negative people.
- Those who have been exposed to the Rh or D antigen

	Rh Blood Types	
Blood type	Rh positive	Rh negative
Erythrocytes	Surface antigen D 	No surface antigen D 
Plasma	No anti-D antibodies	No anti-D antibodies unless exposed to Rh positive blood



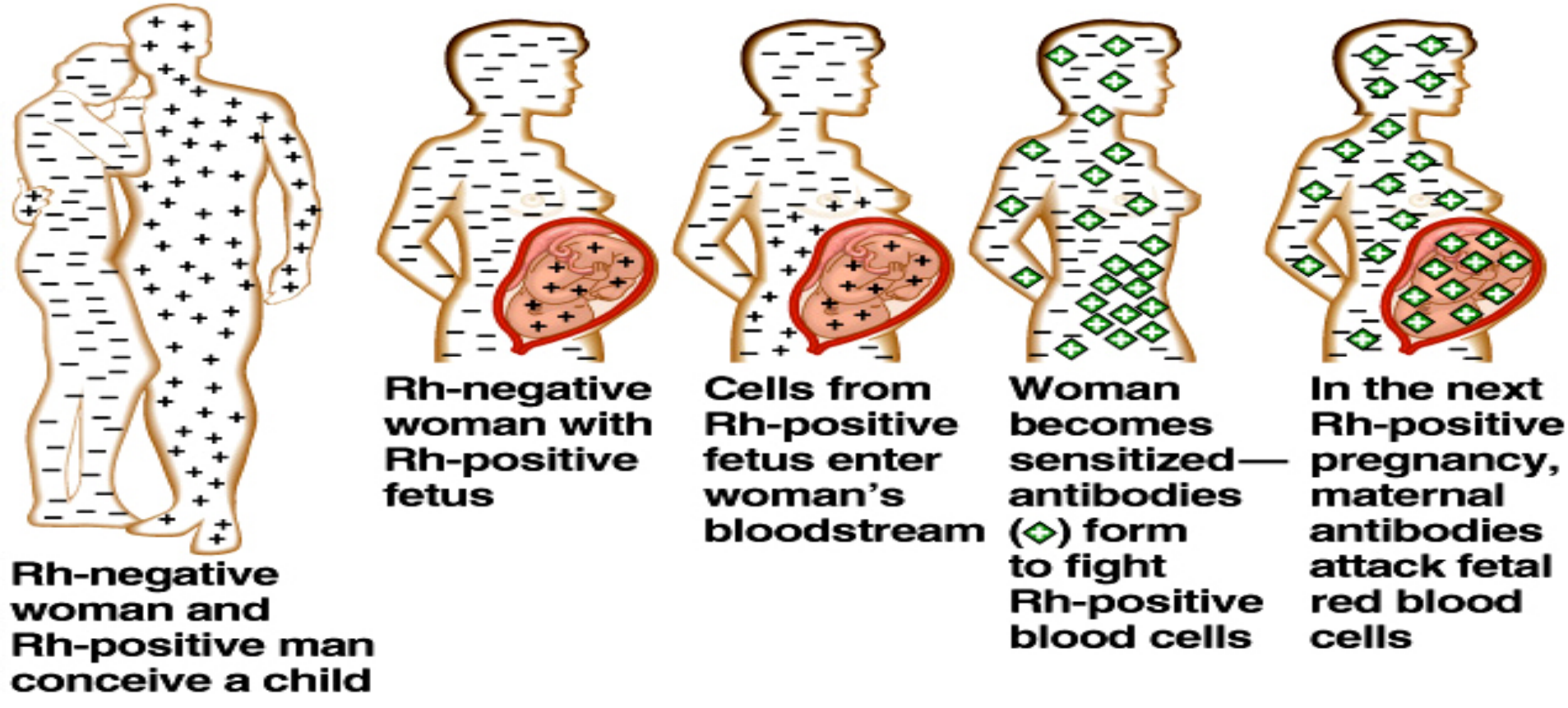
(a) Erythrocyte agglutination

Exposure to Antigens: How?

- The Rh+ve people will never manufacture Anti D antibodies.
- Only Rh – ve individuals can develop these Agglutinins.

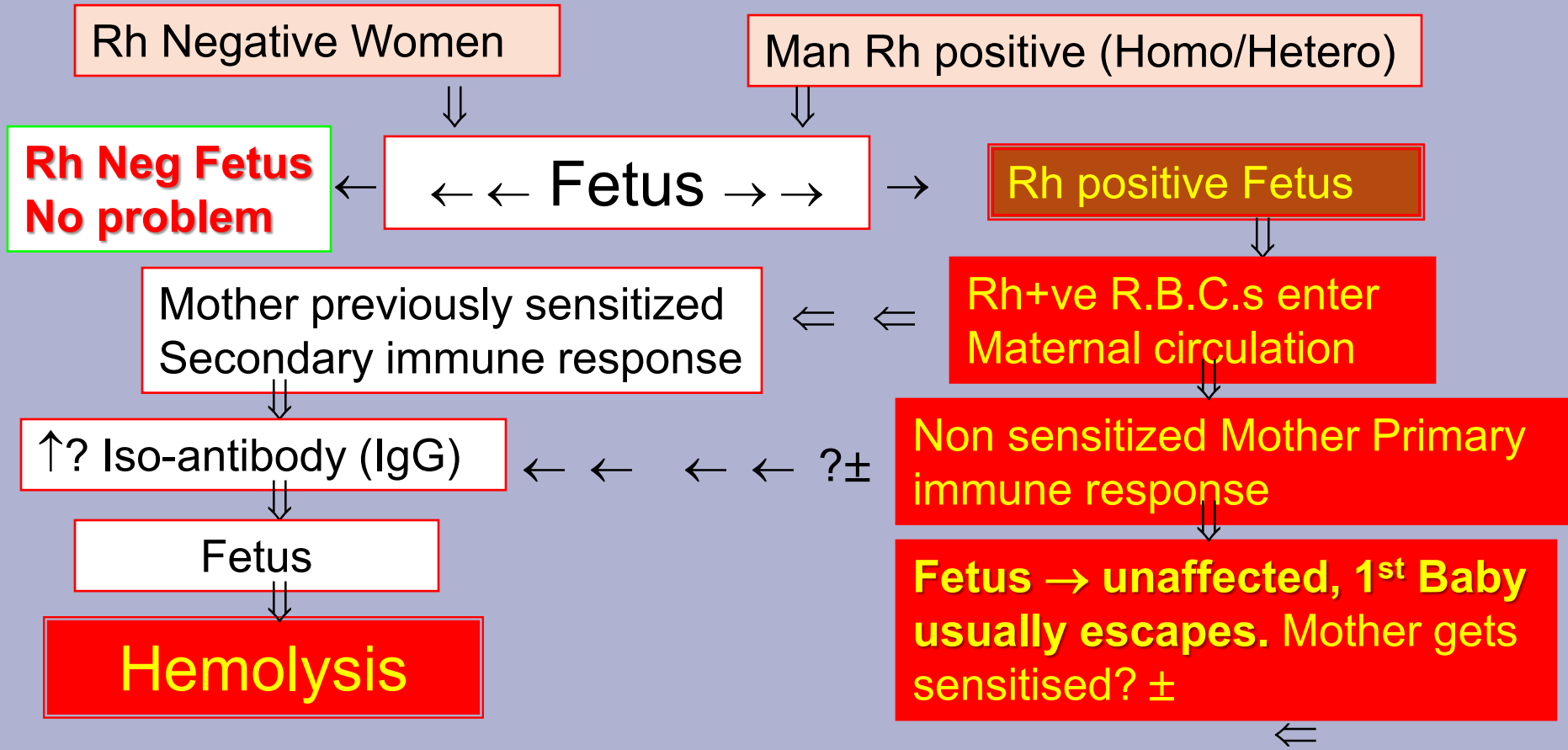
- When these Rh-ve people receive Rh+ve blood by mistake, they get exposed to the antigen.

- Then they will develop the antibody.



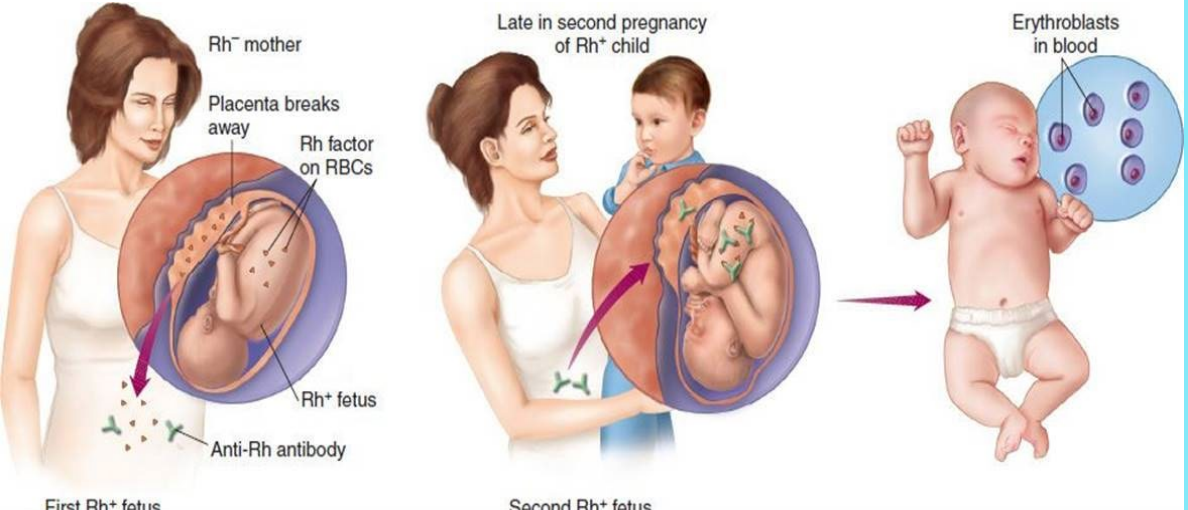
The disease, called **erythroblastosis fetalis** or **hemolytic disease of the newborn,** may be so severe as to kill the fetus or even the newborn infant. It is an example of an antibody-mediated cytotoxicity disorder.

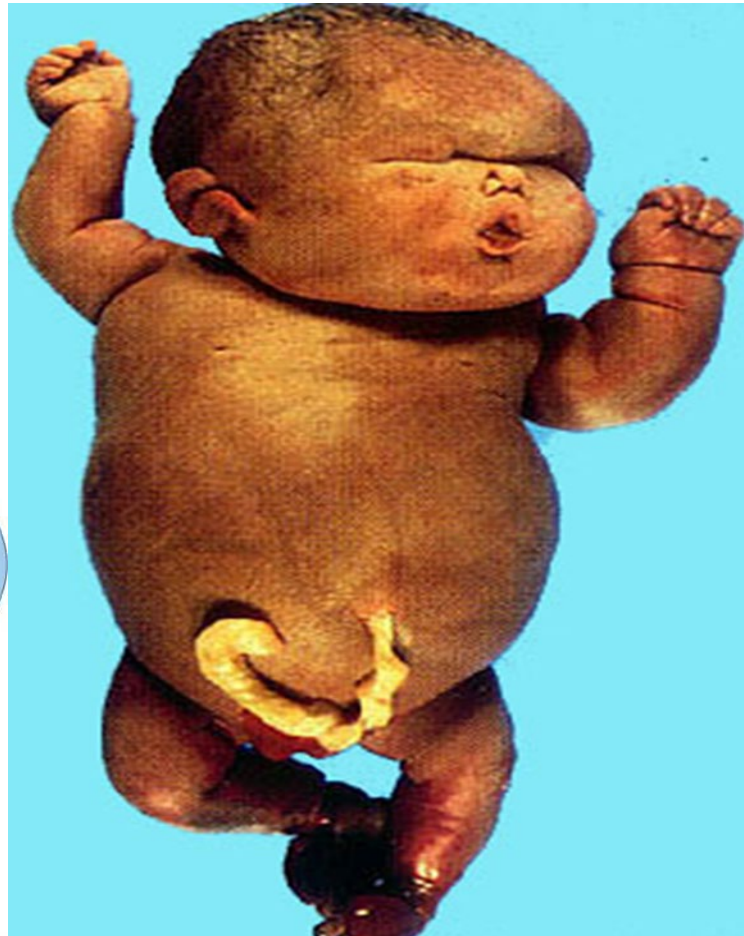
Pathogenesis Of Rh Iso-immunisation

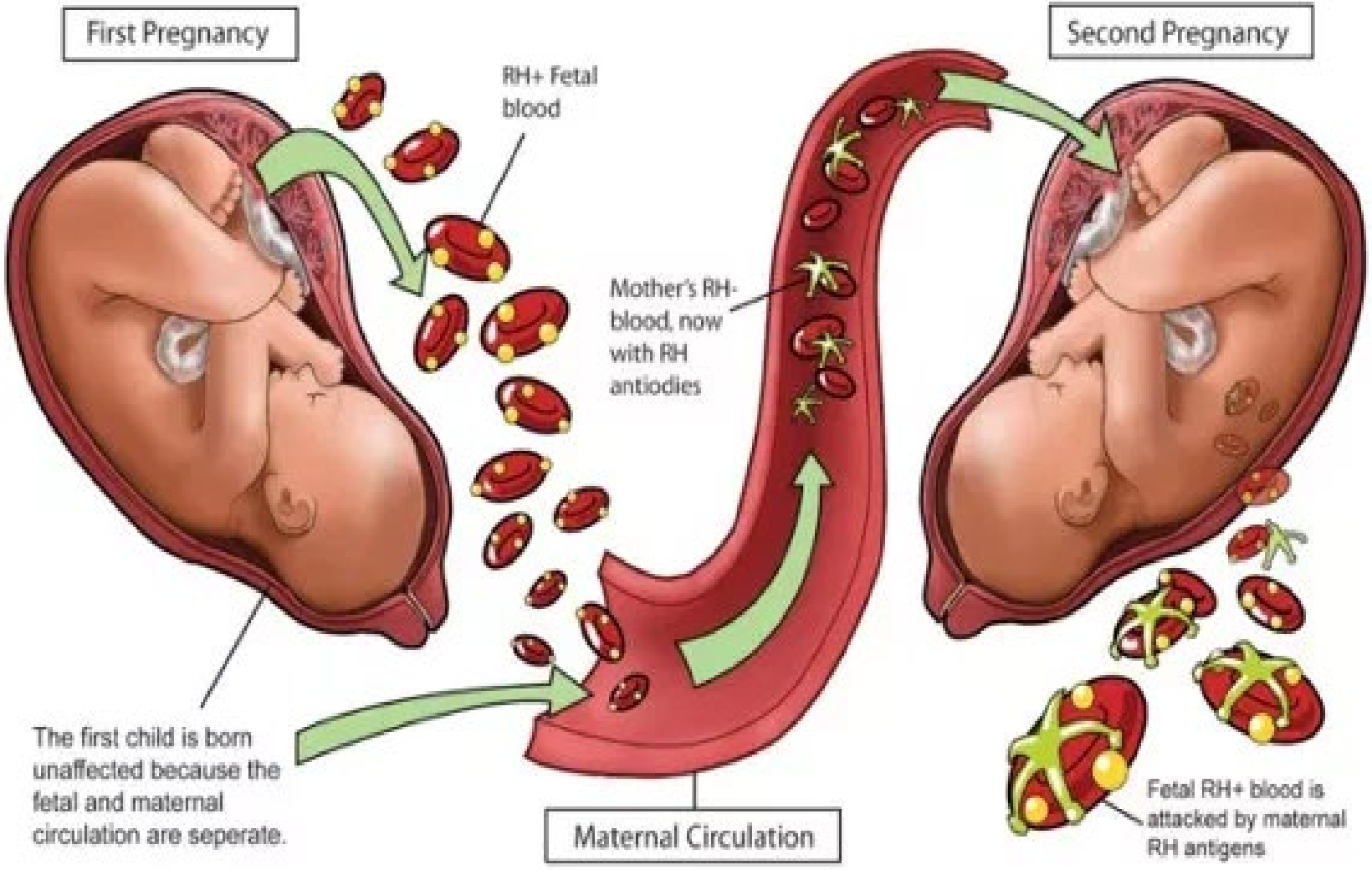


ERYTHROBLASTOSIS FETALIS

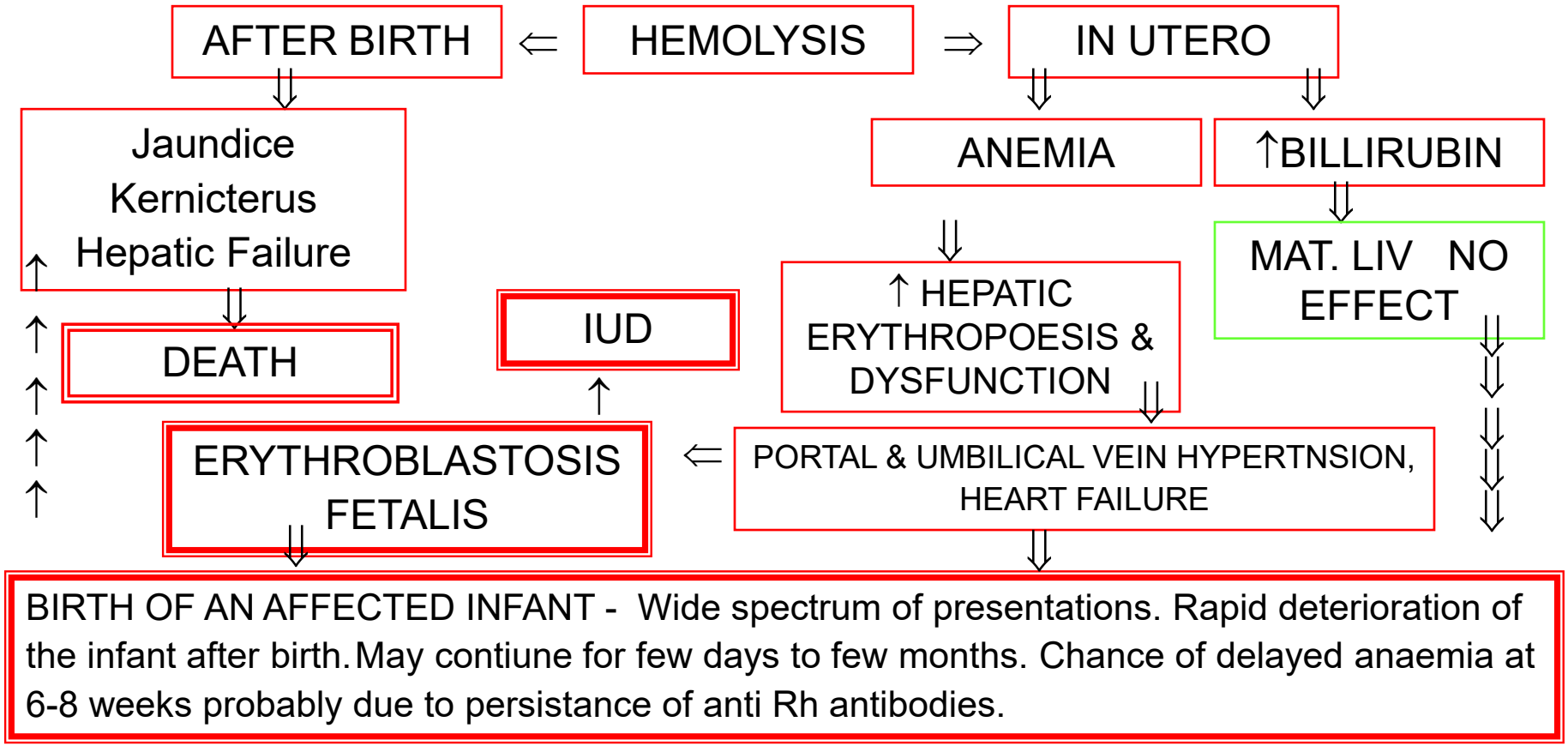
- The second child in such a woman, if also Rh+ve, can develop a disease called as **Erythroblastosis fetalis.**
- This is due to the **Anti D antibodies** developed in the mother.
- **These antibodies traverse through the placenta, enter the fetal circulation and cause agglutination** of the erythrocytes of the fetus.






First Pregnancy
RH+ Fetal blood
Mother's RH- blood, now with RH antiodies
Second Pregnancy
The first child is born unaffected because the fetal and maternal circulation are seperate.
Maternal Circulation
Fetal RH+ blood is attacked by maternal RH antigens

Pathology Of Iso-immunisation



Hemolytic Disease of the Newborn

- The drug RhoGAM can prevent the Rh^- mother from becoming sensitized
- Treatment of hemolytic disease of the newborn involves pre-birth transfusions and exchange transfusions after birth

Transfusion Reactions

- Transfusion reactions occur when mismatched blood is infused
- Donor's cells are attacked by the recipient's plasma agglutinins causing:
  - **Diminished oxygen-carrying capacity**
  - **Clumped cells that impede blood flow**
  - **Ruptured RBCs that release free hemoglobin into the bloodstream**

Circulating hemoglobin precipitates in the kidneys and causes renal failure

Blood Typing

Blood type being tested	RBC agglutinogens	Serum Reaction	
		Anti-A	Anti-B
AB	A and B	+	+
B	B	–	+
A	A	+	–
O	None	–	–

Plasma Volume Expanders

- When shock is imminent from low blood volume, volume must be replaced
- **Plasma or plasma expanders can be administered**

Aging changes in the blood

- The properties of blood change as we grow older.
- It is thought that these changes might contribute to the increased incident of clot formation and atherosclerosis in older people.
- Some of the most prominent findings on these changes include:
  - Rise in fibrinogen
  - Rise in blood viscosity
  - Rise in plasma viscosity
  - Increased red blood cell rigidity
  - Increased formation of fibrin degradation products
  - Earlier activation of the coagulation system