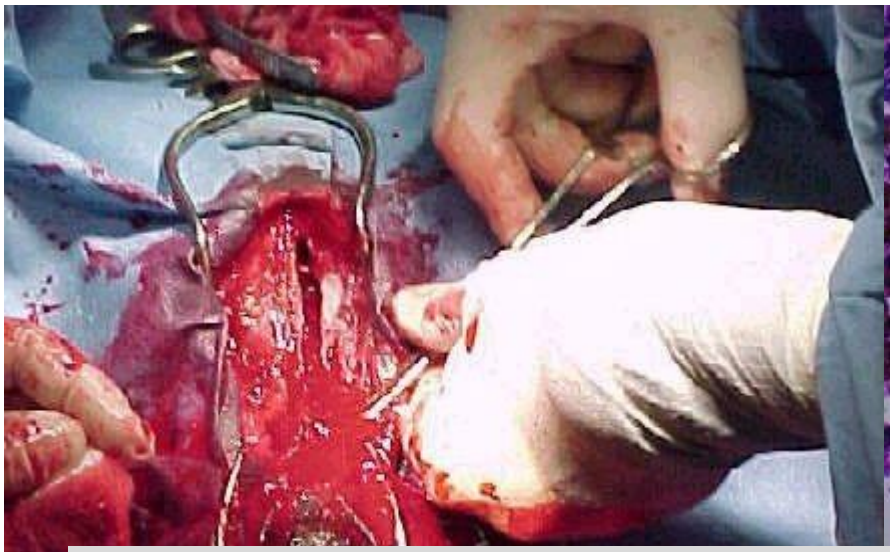
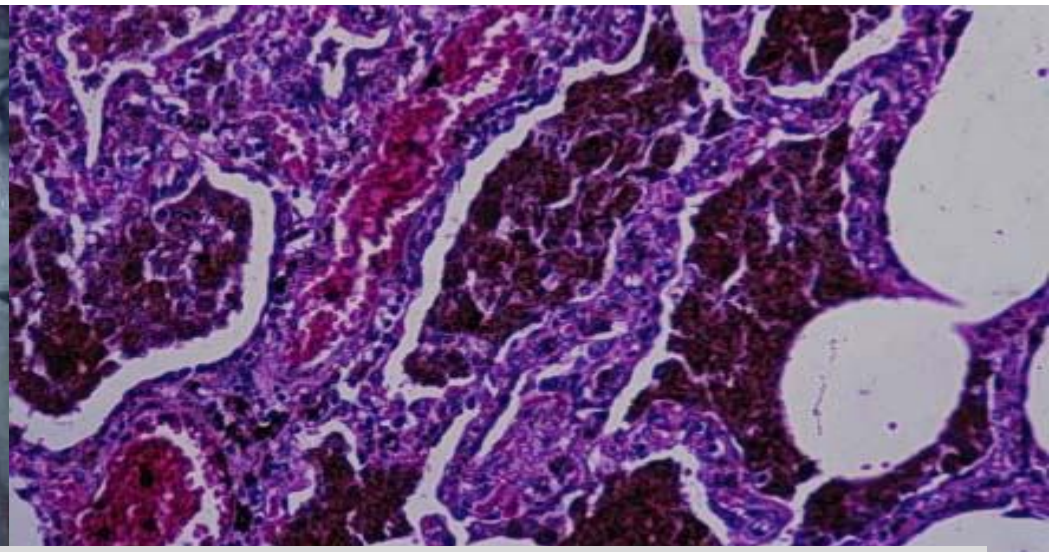
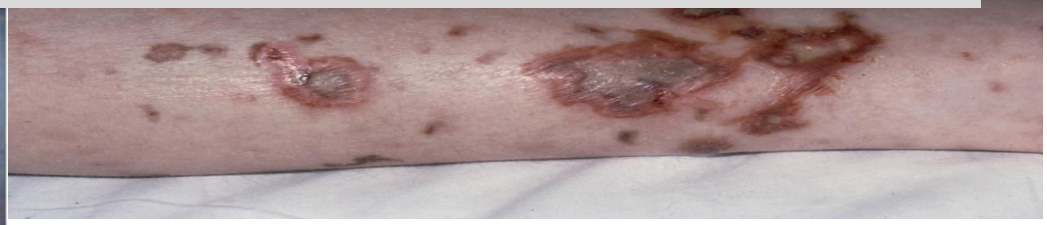




Hemostasis and coagulation



Danil Hammoudi.MD

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

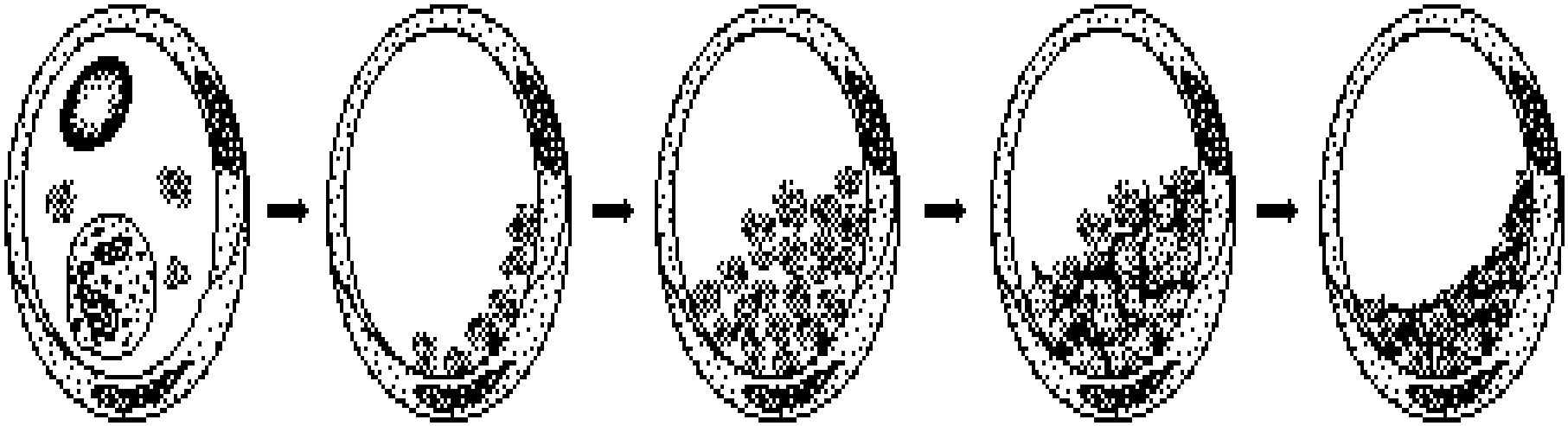
- Primary Hemostasis
  - Platelet Plug Formation
  - Dependent on normal platelet number & function
  - Initial Manifestation of Clot Formation

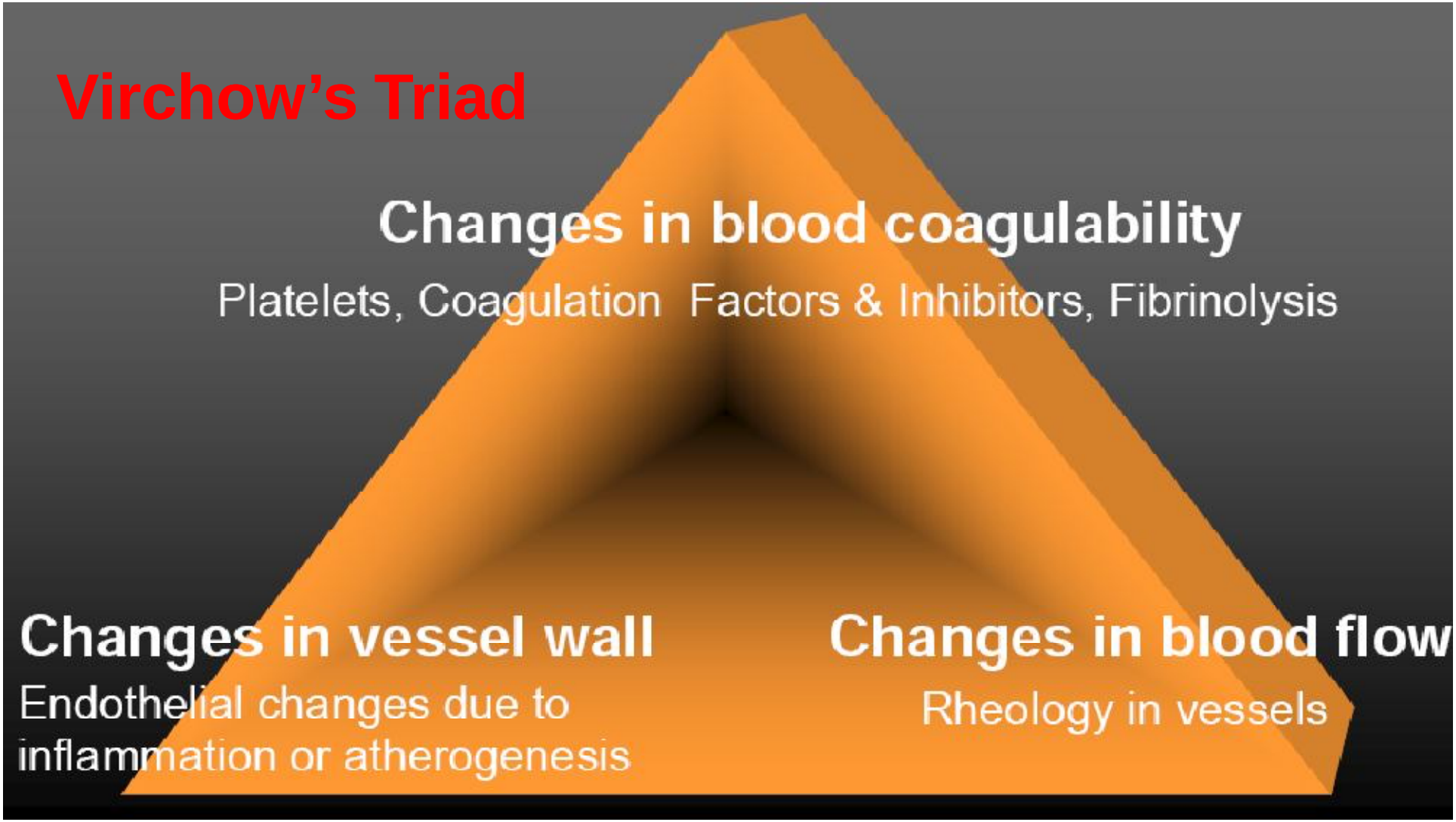
Secondary Hemostasis

- Activation of Clotting Cascade ☒ Deposition & Stabilization of Fibrin

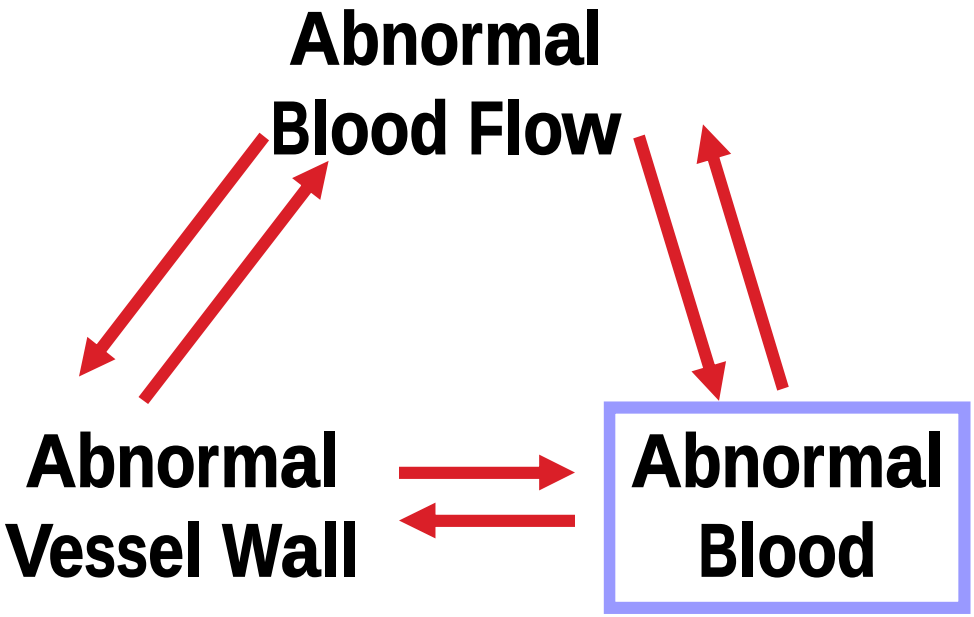
Tertiary Hemostasis

- Dissolution of Fibrin Clot
- Dependent on Plasminogen Activation

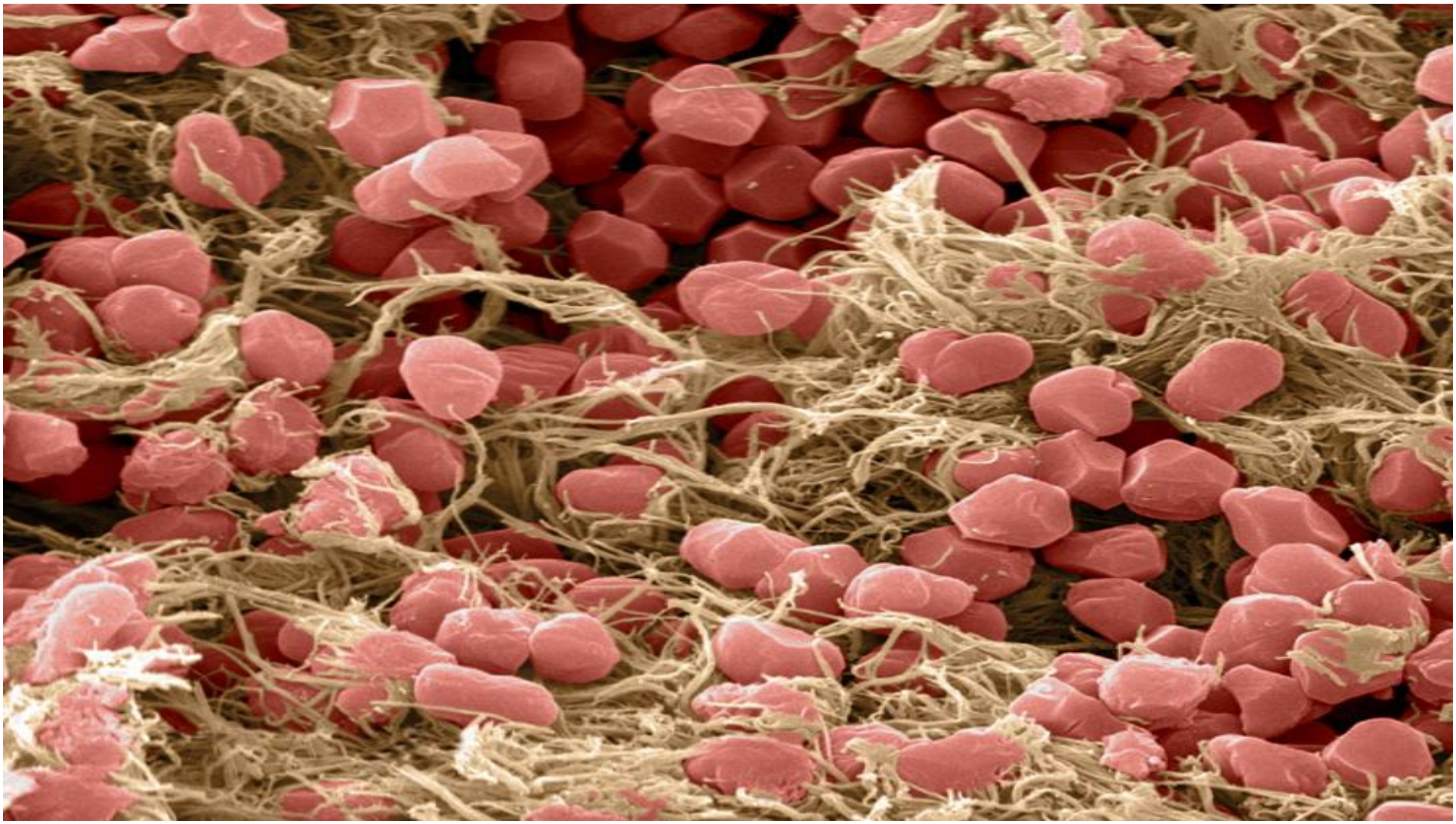

Circulating platelets; endothelial damage
Platelet adhesion
Platelet aggregation
Platelet-Fibrin plug
Clot retraction


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

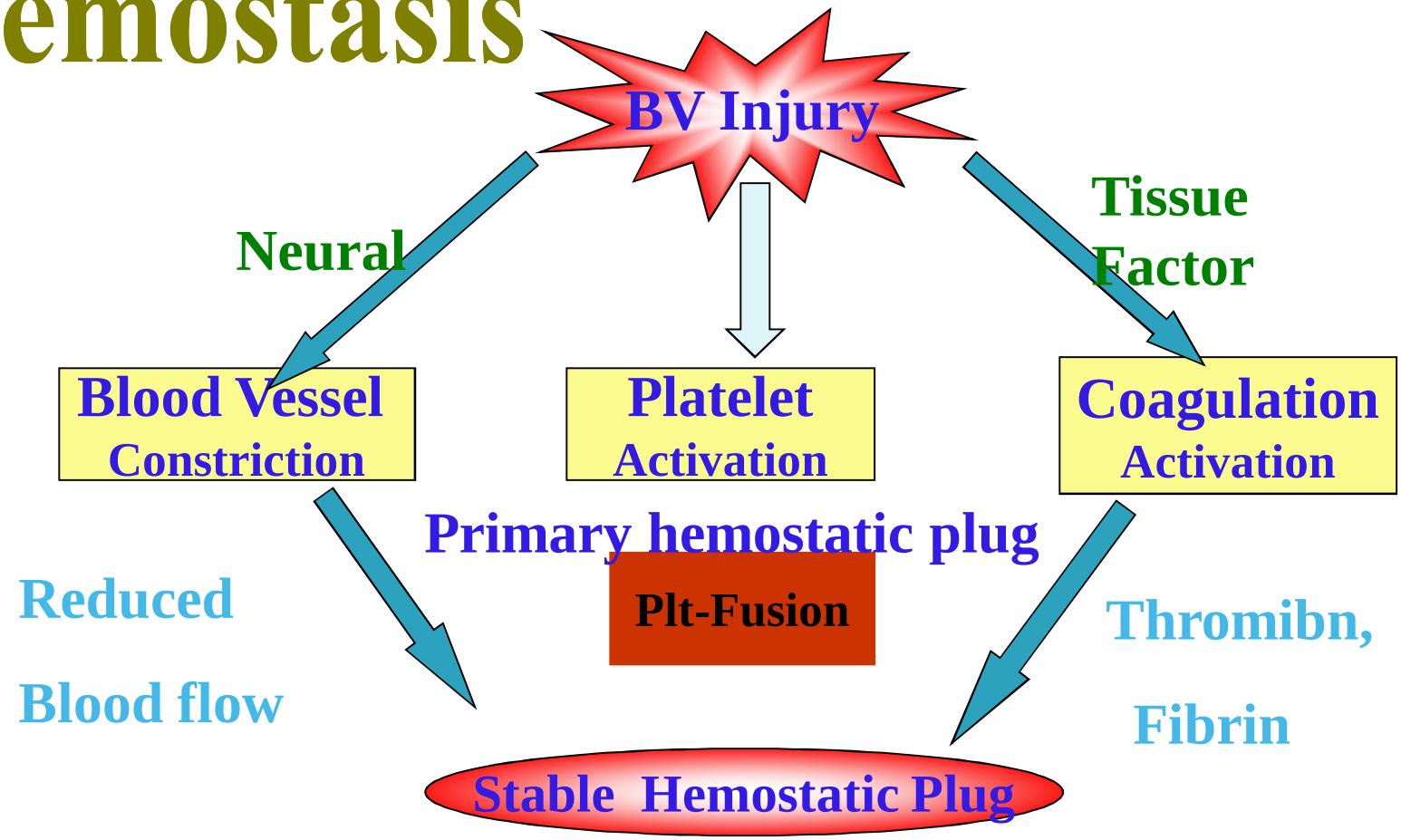
- Acquired antibodies to coagulation factors
- Inhibitor directed
  - Against Factor VIII
  - Antiphospholipid

Genetic

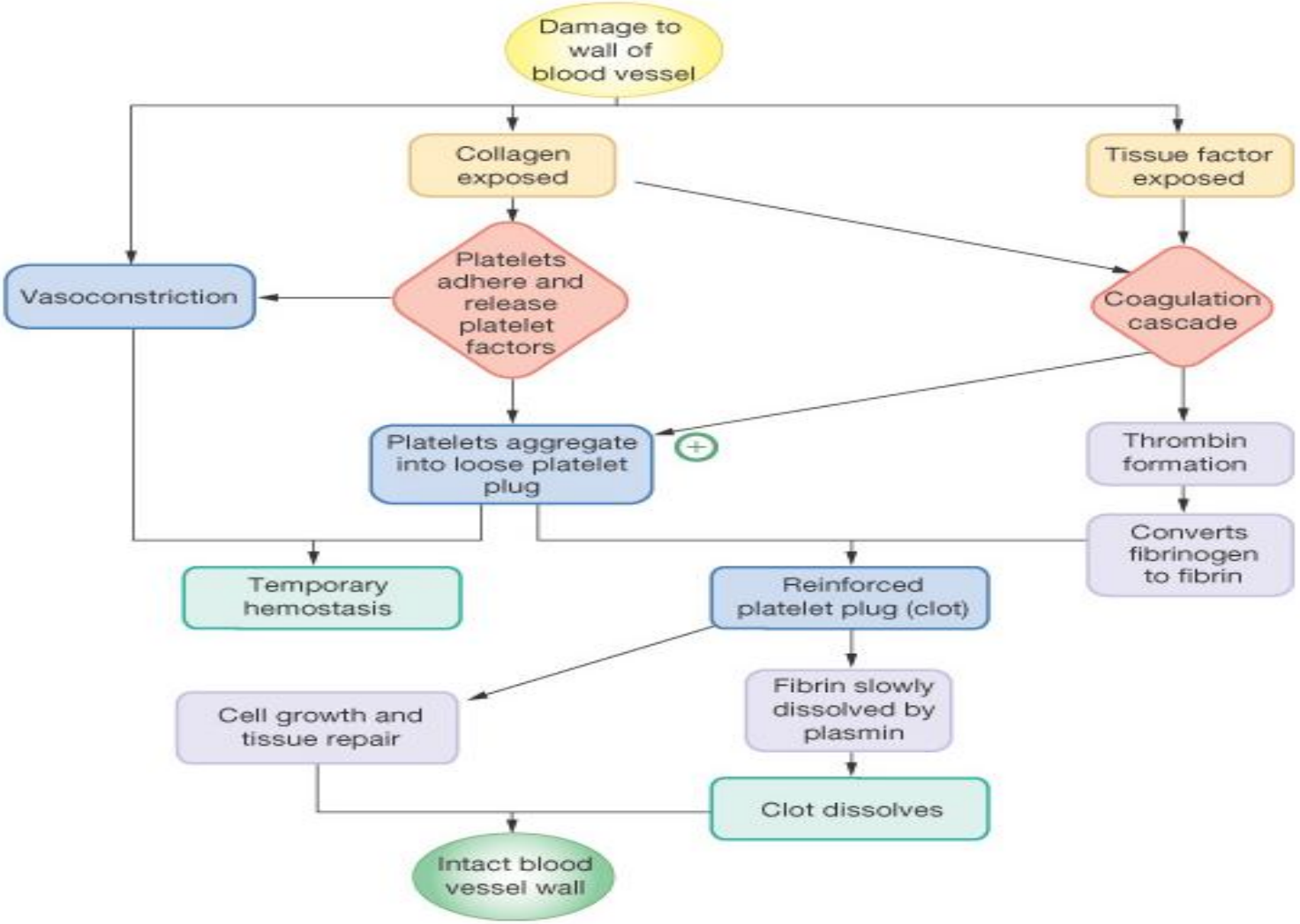
Lack of coagulation factor protien producing genes

- •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

Hemostasis

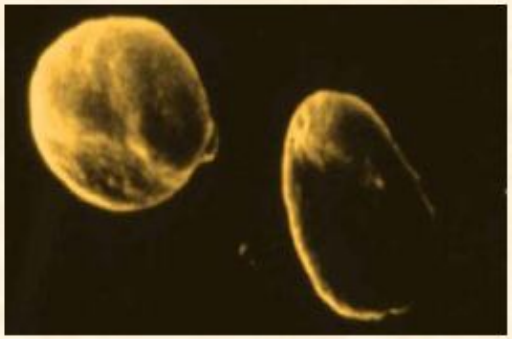

BV Injury
Neural
Tissue Factor
Blood Vessel Constriction
Platelet Activation
Coagulation Activation
Primary hemostatic plug
Plt-Fusion
Reduced Blood flow
Thromibn, Fibrin
Stable Hemostatic Plug

Overview of Hemostasis: Clot Formation & Vessel Repair

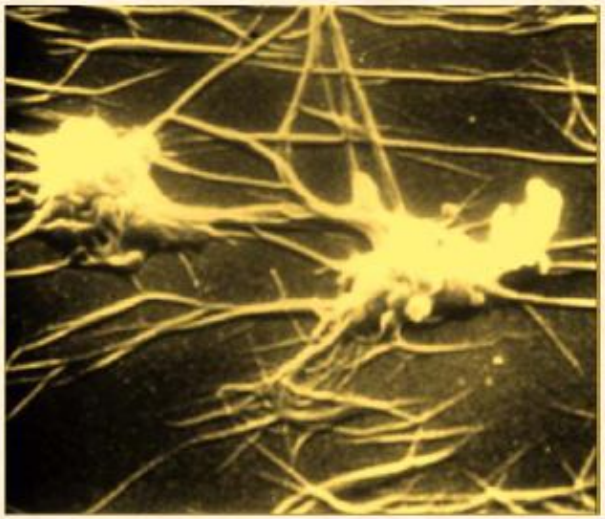


Hemostasis

- 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)**



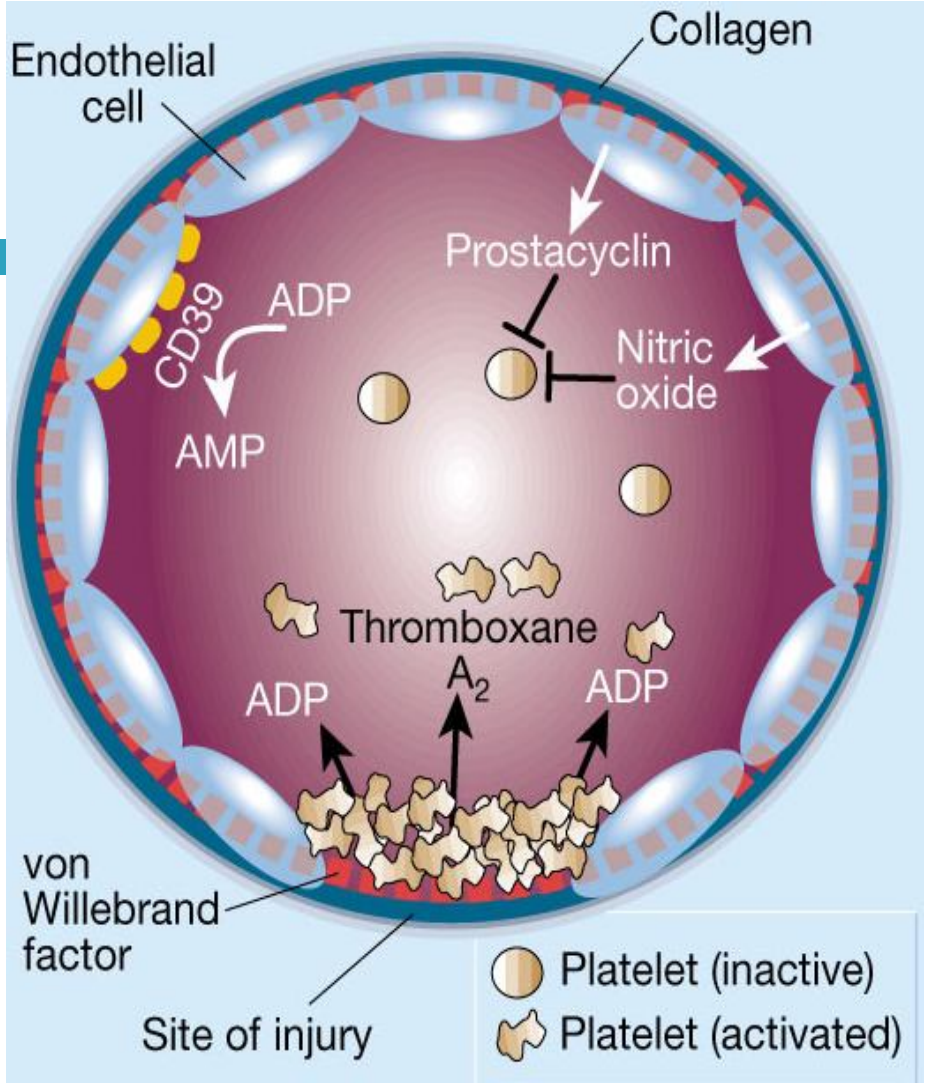
Resting platelets

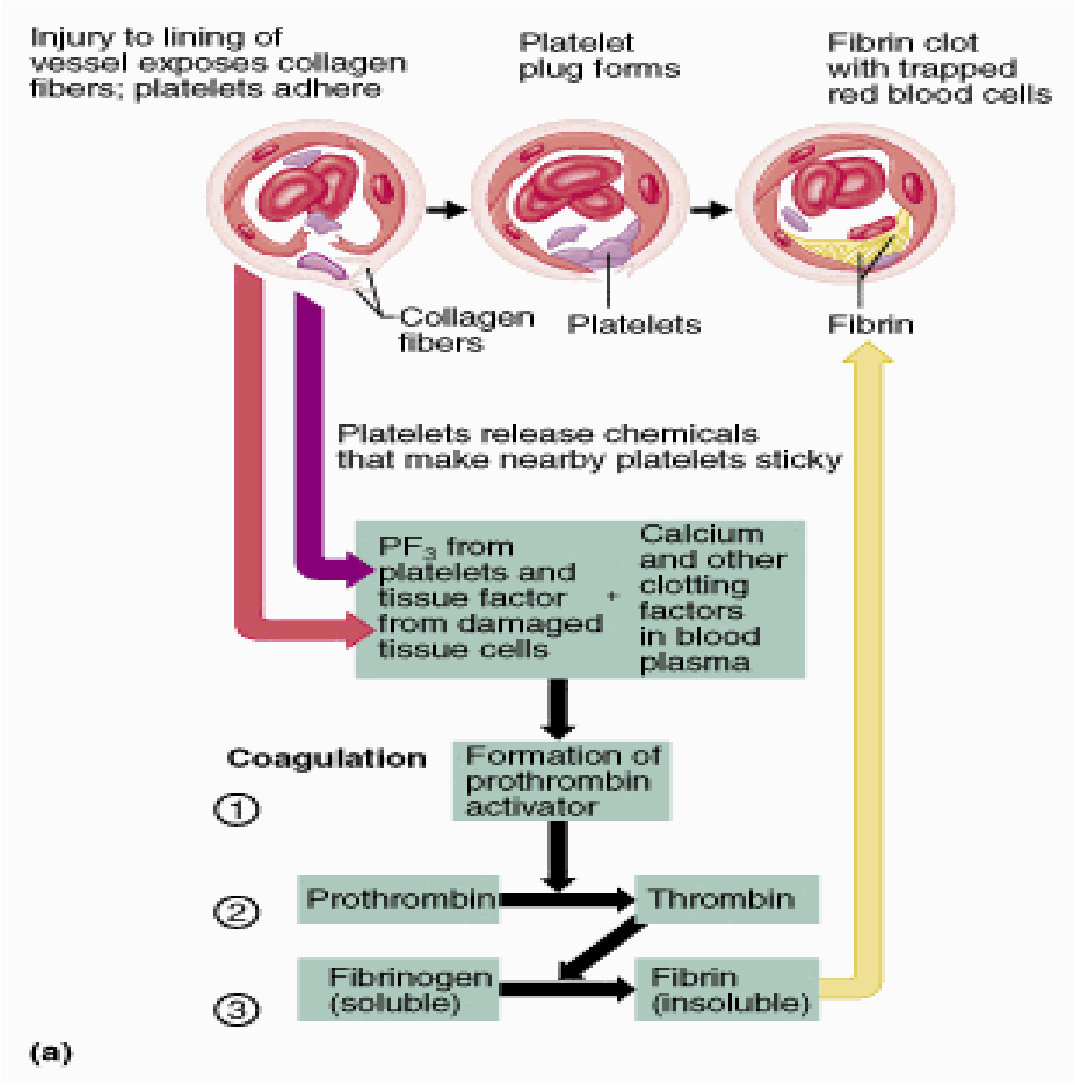


Activated platelets

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**





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

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)

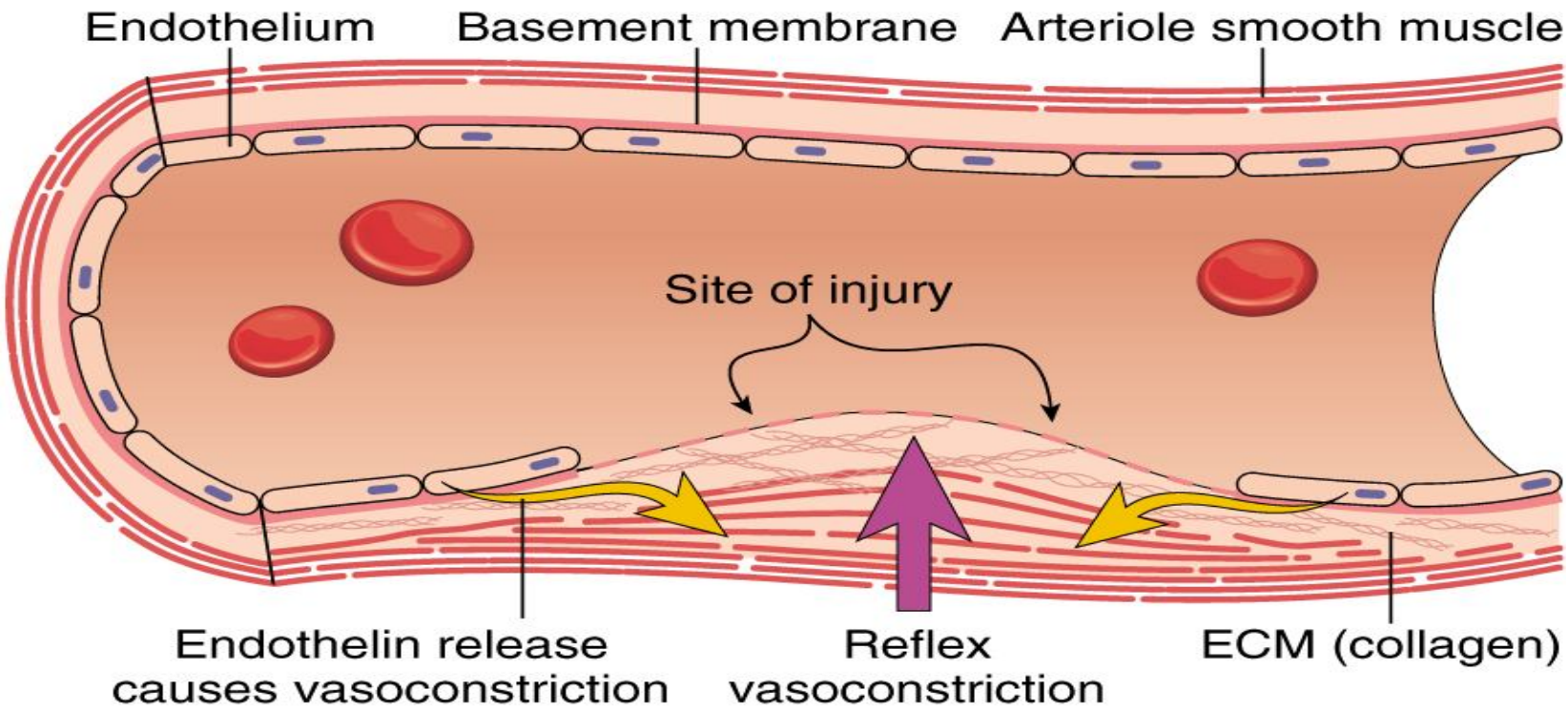
*__THROMBOMODULIN__: changes specificity of thrombin (activates protein C , which inactivates factors Va and VIIIa

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

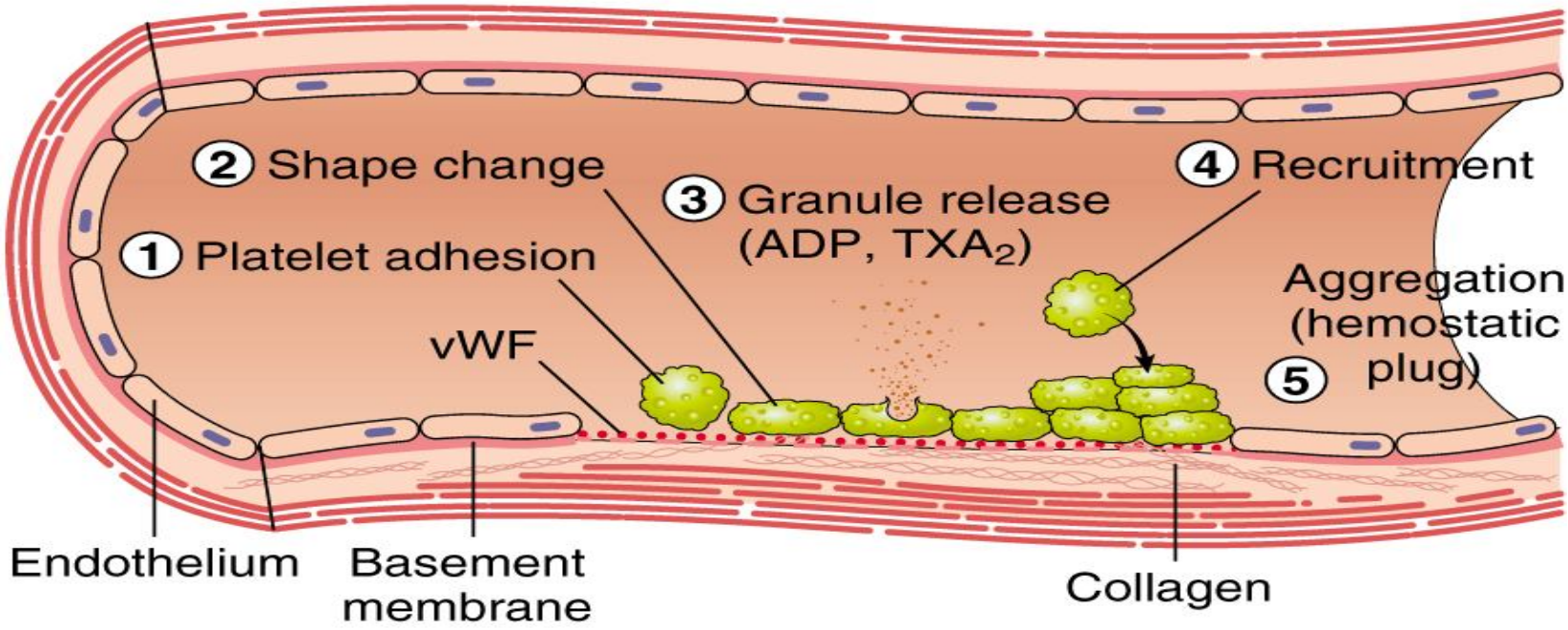
Prothrombotic Properties of the Endothelium

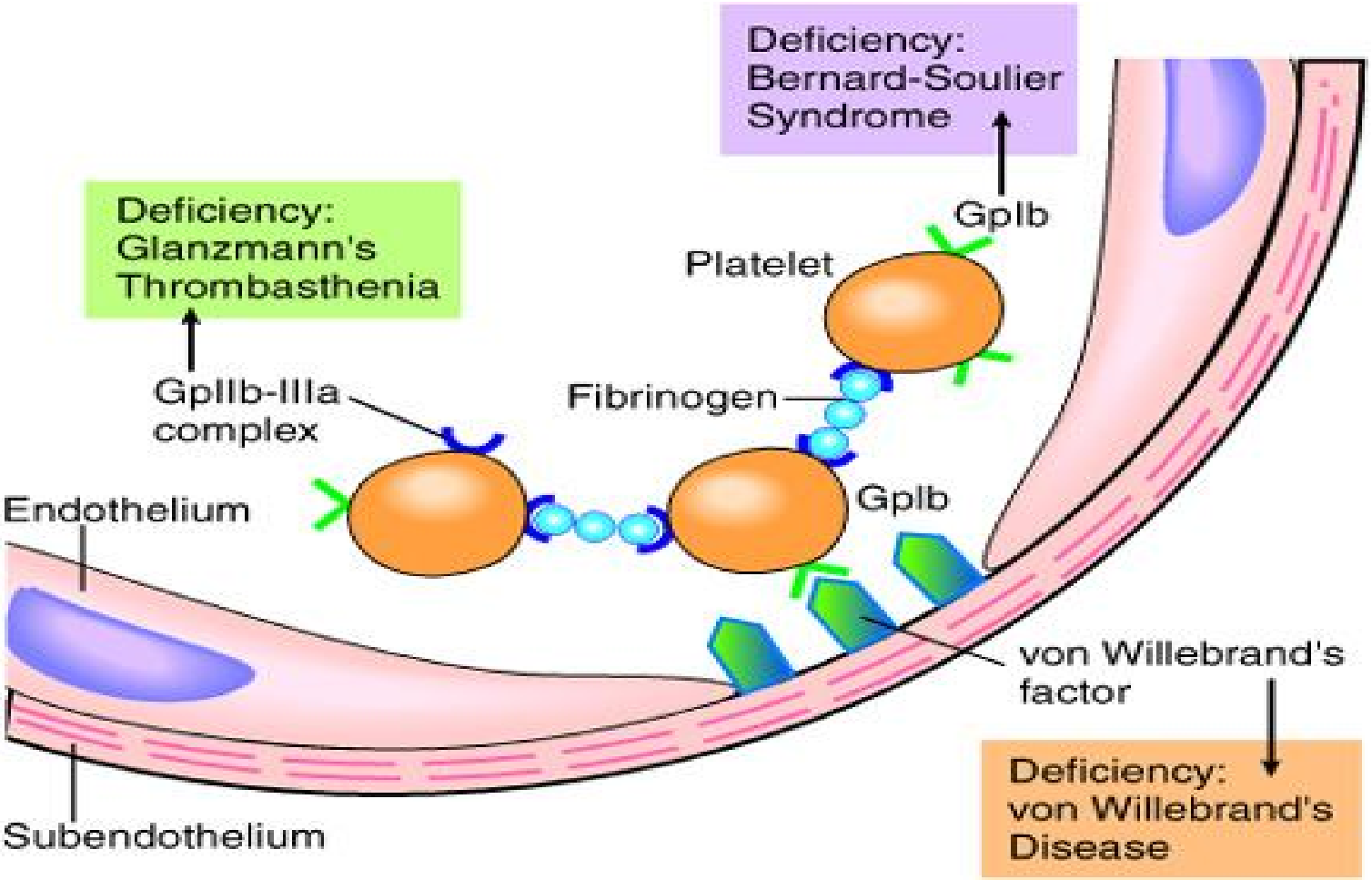
- Synthesis of von Willebrand factor
- Release of tissue factor
- Production of plasminogen activator inhibitors (PAI)
- Membrane phospholipids bind and facilitate activation of clotting factors via Ca bridges

A. VASOCONSTRICTION

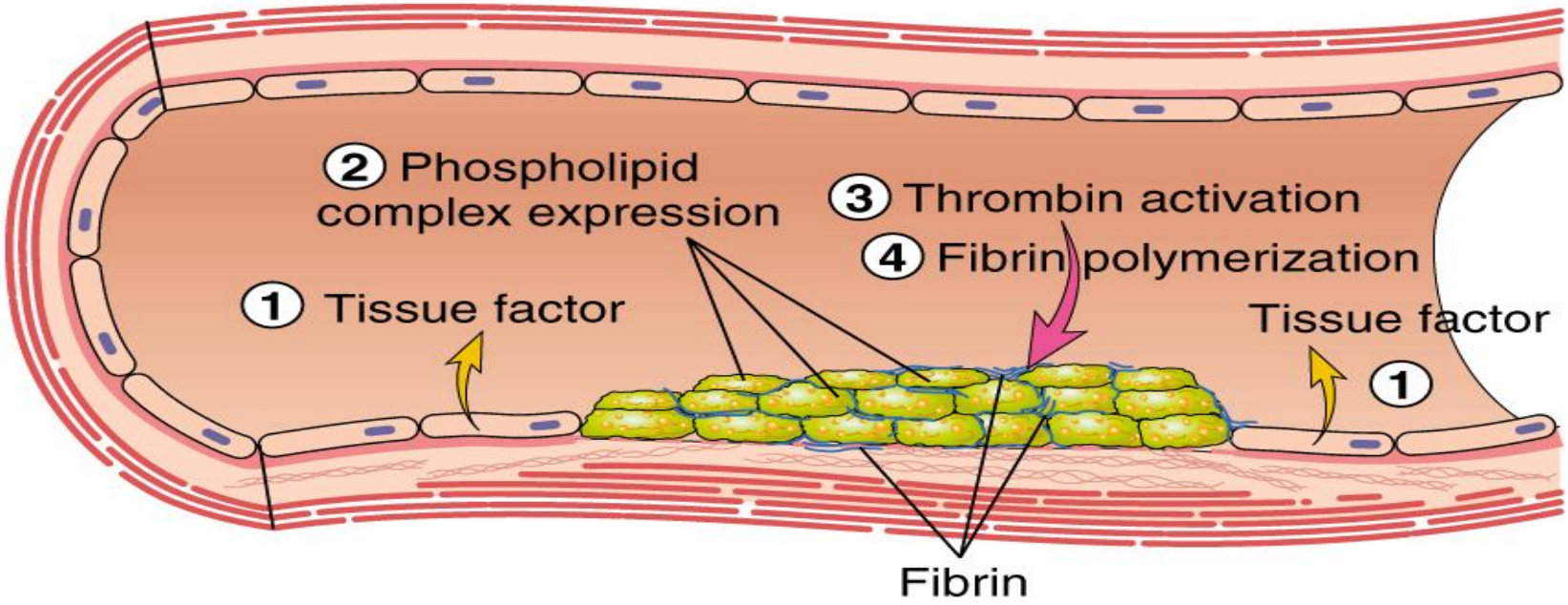


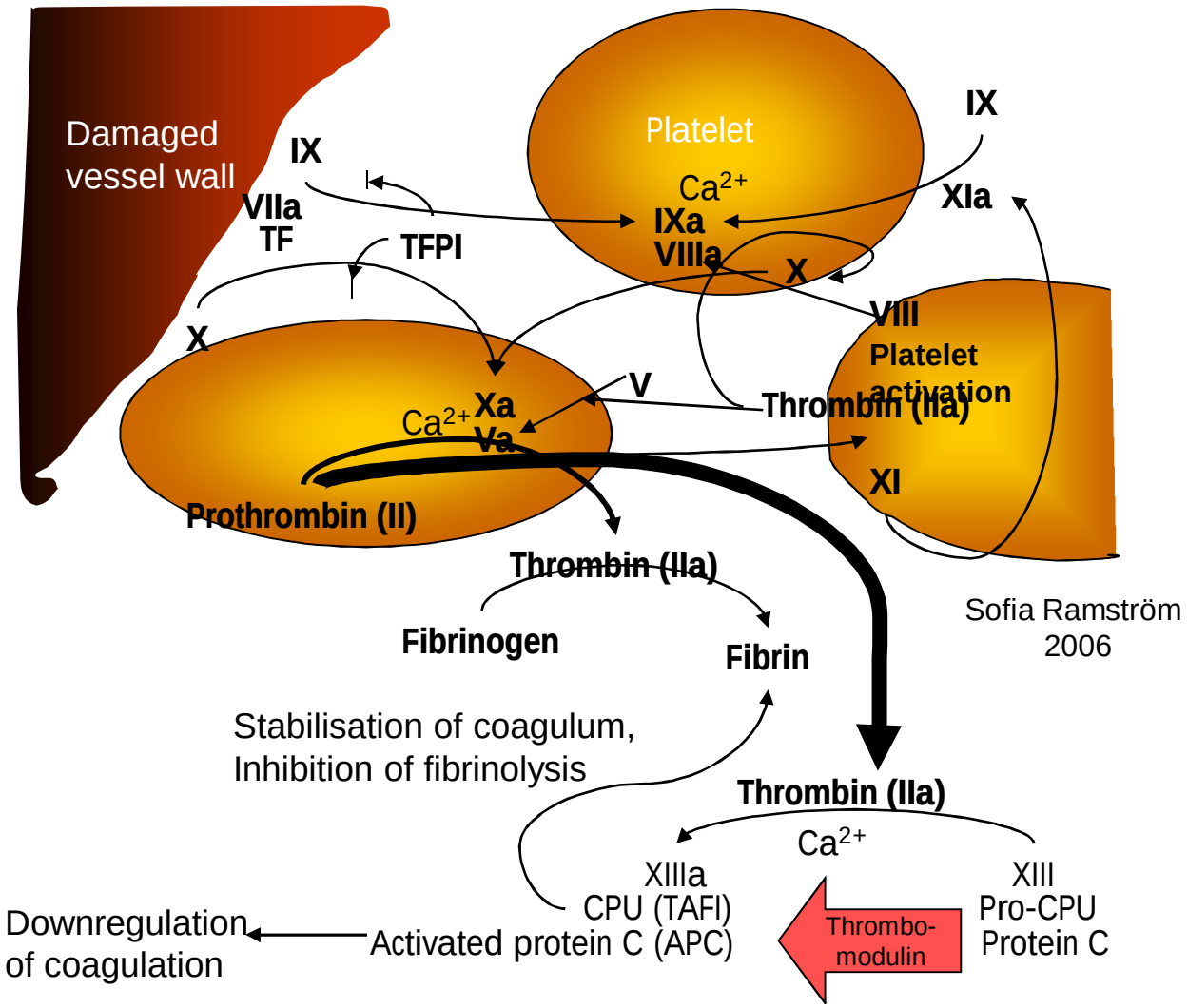
B. PRIMARY HEMOSTASIS




Deficiency:
Bernard-Soulier
Syndrome
GpIb
Platelet
Deficiency:
Glanzmann's
Thrombasthenia
GpIIb-IIIa
complex
Fibrinogen
GpIb
Endothelium
von Willebrand's
factor
Deficiency:
von Willebrand's
Disease
Subendothelium

C. SECONDARY HEMOSTASIS

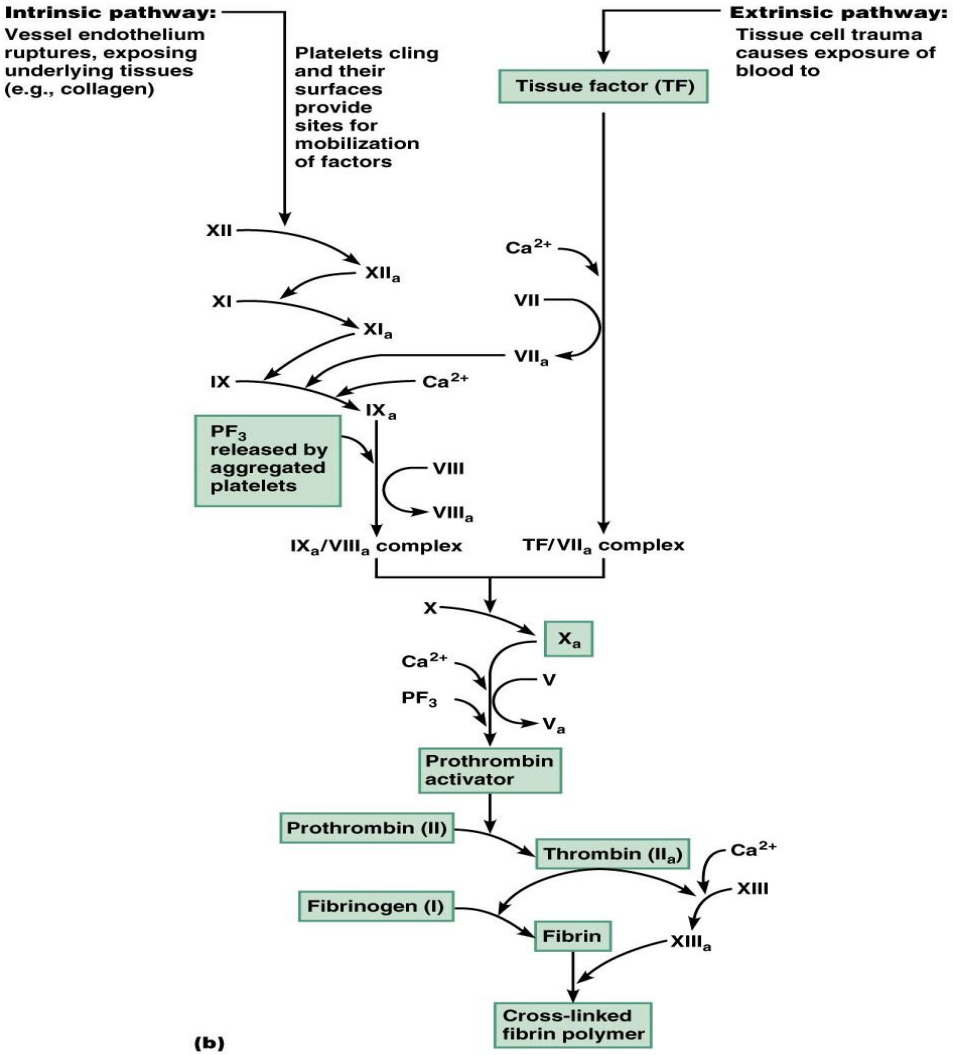


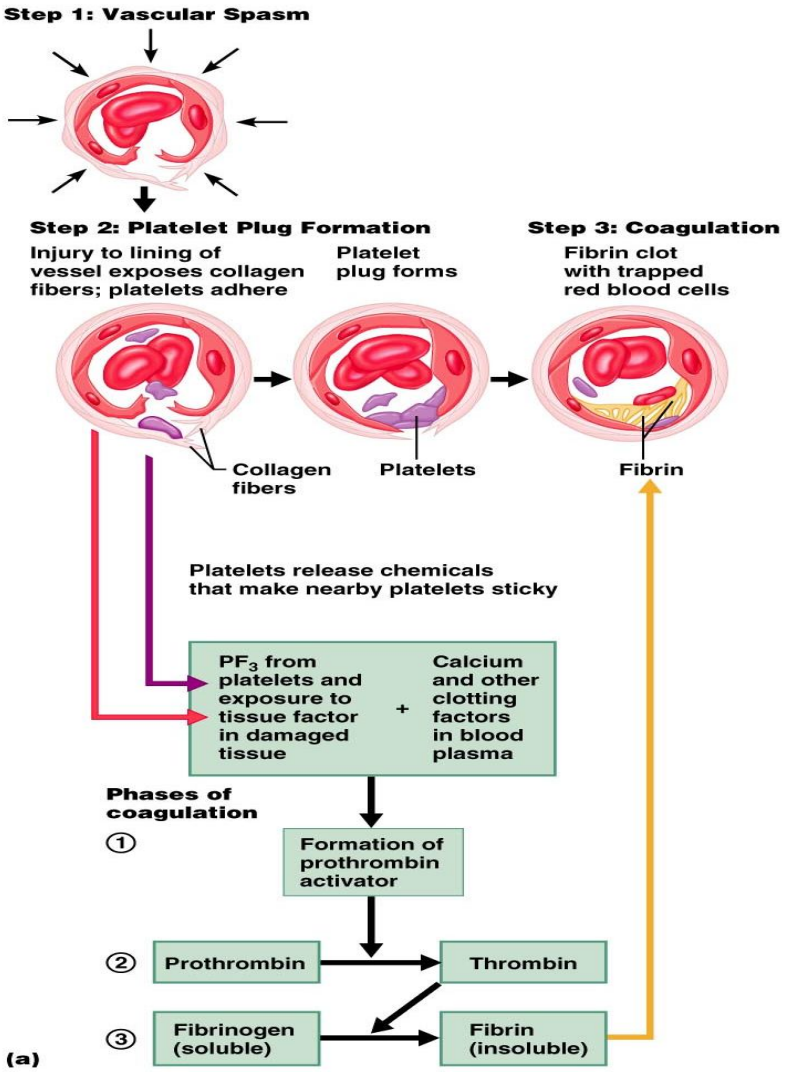

Damaged vessel wall
IX
VIIa
TF
TFPI
X
Platelet
Ca2+
IXa
VIIIa
X
IX
XIa
VIII
Platelet activation
Thrombin (IIa)
V
Ca2+
Xa
Va
Prothrombin (II)
XI
Thrombin (IIa)
Fibrinogen
Fibrin
Sofia Ramström 2006
Stabilisation of coagulum,
Inhibition of fibrinolysis
Thrombin (IIa)
Ca2+
XIIIa
CPU (TAFI)
XIII
Pro-CPU
Protein C
Thrombo-modulin
Downregulation of coagulation
Activated protein C (APC)

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**

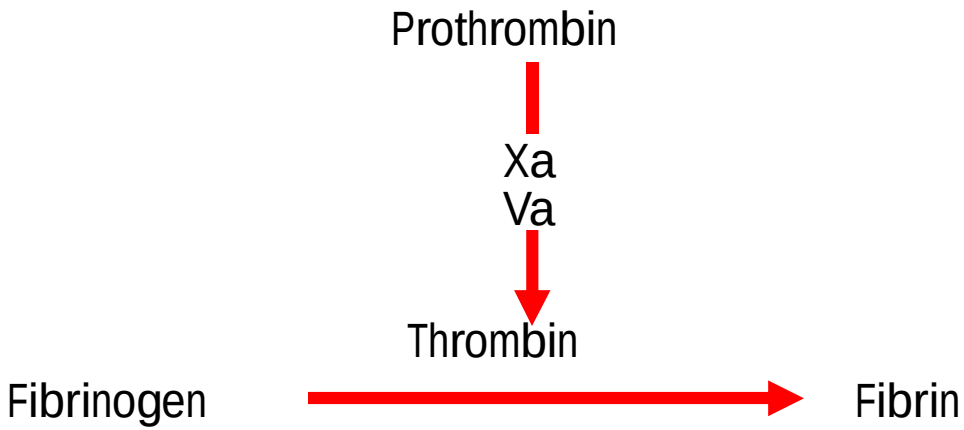
Coagulation

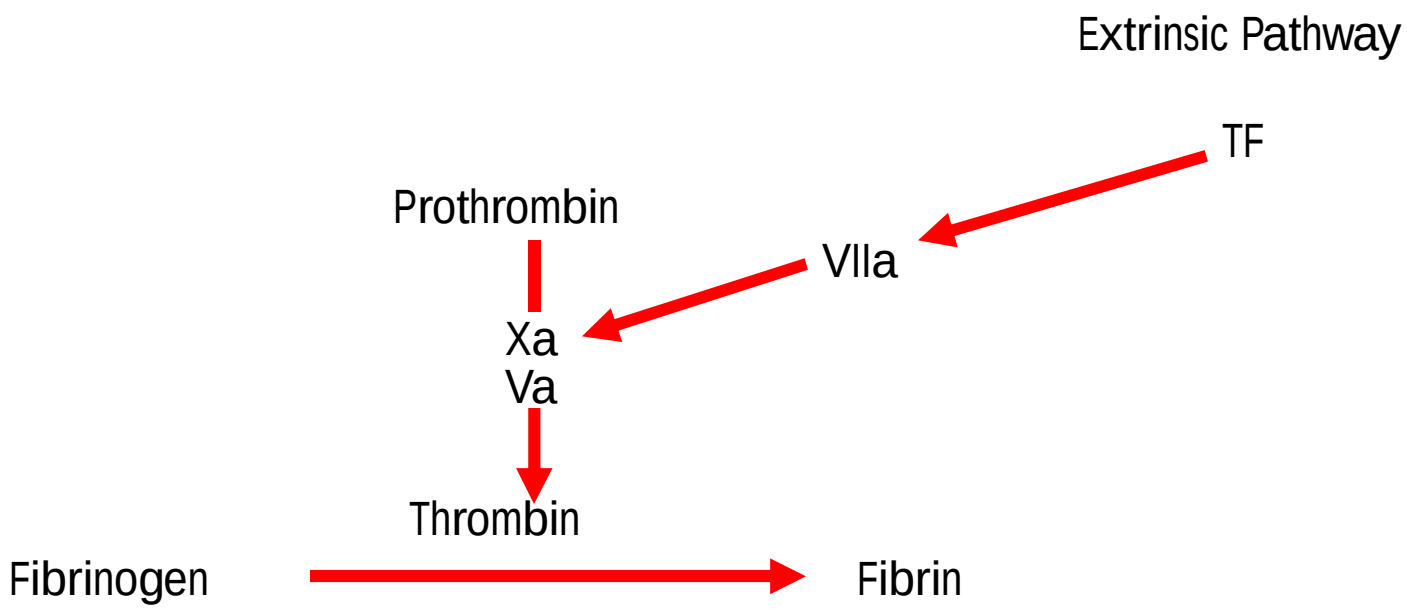


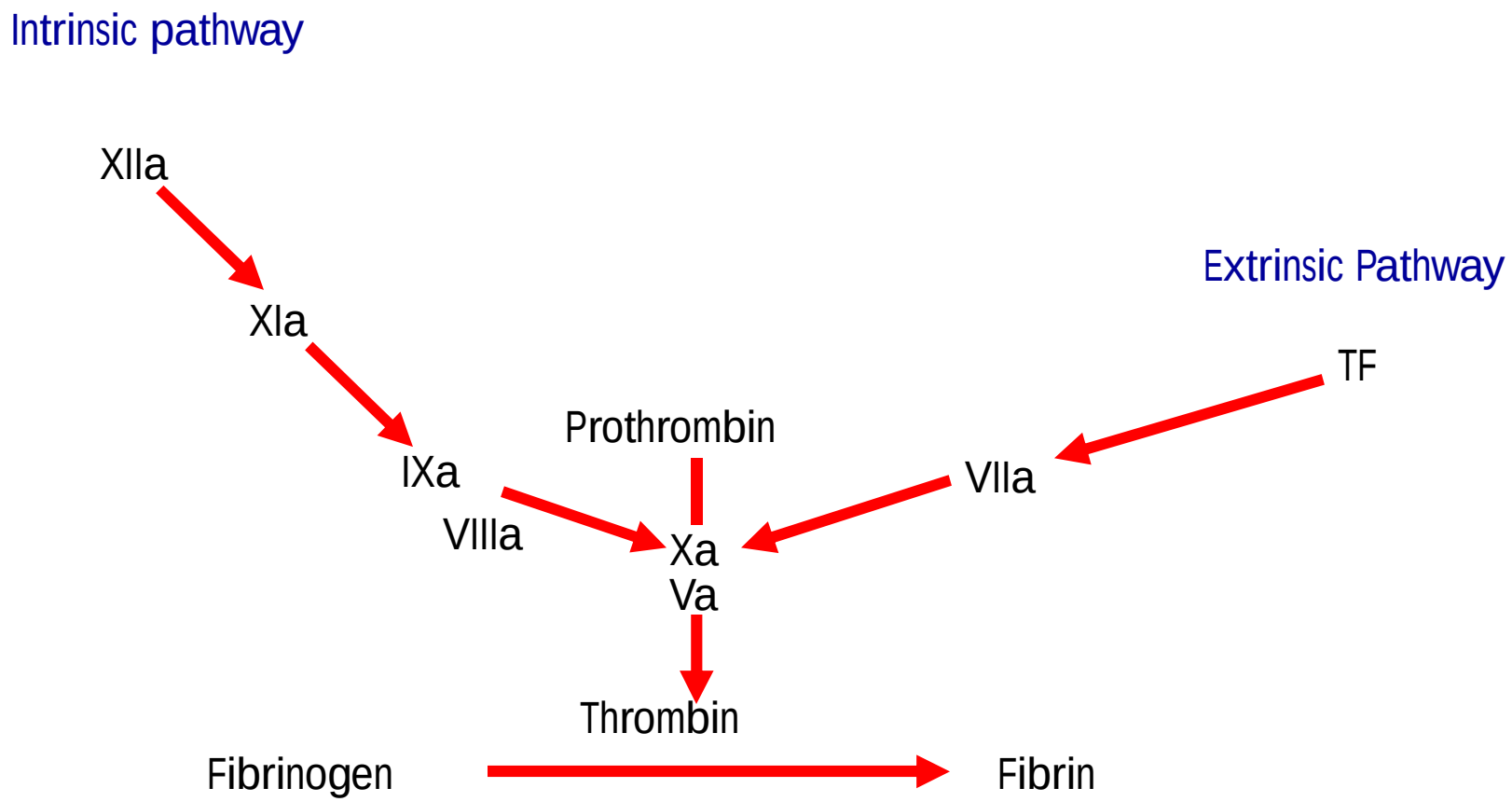


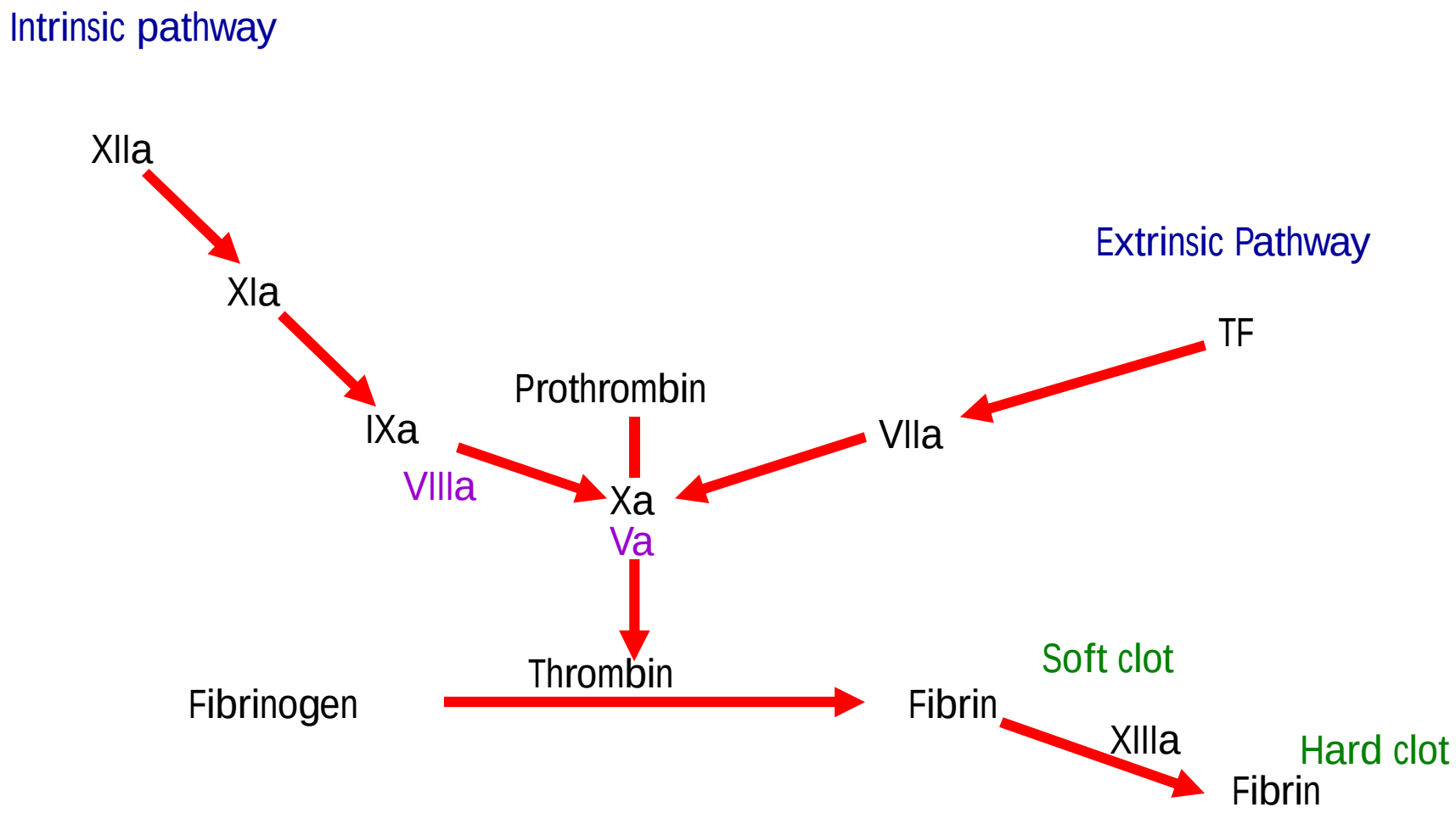

Fibrinogen
Fibrin

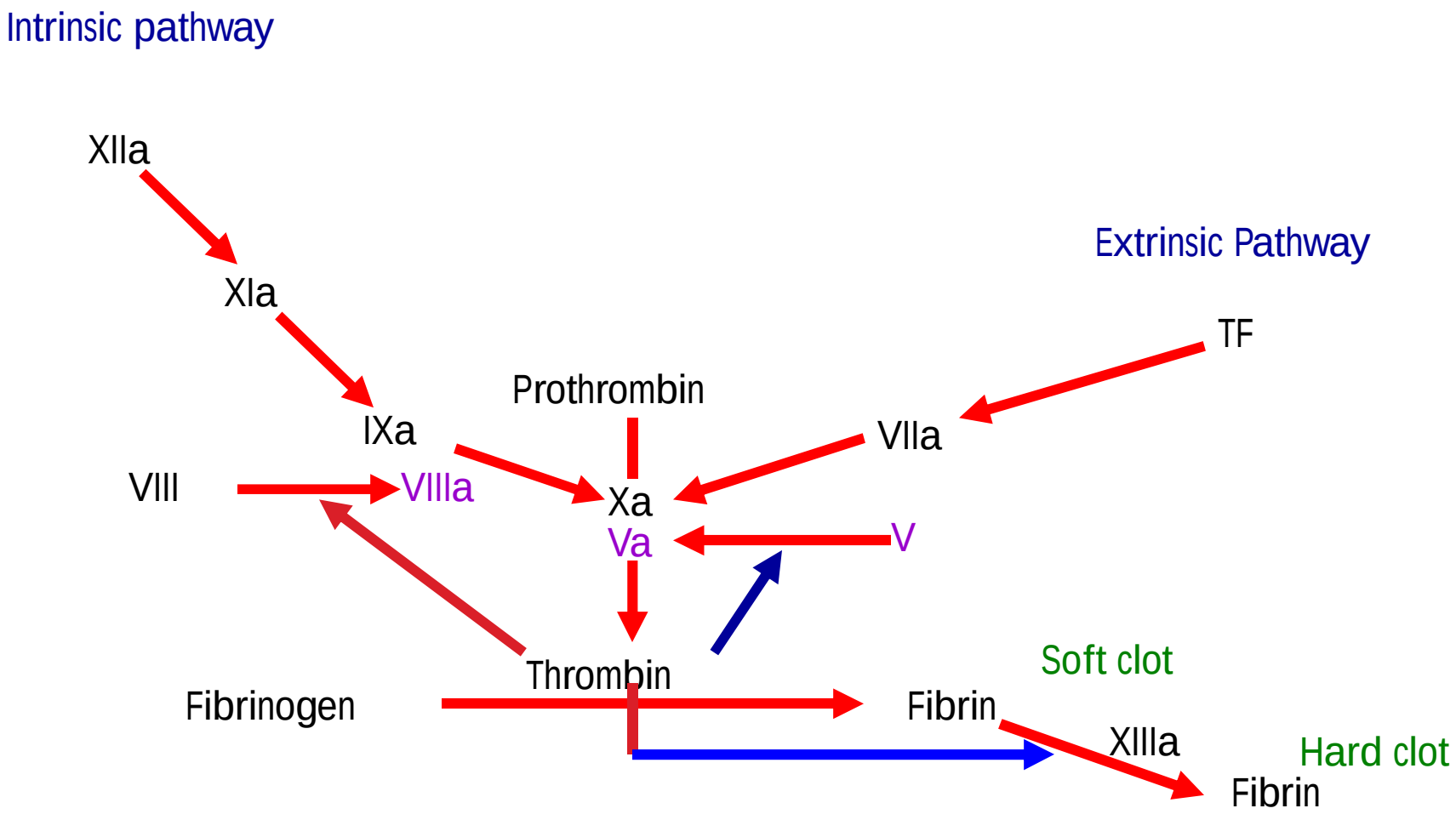

Thrombin
Fibrinogen
Fibrin


Prothrombin
Xa
Va
Thrombin
Fibrinogen
Fibrin


Extrinsic Pathway
TF
Prothrombin
VIIa
Xa
Va
Thrombin
Fibrinogen
Fibrin


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


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
VIIIa
Prothrombin
Xa
Va
V
Extrinsic Pathway
TF
VIIa
Thrombin
Fibrinogen
Fibrin
Soft clot
XIIIa
Hard clot
Fibrin

CLOT-ED

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)

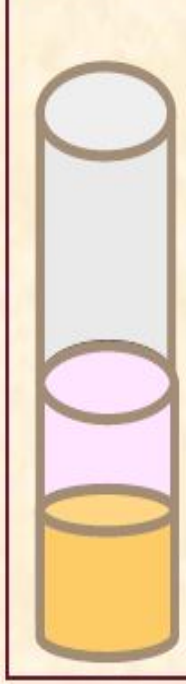
Factor I (fibrinogen) is converted to fibrin

- 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*.

$$\mathrm{INR} = \left(\frac{\mathrm{PT}_{\mathrm{test}}}{\mathrm{PT}_{\mathrm{normal}}} \right)^{\mathrm{ISI}}$$

CLOT-ED

Prothrombin Time (PT)



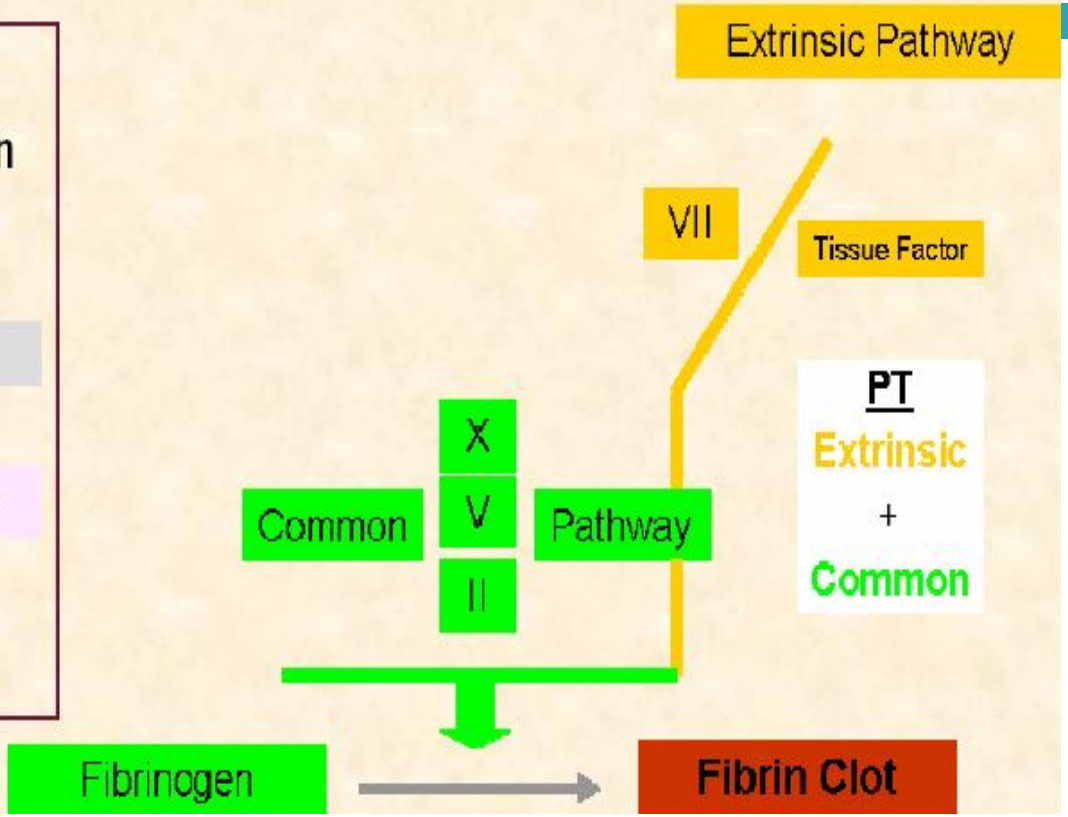
Time for clot formation

~ 12 seconds

Incubate at 37 °C for ~3 minutes

0.1 ml Thromboplastin + Ca^{++}

0.1 ml Plasma

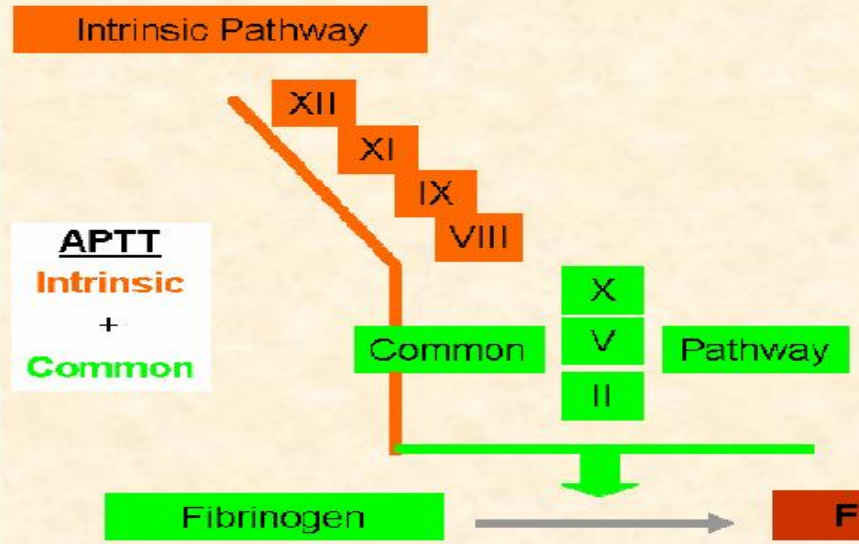


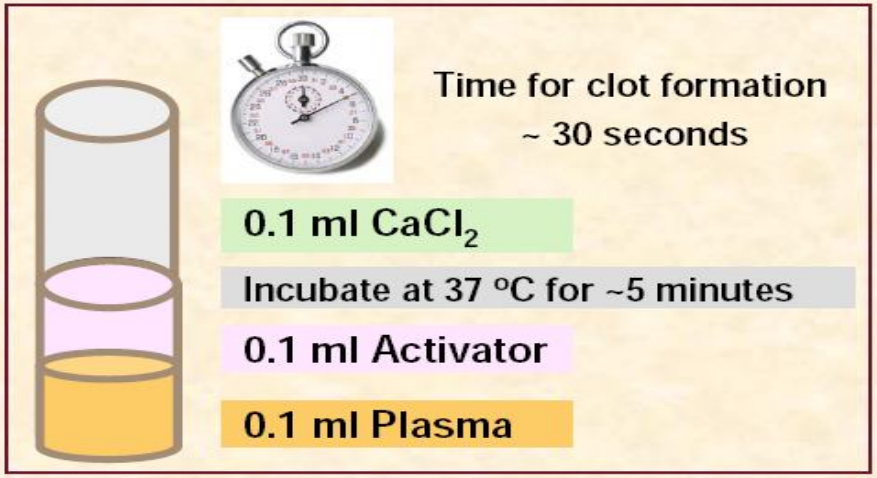
- The **partial thromboplastin time** (PTT) or **activated partial thromboplastin time** (**aPTT** or **APTT**)
- is a performance indicator measuring the efficacy of both the **"intrinsic"** (now referred to as the contact activation pathway) and the common coagulation pathways.

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

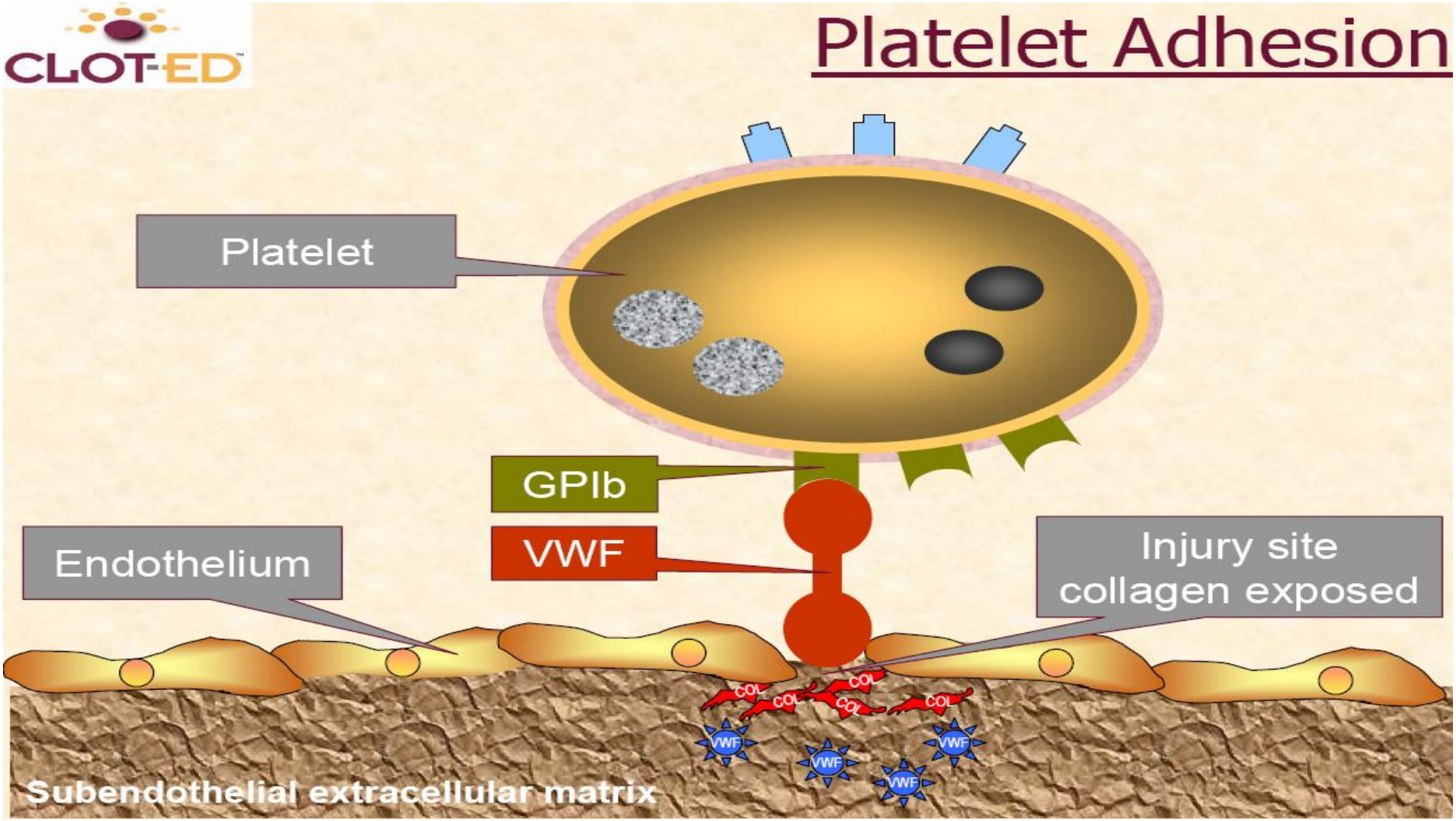
- Activator to convert FXII to FXIIa
- Phospholipid (replaces "in vivo" platelet surface on which coagulation reactions occur)
- $CaCl_2$ – used to reintroduce calcium ions that were chelated by sodium citrate
- Referred to as "partial thromboplastin" since no Tissue Factor is used
  - Two-stage assay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

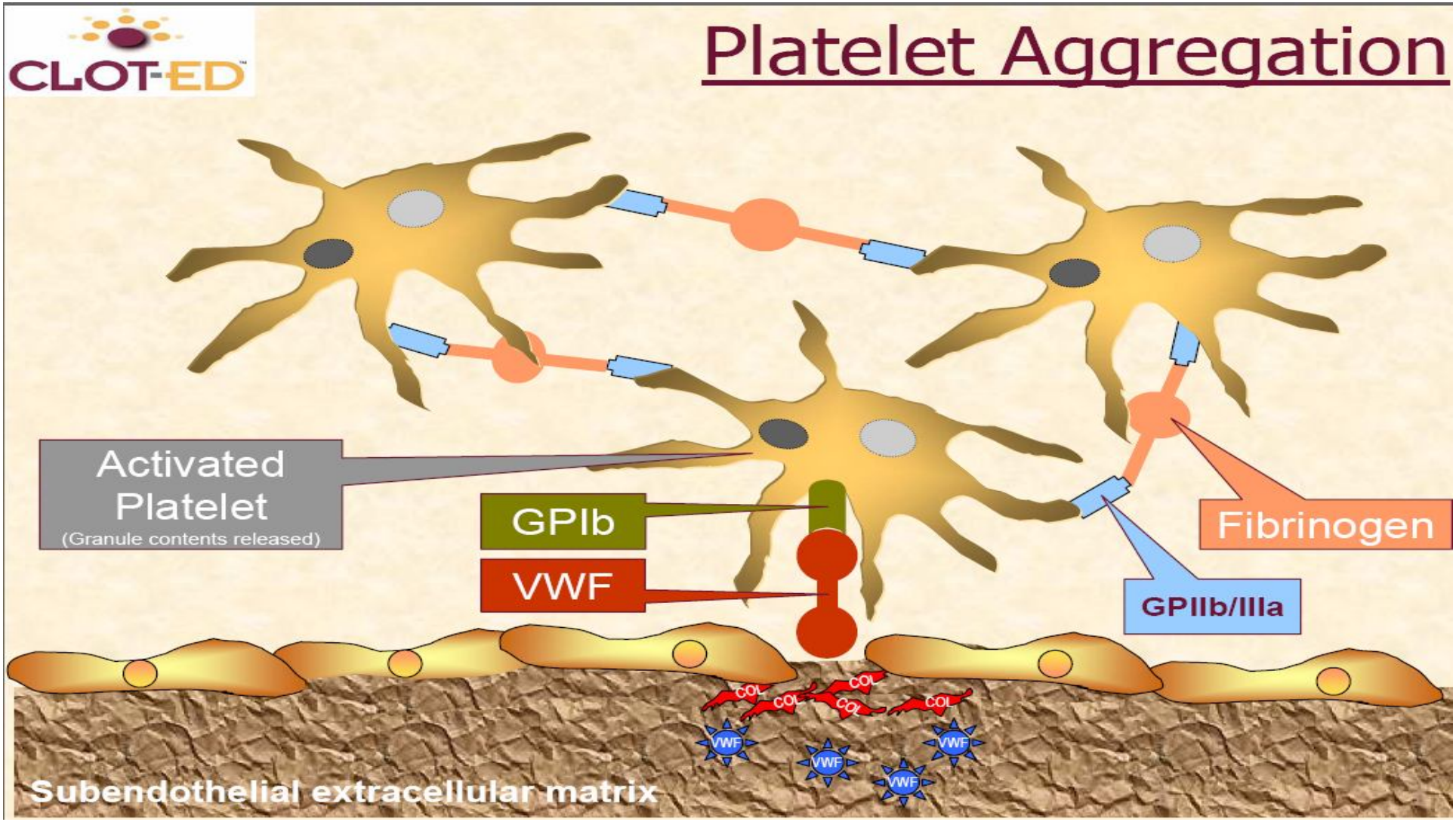
- Prolonged APTT may indicate:

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




CLOT-ED
Platelet Aggregation
Activated Platelet
(Granule contents released)
GPIb
VWF
Fibrinogen
GPIIb/IIIa
COL
VWF
Subendothelial extracellular matrix

Hemostasis: Vasoconstriction & Plug Formation

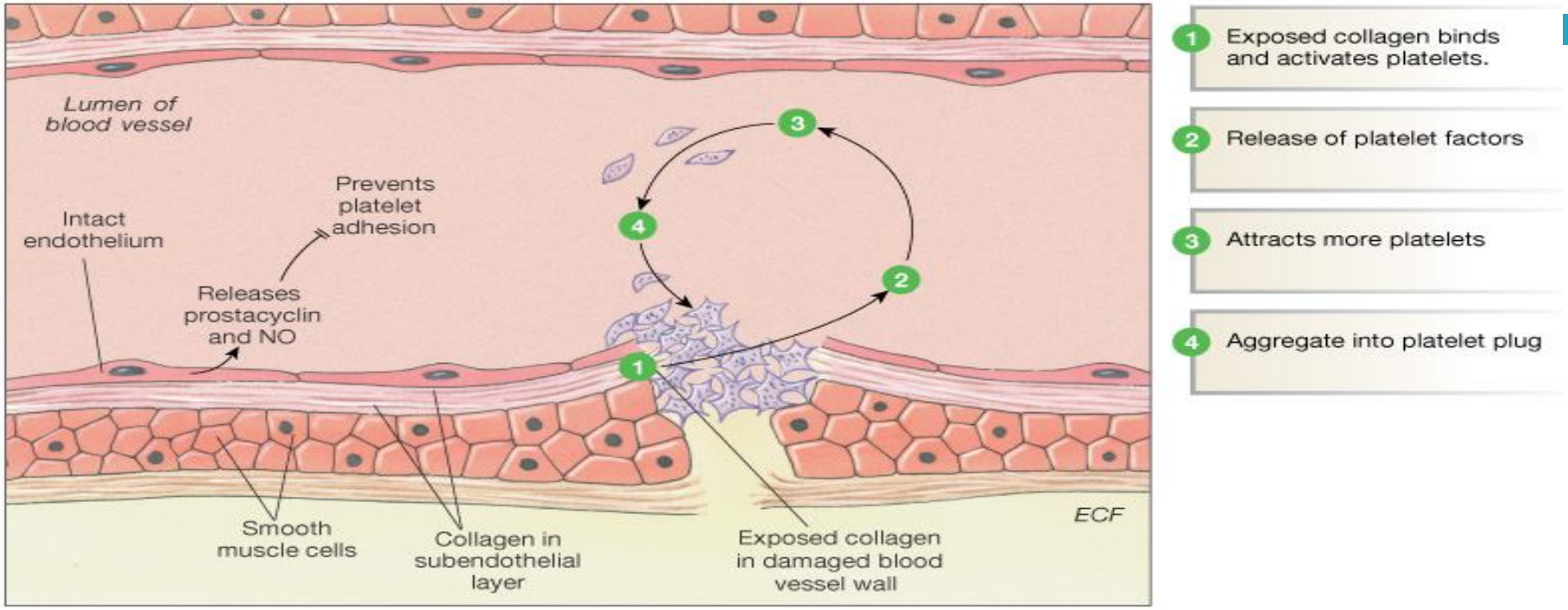


Figure 16-12: Platelet plug formation

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

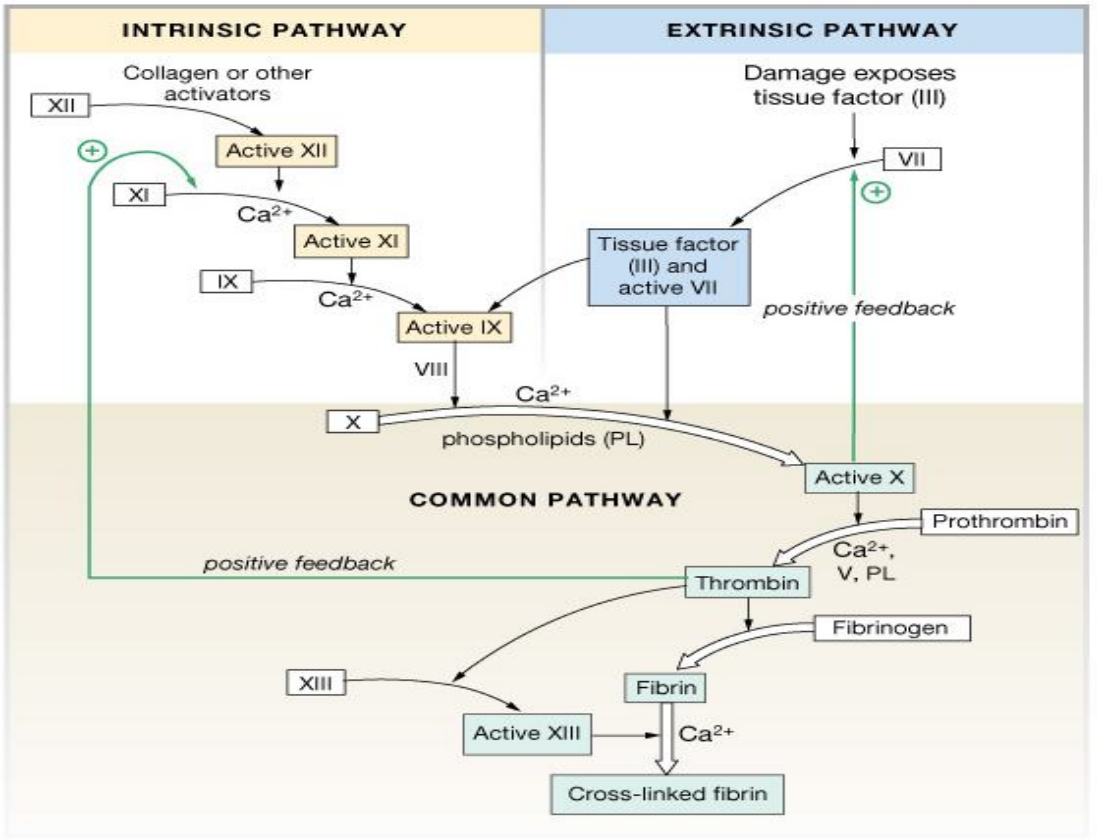


Figure 16-13: The coagulation cascade

Dissolving the Clot and **Anticoagulants**

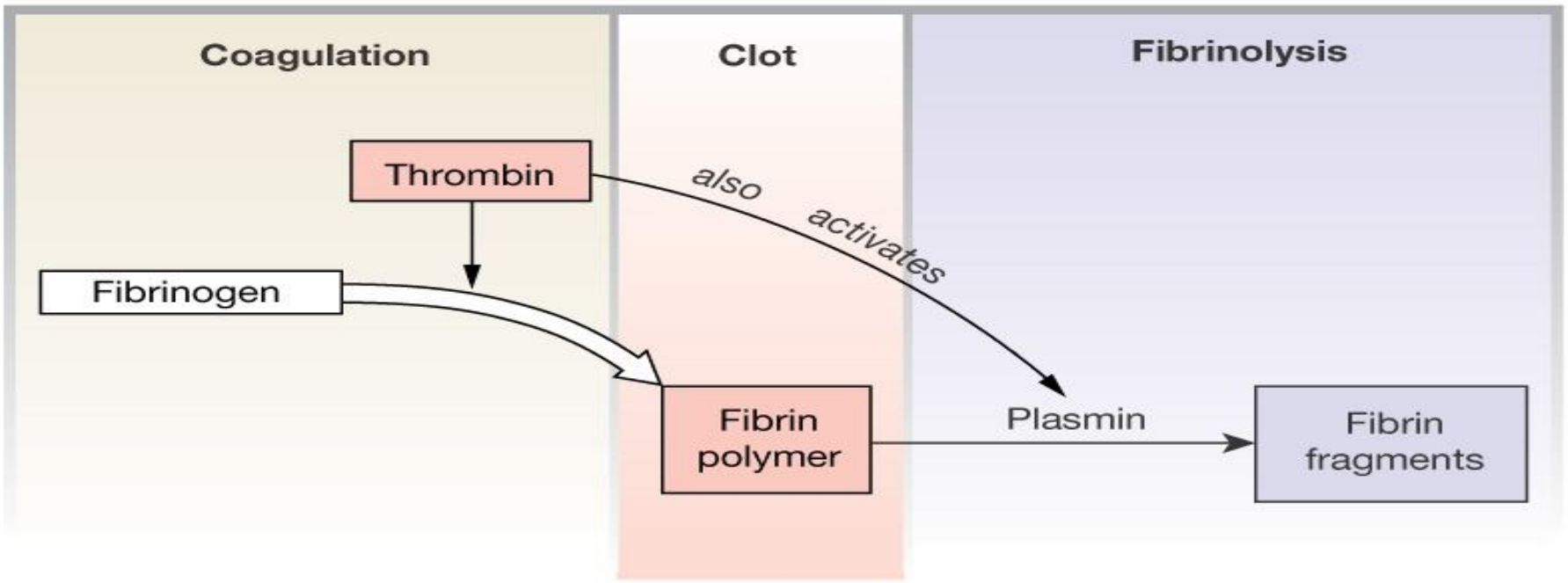


Figure 16-14: Coagulation and fibrinolysis

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

Platelet Disease

Mucosal/cutaneous bleeding

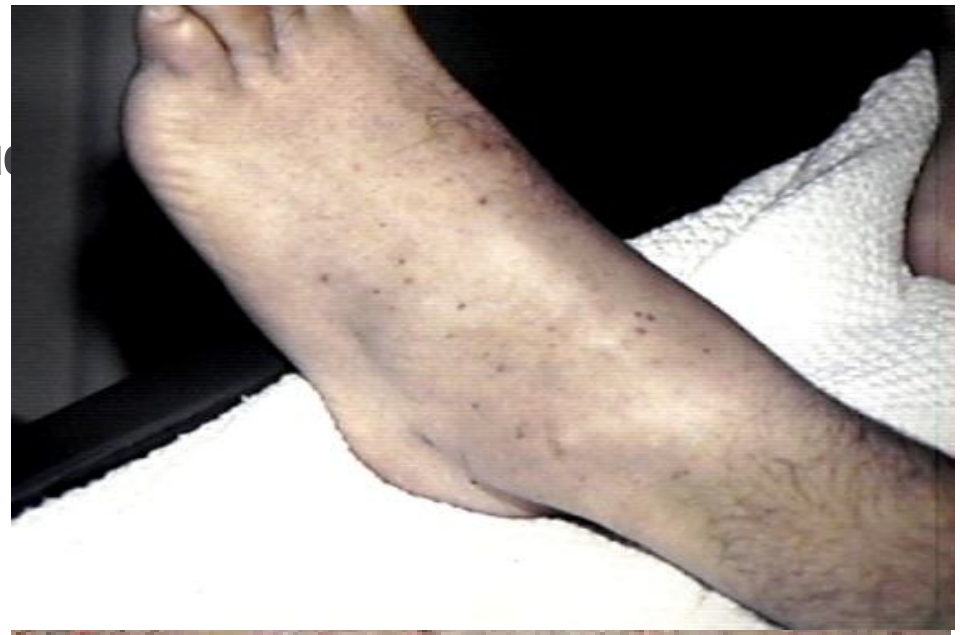
Lack vessel protection by submucosal tissue

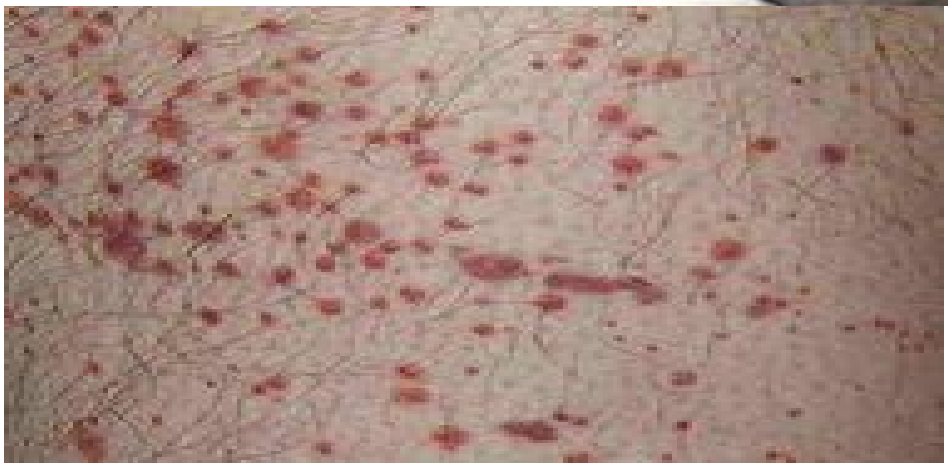
Bleed immediately after vascular trauma

- **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

1. **Low platelets -<**50,000/micrL(Thrombocytopenia most common acquired Diathesis)
2. **Normal Platelet Count and PT, aPTT + mucocutaneous bleeds**

- **Platelet dysfunction**
  - Qualitative disorders
  - Morphology
  - Aggregation/Function
  - Common acquired causes of dysfunction
    - Aspirin, NSAID, Beta-lactam antibiotics
    - Uraemia
    - myeloproliferative and myelodysplastic syndromes.
  - Uncommon causes of dysfunction
    - Bernard-Soulier syndrome
    - Glanzmann's thrombasthenia
- **Platelet aggregation**
    - Wiskott-Aldrich syndrome

von Willebrand's disease (vWD) –XIII

Other disorders

Factor XIII deficiency (Bleeding delayed by 24-36hr)

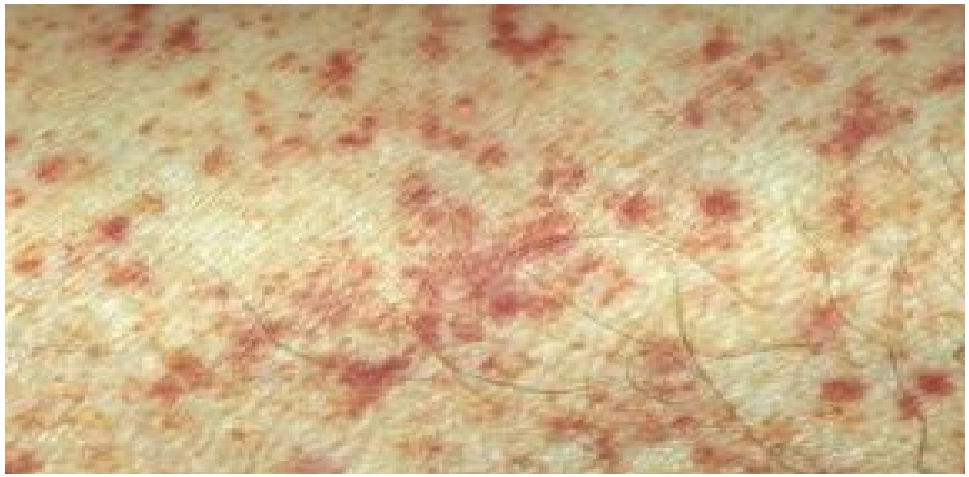
alpha-2 antiplasmin deficiency - plasminogen activator inhibitor-1 deficiency

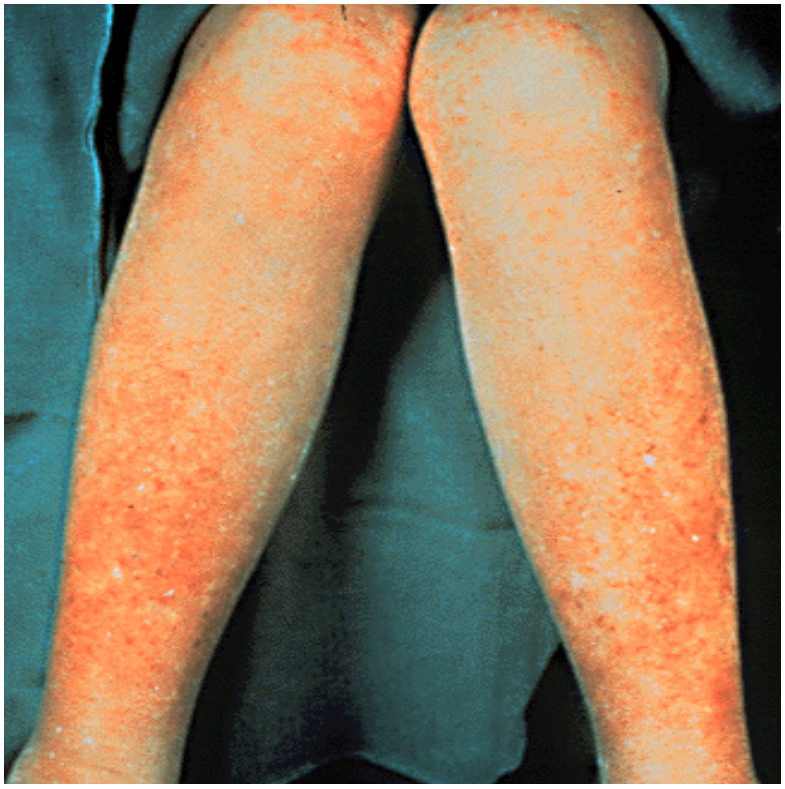
Role of PT, PTT: Warfarin, Heparin Monitoring

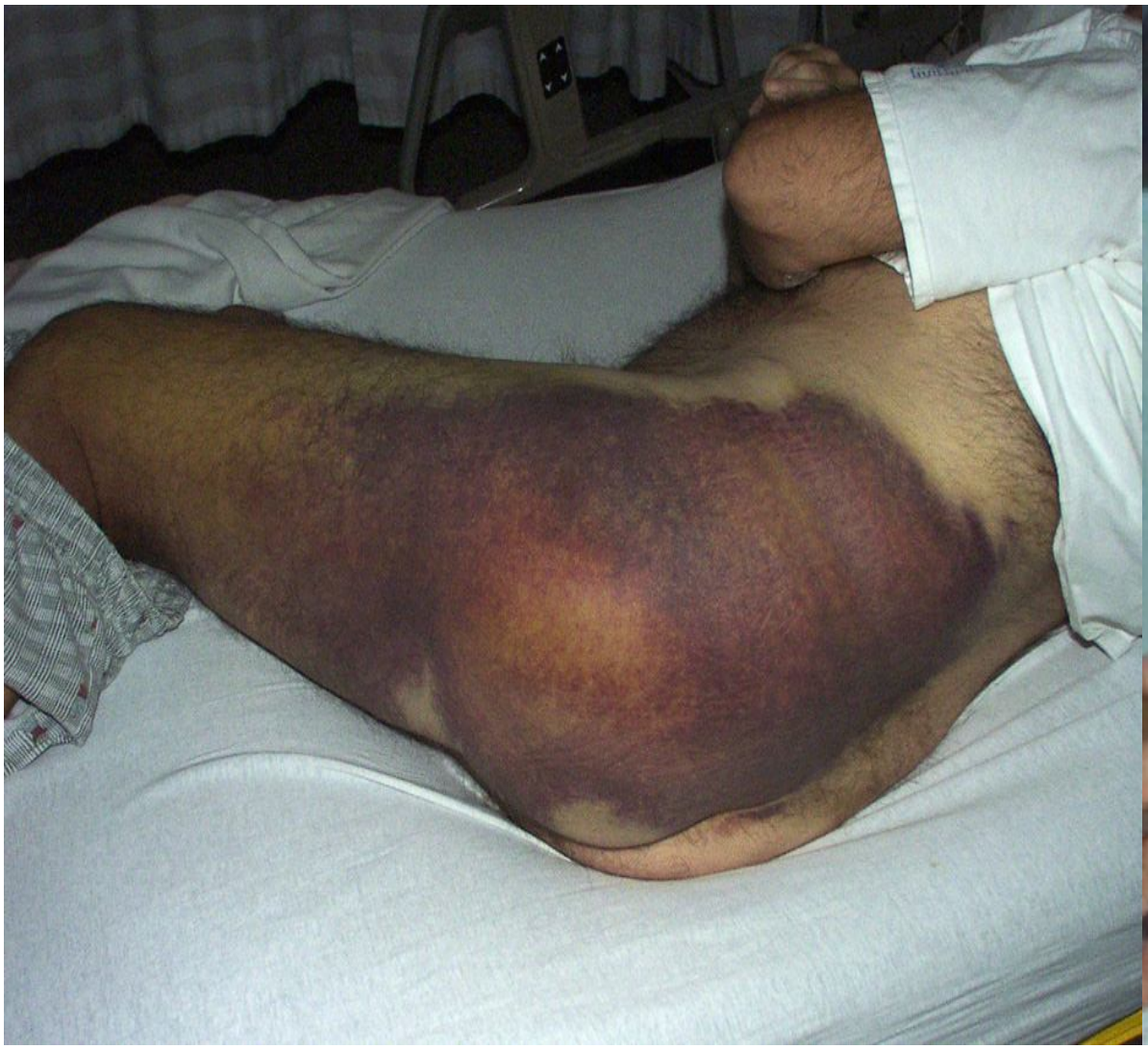
Anticoagulant	PT	PTT
Low dose Heparin	Normal	Prolonged
High dose heparin	Prolonged	Prolonged
Low dose warfarin	Prolonged	Normal
High dose warfarin	Prolonged	Prolonged

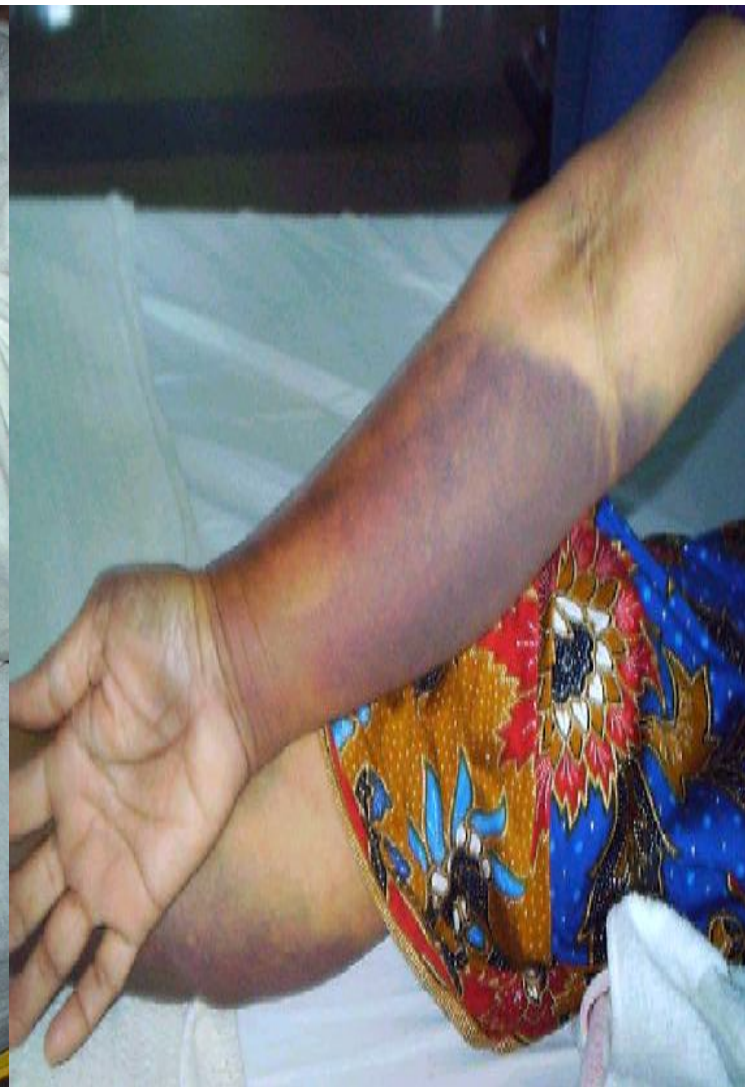
Physical Examination

- **Petechiae**





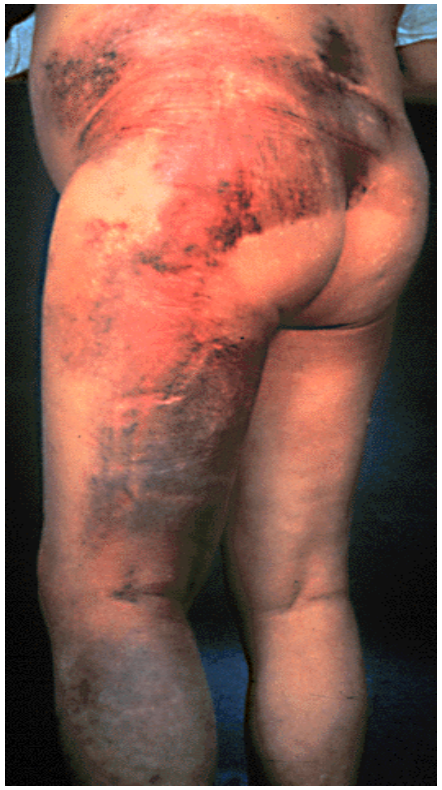


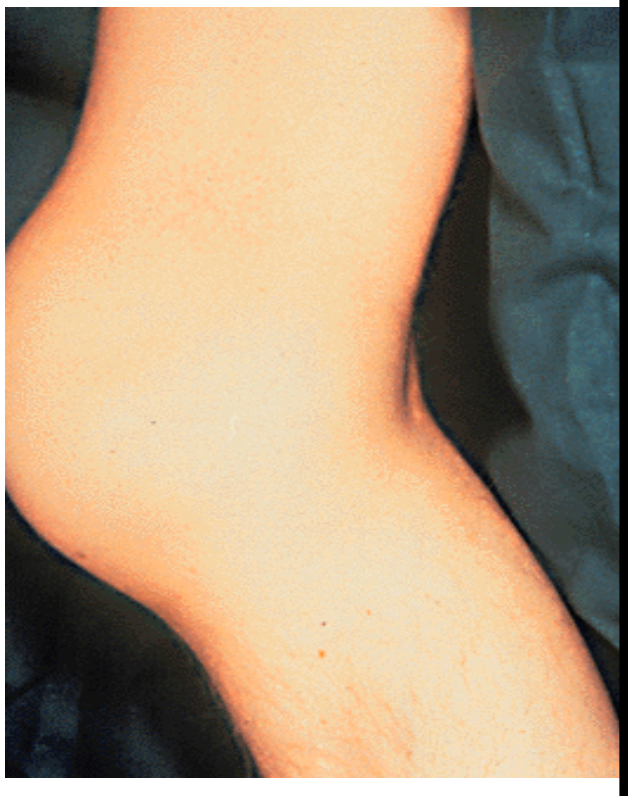


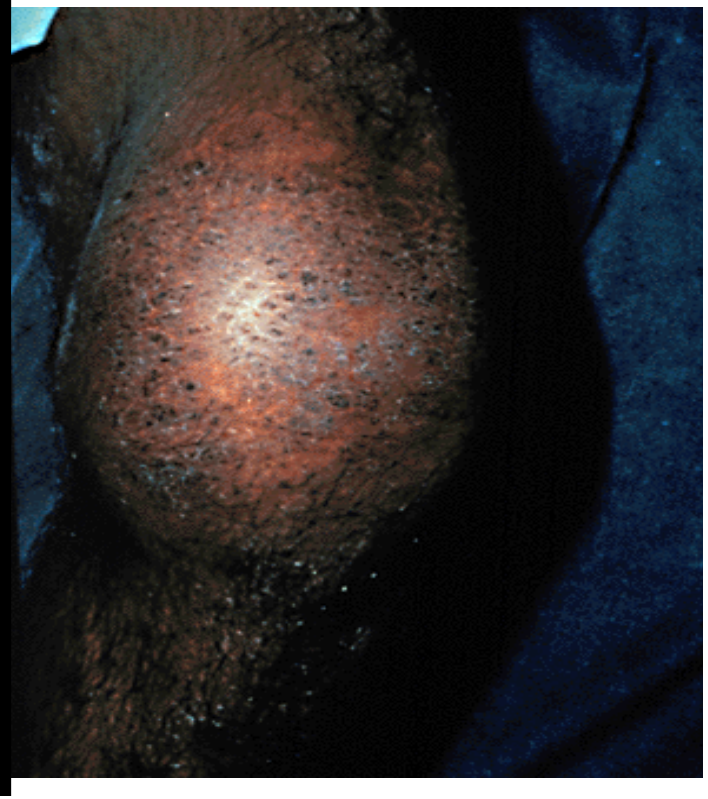
Physical Examination

Ecchymoses

Hemarthrosis







52 year old male

Severe Hemophilia

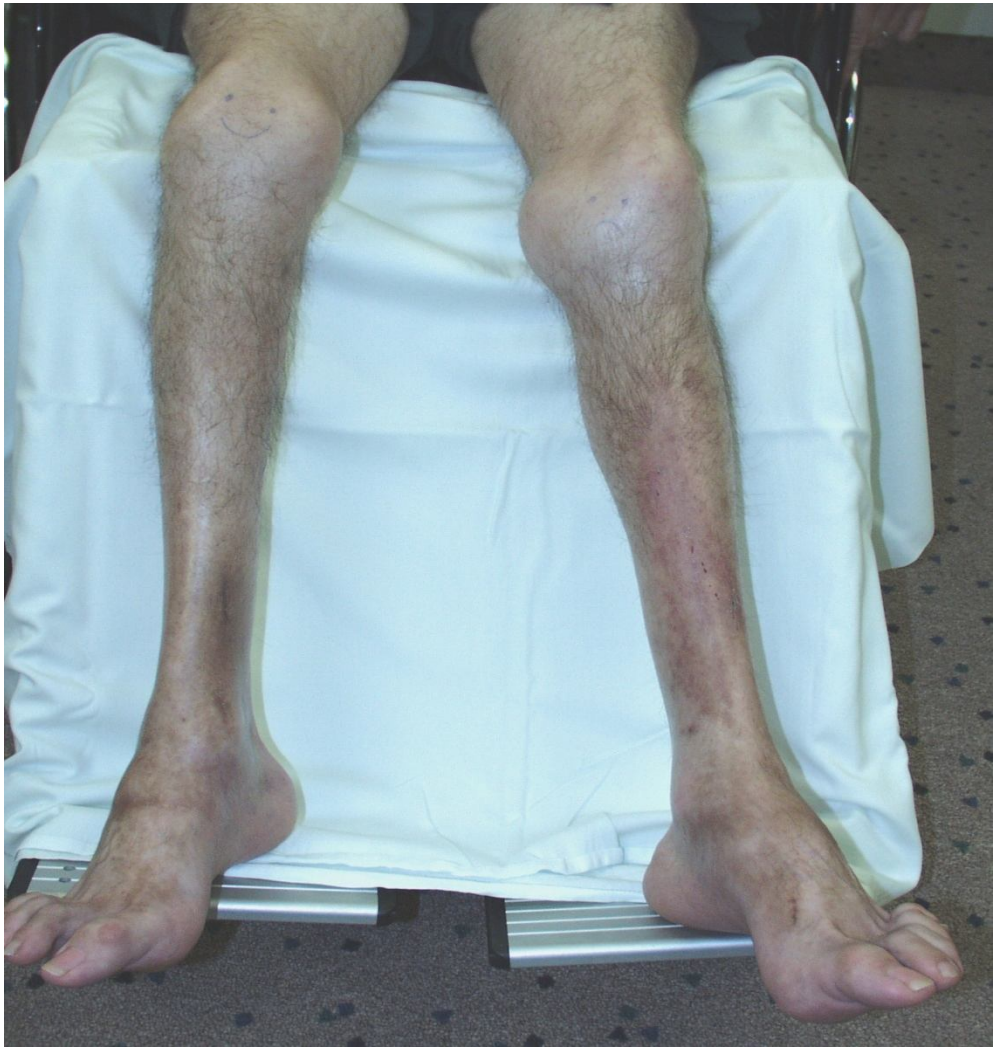
Now bleeds 3x month

Severe muscle wasting

Joint immobility

Atrophic skin changes

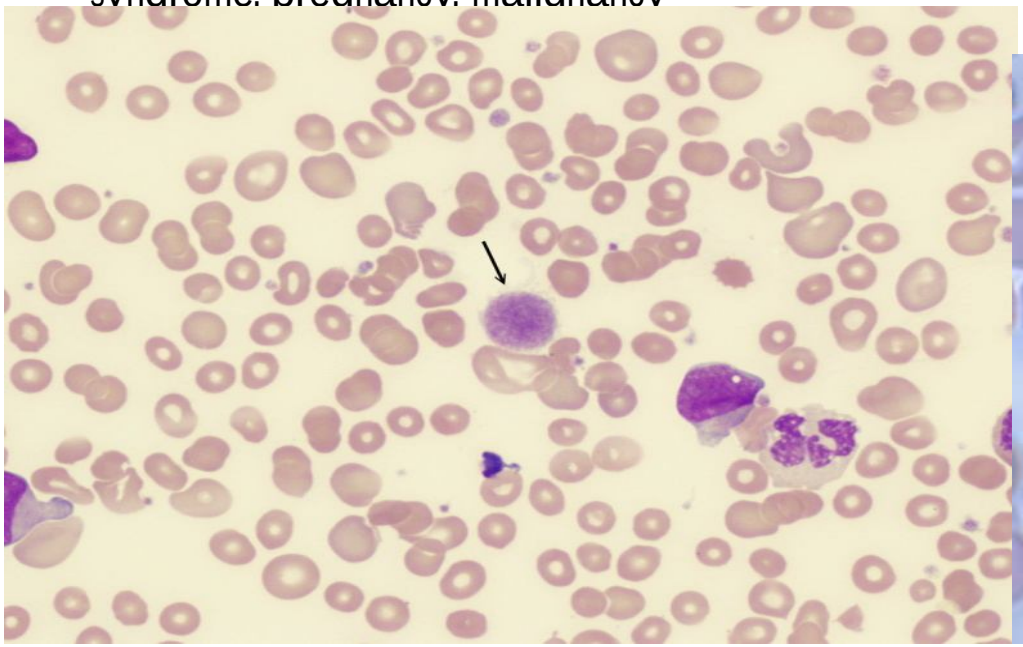
HIV and HCV +ve



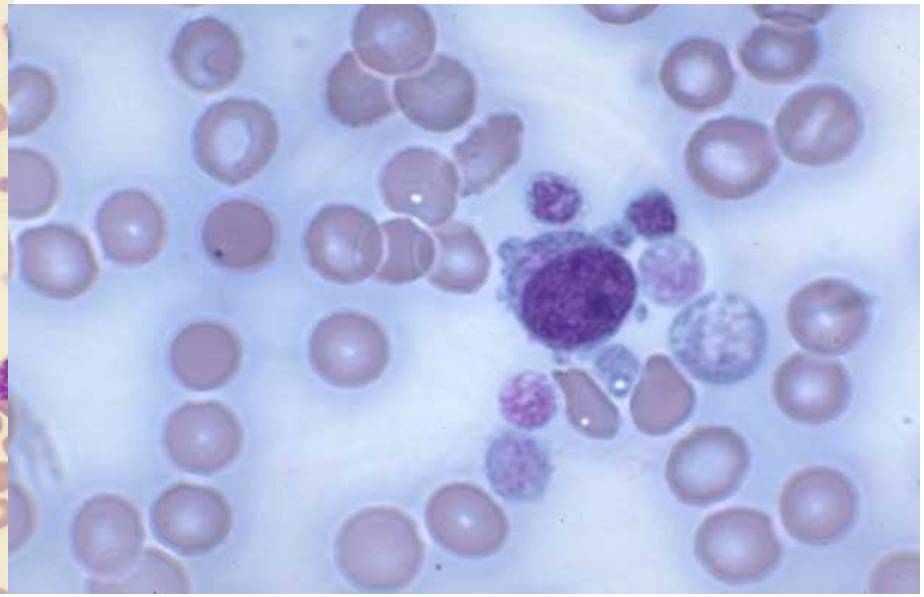
Platelet: Side note

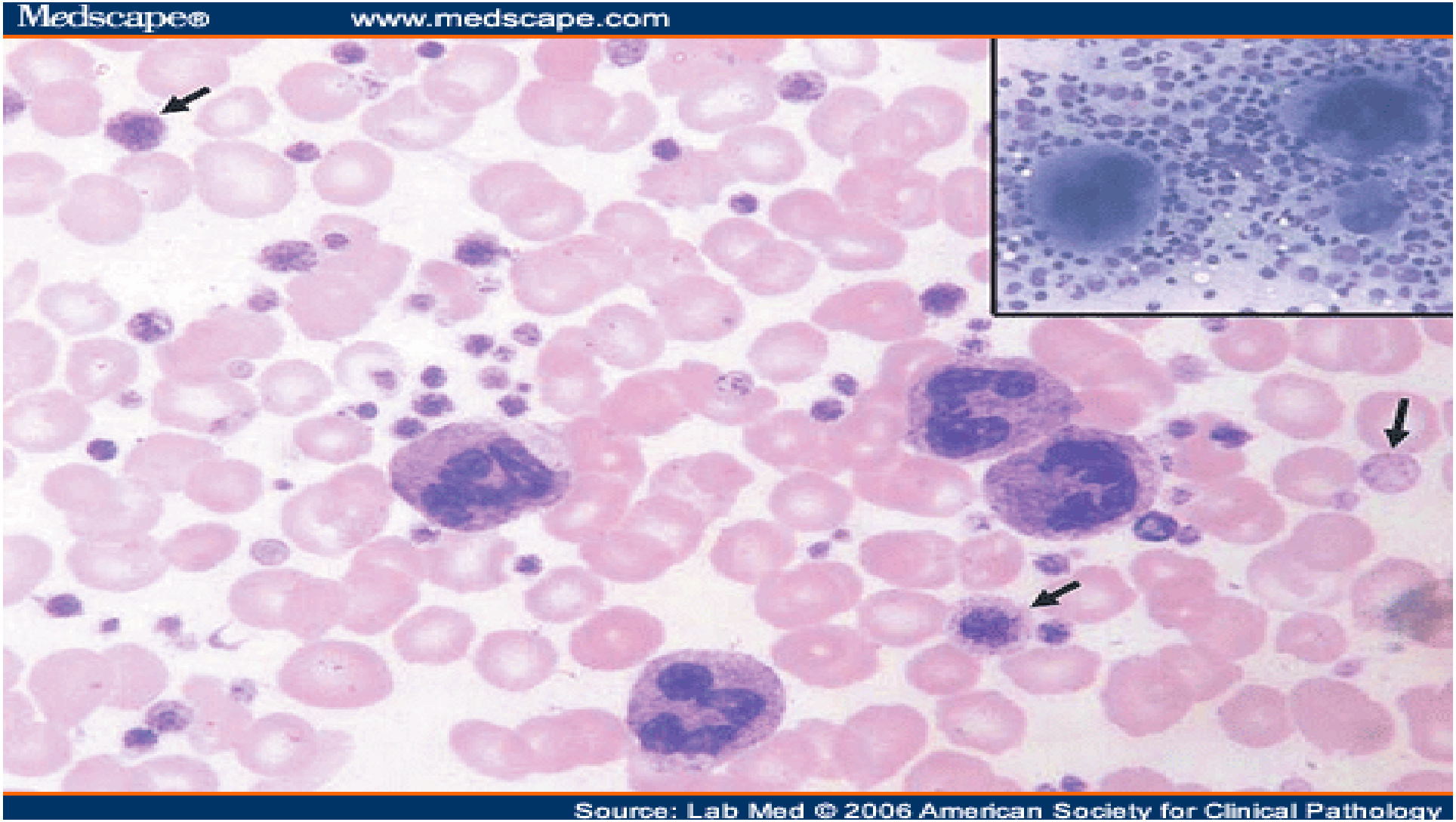
Seen in conditions with increased need and/or destruction

- Giant platelets
  - May Hegglin anomaly, Bernard-Soulier syndrome, pregnancy, malignancy



- Micromegakaryocytes= Dwarf Megs
- Seen in malignant disorders such as CML and MDS




Medscape®
www.medscape.com
Source: Lab Med © 2006 American Society for Clinical Pathology

The Best Screening Test For Bleeding is HISTORY

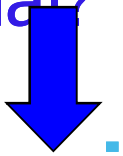
No History of Bleeding:

No further testing Is required

History of Bleeding:

Is it congenital or acquired bleeding?

Which component of hemostasis is abnormal?



Phase II: Screening Tests

Phase II

Screening for the plt count

1) Automated platelet count

Mean Platelet volume (MPV)
Plateletocrit (PCT)
Platelet Distribution width (PDW)

2) Peripheral blood film.

Giant platelets
Platelet clumps

Screening for the plt function

Coagulation screening:

4) PT / INR

5) APTT

Physiologic Inhibitors of coagulation

- **Antithrombin III** (serpin)
- **Activated Protein C + protein S**
  - Inactivates Va and VIIIa (via proteolysis)
  - NB: Factor V Leiden
- **Thrombomodulin (EC glycoprotein)**
  - Binds to thrombin
  - Decreases ability to produce fibrin
  - Increases ability to activate Protein C

Role of vitamin K

Play key roles in the regulation of three physiological processes:

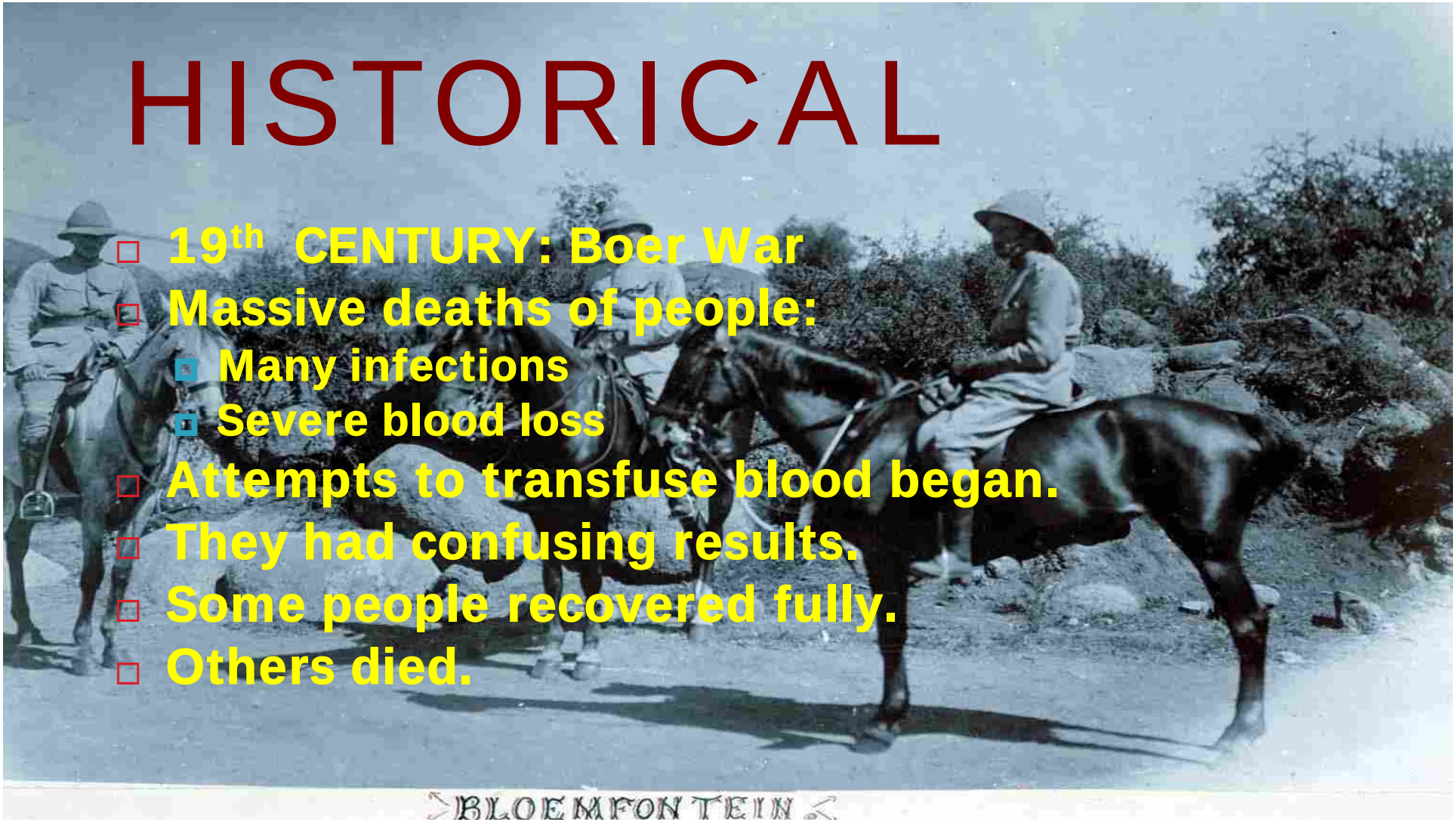
- Blood coagulation: (prothrombin (factor II), factors VII, IX, X, protein C, protein S, and protein Z).

- 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.

HISTORICAL



- 19th CENTURY: Boer War
- Massive deaths of people:
  - Many infections
  - Severe blood loss
- Attempts to transfuse blood began.
- They had confusing results.
- Some people recovered fully.
- Others died.

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%

Blood Transfusions

- Whole blood transfusions are used:
  - When blood loss is substantial
  - In treating thrombocytopenia
- Packed red cells (cells with plasma removed) are used to treat anemia

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

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

ABO Blood Groups

- 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.

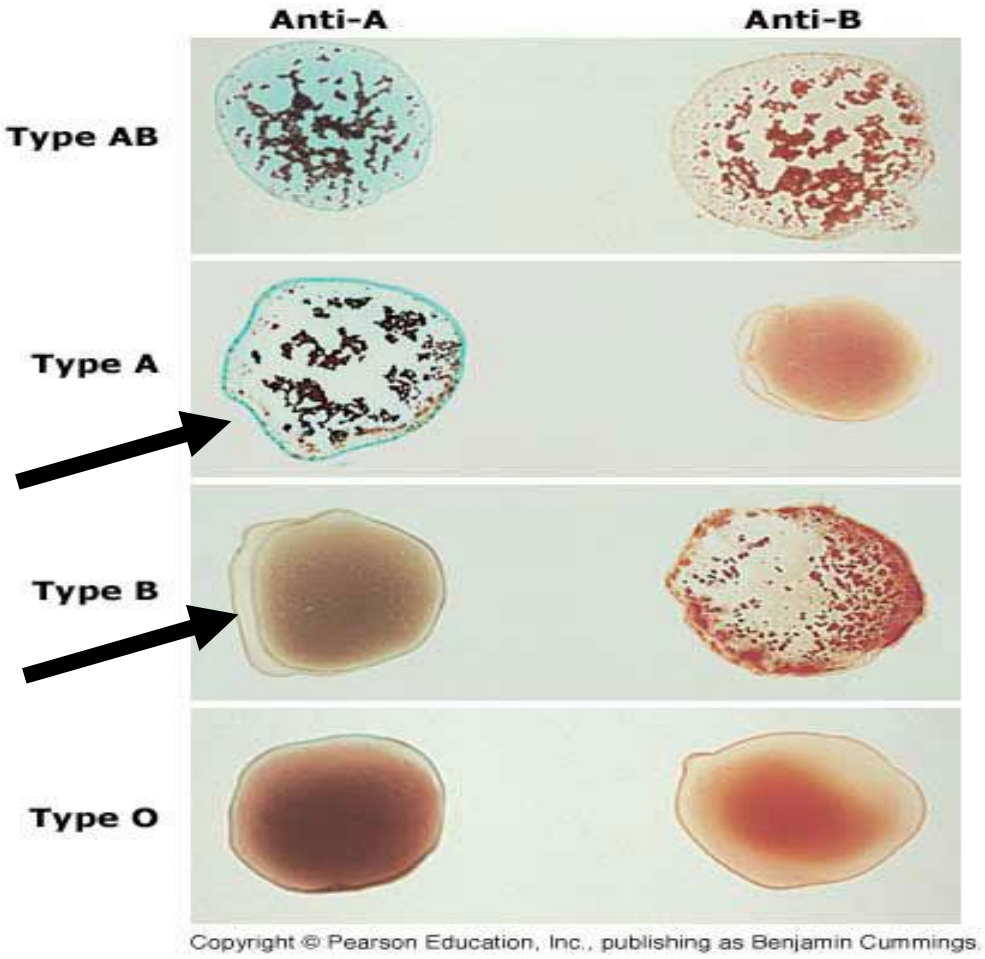
Some individuals will have Erythrocytes with an agglutinogen called as “A”.

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...

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

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*

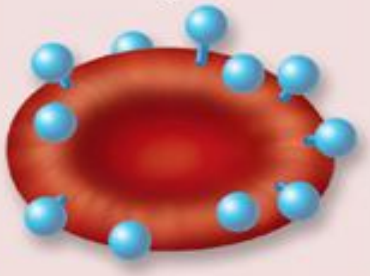
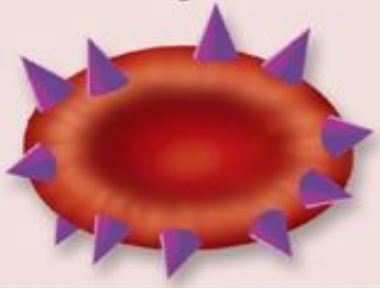
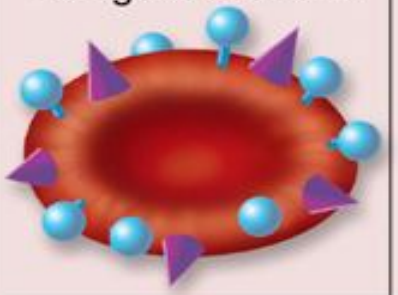
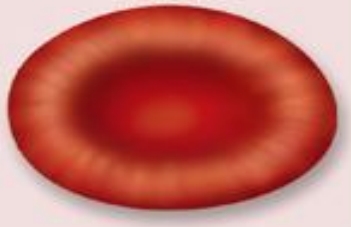
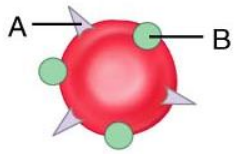
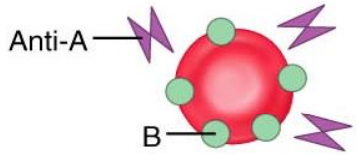
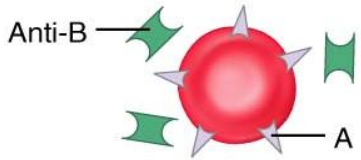
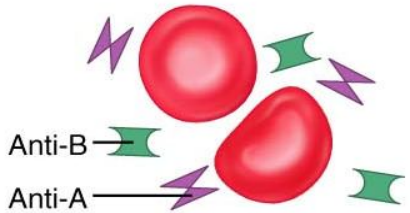
Erythrocytes	Antigen A 	Antigen B 	Antigens A and B 	Neither antigen A nor B 
Plasma	Anti-B antibodies 	Anti-A antibodies 	Neither anti-A nor anti-B antibodies	Both anti-A and anti-B antibodies 
Blood type	**Type A** Erythrocytes with type A surface antigens and plasma with anti-B antibodies	**Type B** 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

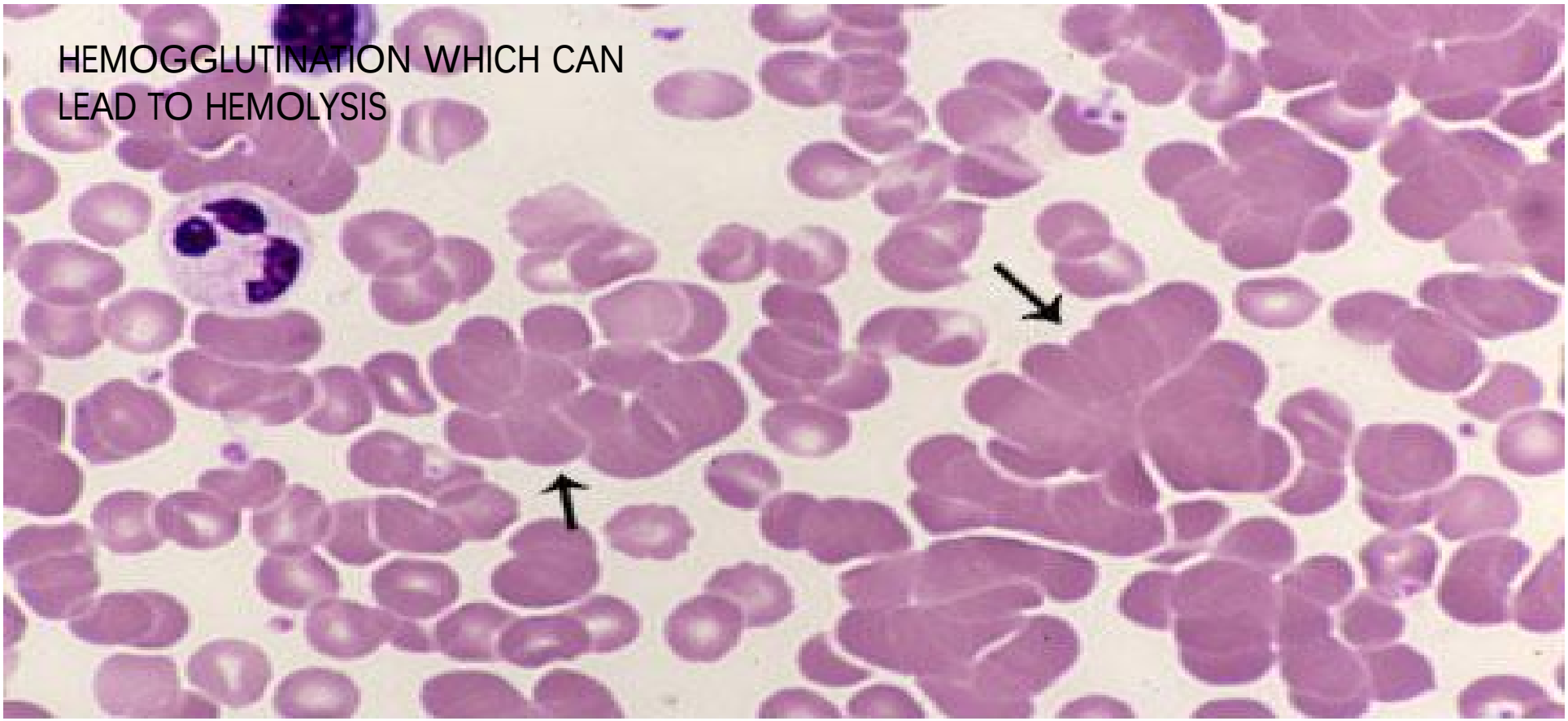
TABLE 17.4 ABO Blood Groups

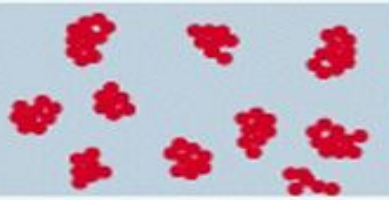
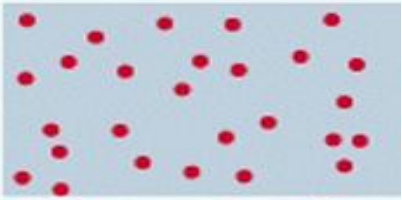
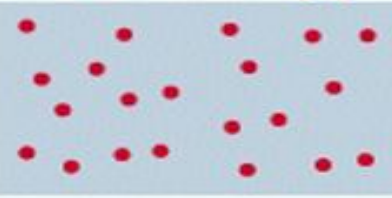
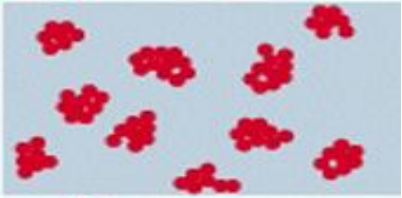
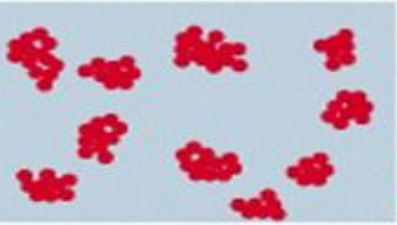
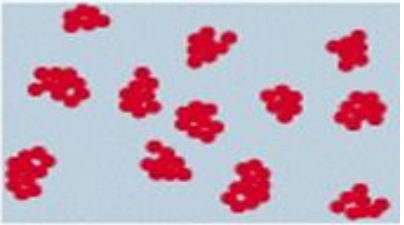
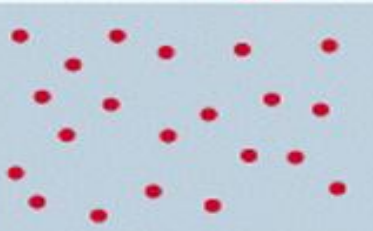
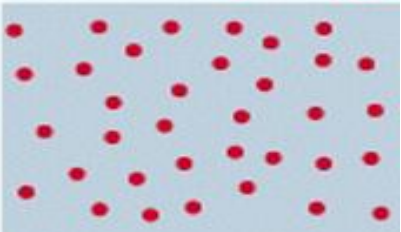
BLOOD GROUP	FREQUENCY (% U.S. POPULATION)				RBC ANTIGENS (AGGLUTINOGENS)	ILLUSTRATION	PLASMA ANTIBODIES (AGGLUTININS)	BLOOD THAT CAN BE RECEIVED
	WHITE	BLACK	ASIAN	NATIVE AMERICAN				
AB	4	4	5	<1	A B		None	A, B, AB, O (Universal recipient)
B	11	20	27	4	B		Anti-A (a)	B, O
A	40	27	28	16	A		Anti-B (b)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

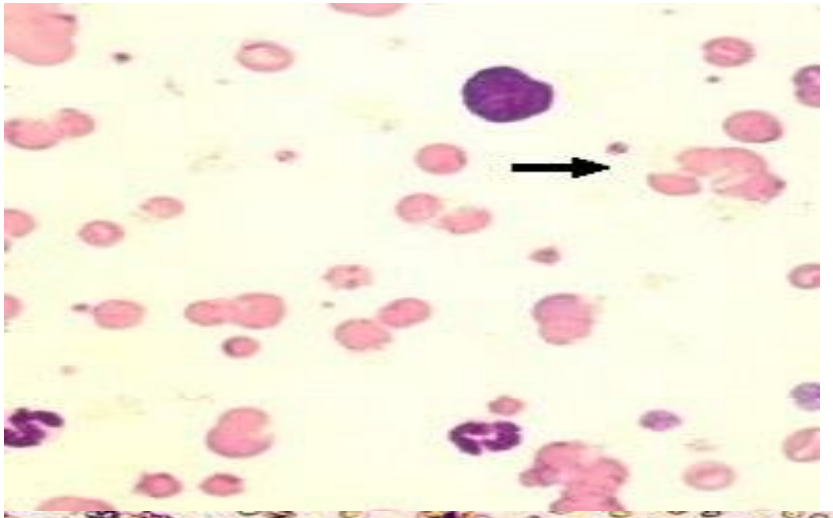
HEMAGGLUTINATION

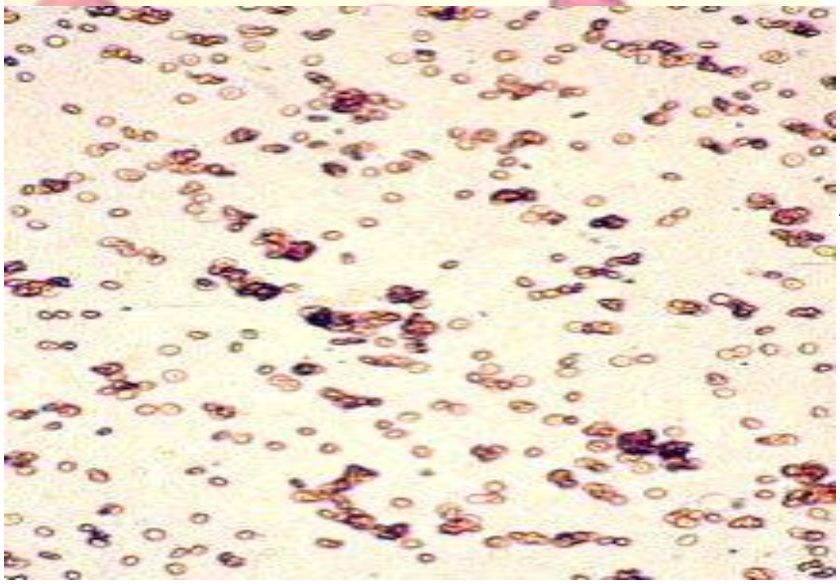
- **Agglutination or clumping is seen whenever the respective agglutinogens and agglutinins are mixed.**
- **Agglutinogen A + Agglutinin Alpha = Agglutination.**
- **Agglutinogen B + Agglutinin Beta = Agglutination.**
- **Both agglutinogens + Both antisera = Agglutination.**
- **No agglutinogens = No agglutination.**

HEMOGGLUTINATION WHICH CAN LEAD TO HEMOLYSIS



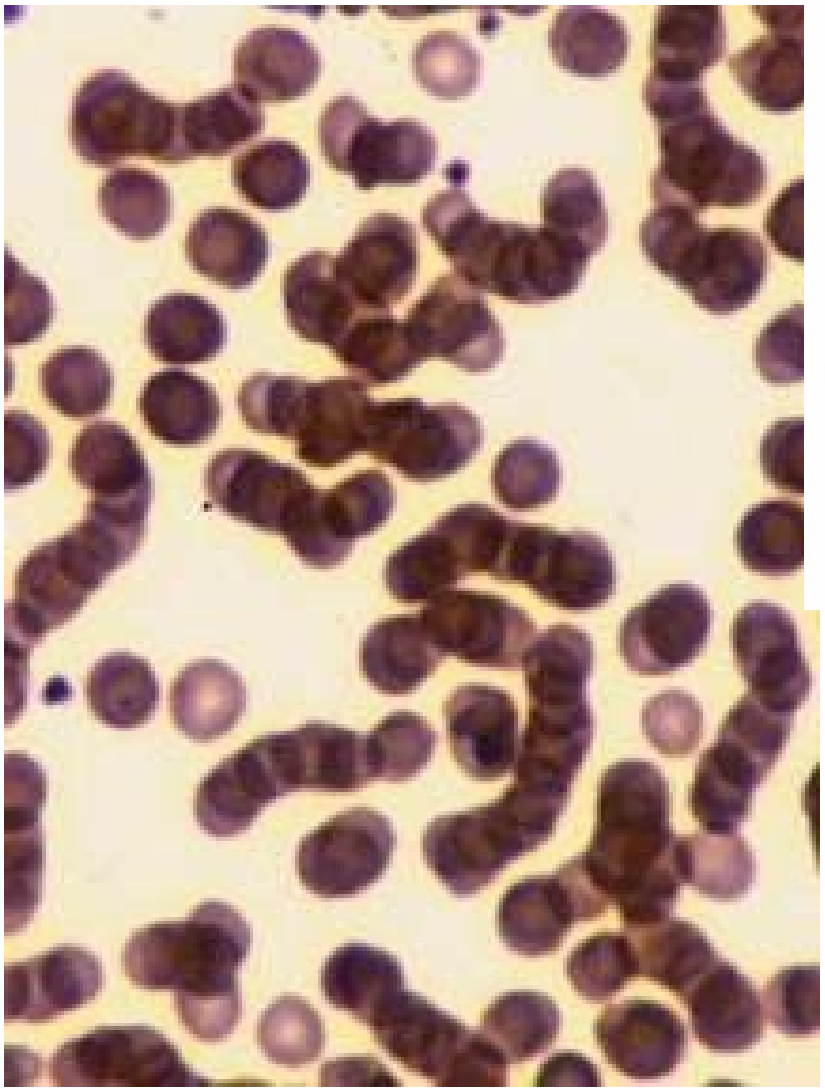
Blood type of cells	Genotype	Antibodies made by body	Reaction to added antibodies	
			Anti-A	Anti-B
A	I^AI^A or I^AI^O	Anti-B		
B	I^BI^B or I^BI^O	Anti-A		
AB	I^AI^B	Neither anti-A nor anti-B		
O	I^OI^O	Both anti-A and anti-B		



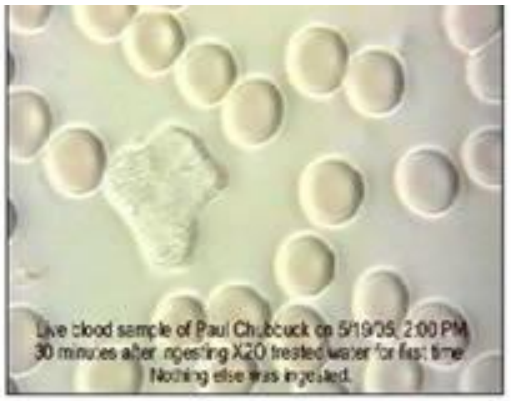


The observation of red blood cell agglutination (also referred to as autoagglutination) must be distinguished from rouleaux formation which is a physiological phenomenon. The presence of antibodies (usually IgM) on the surface of red blood cells is responsible for the phenomenon of autoagglutination. Agglutination can be observed during immune-mediated hemolytic anemia, but also during 'cryoglobulinemia' (a far more rare condition).

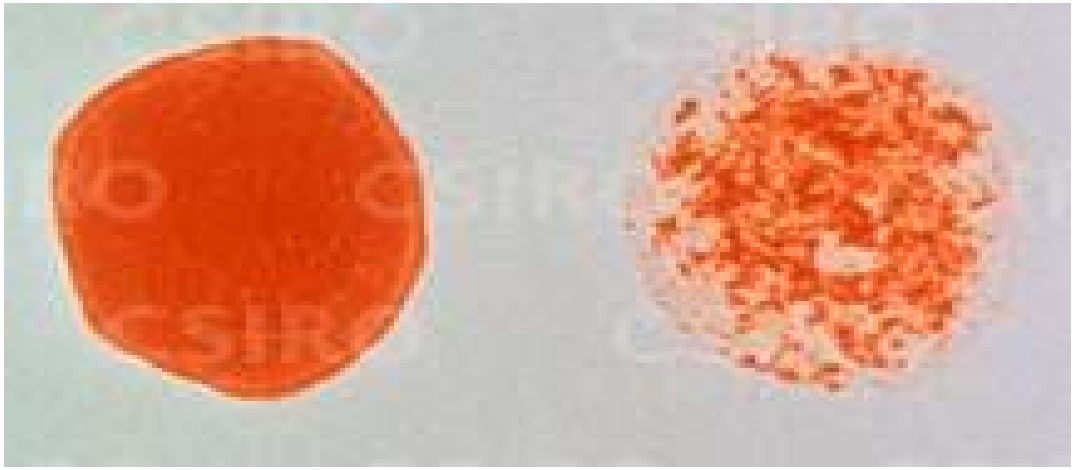
Agglutinating red blood cells resemble grapelike clusters whereas red blood cells in rouleaux formation resemble a stack of coins.
In order to clearly distinguish erythrocyte agglutination from rouleaux formation, a simple saline test can be performed.



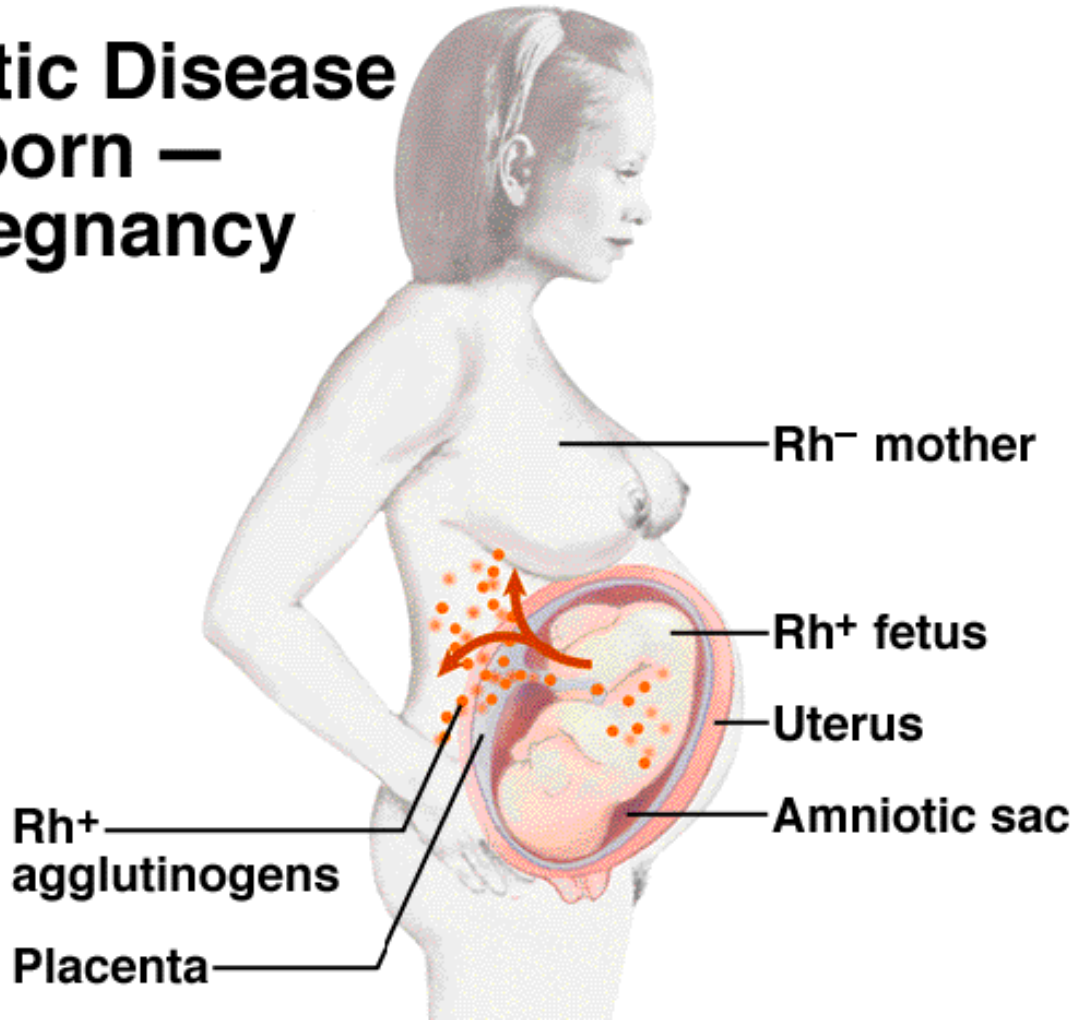
Actual blood sample taken during a demonstration showing red blood cells glued together lacking oxygen.



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.

Rh Blood Groups

- **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

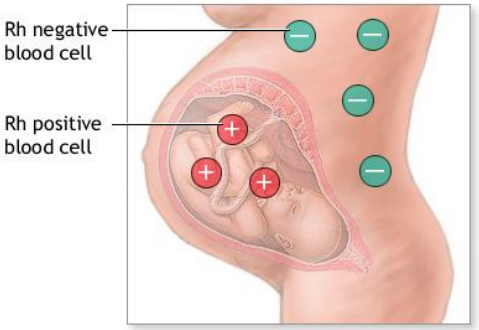
ABO System & Pregnancy

- 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

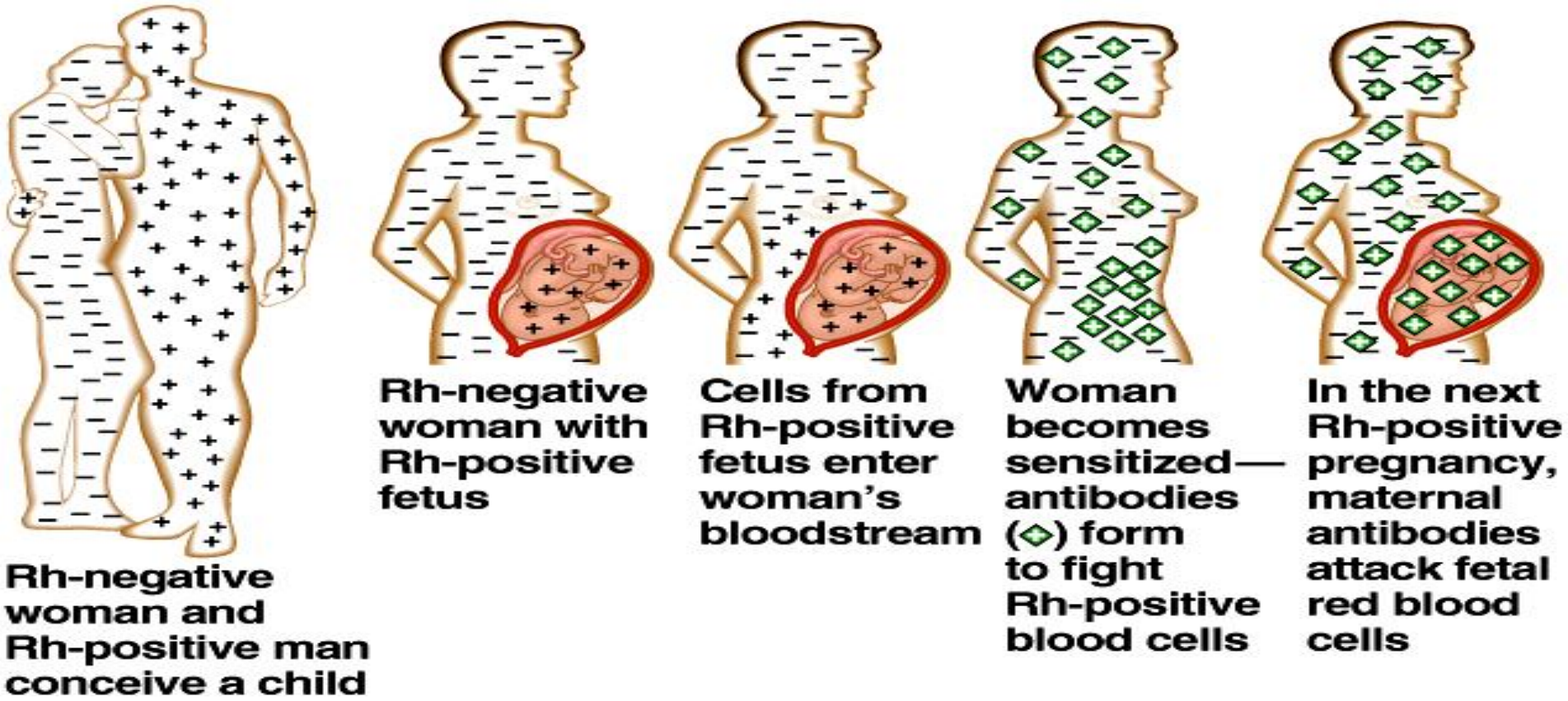


- **Anti-D agglutinins or antibodies do not occur naturally.**
- **They are produced by the Immune systems as and when it is exposed to the D antigens.**
- **So these Anti D agglutinins are found only in some of the Rh Negative people.**

- **Those who have been exposed to the Rh or D antigen**

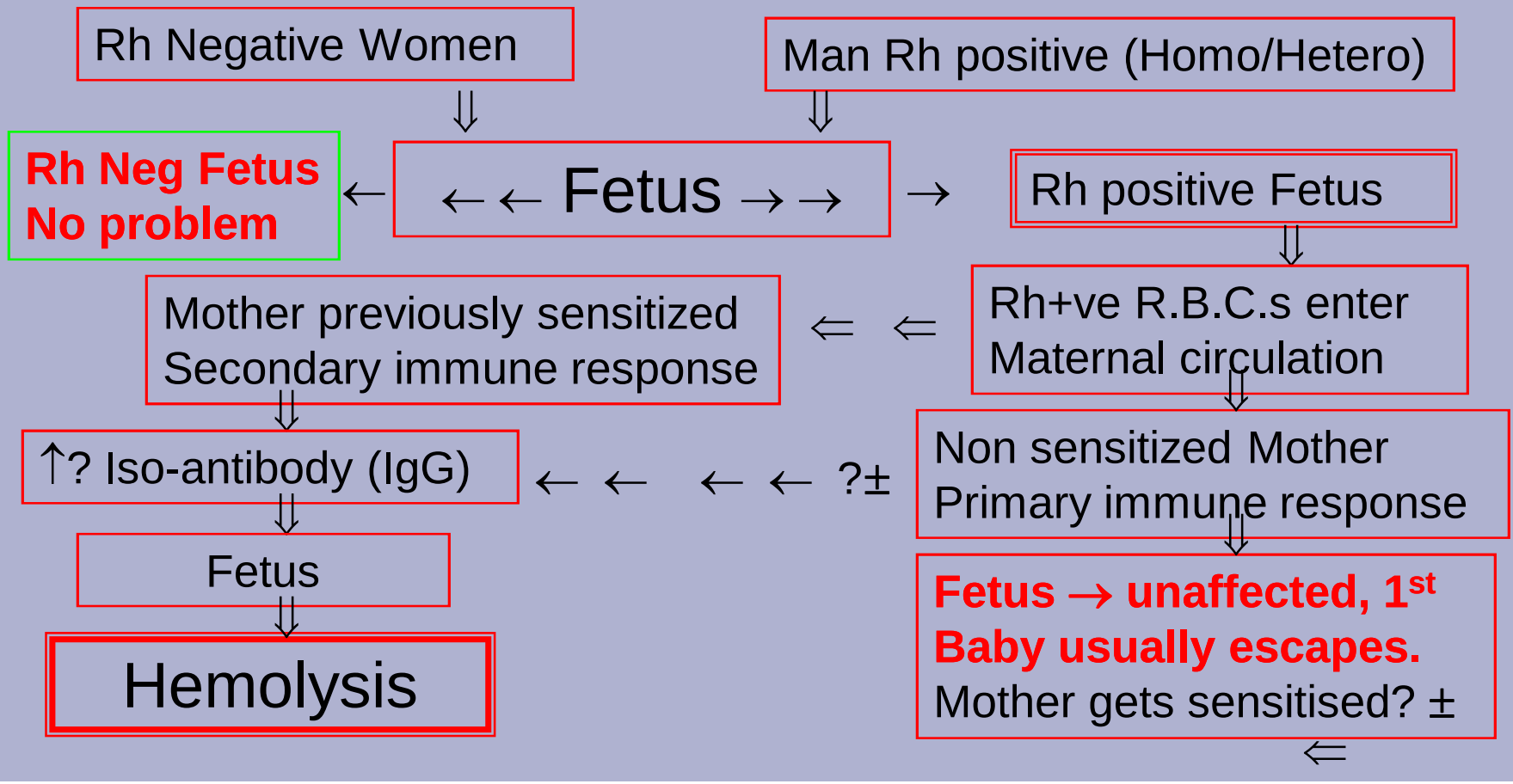
Exposure to Antigens: How?

- 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.



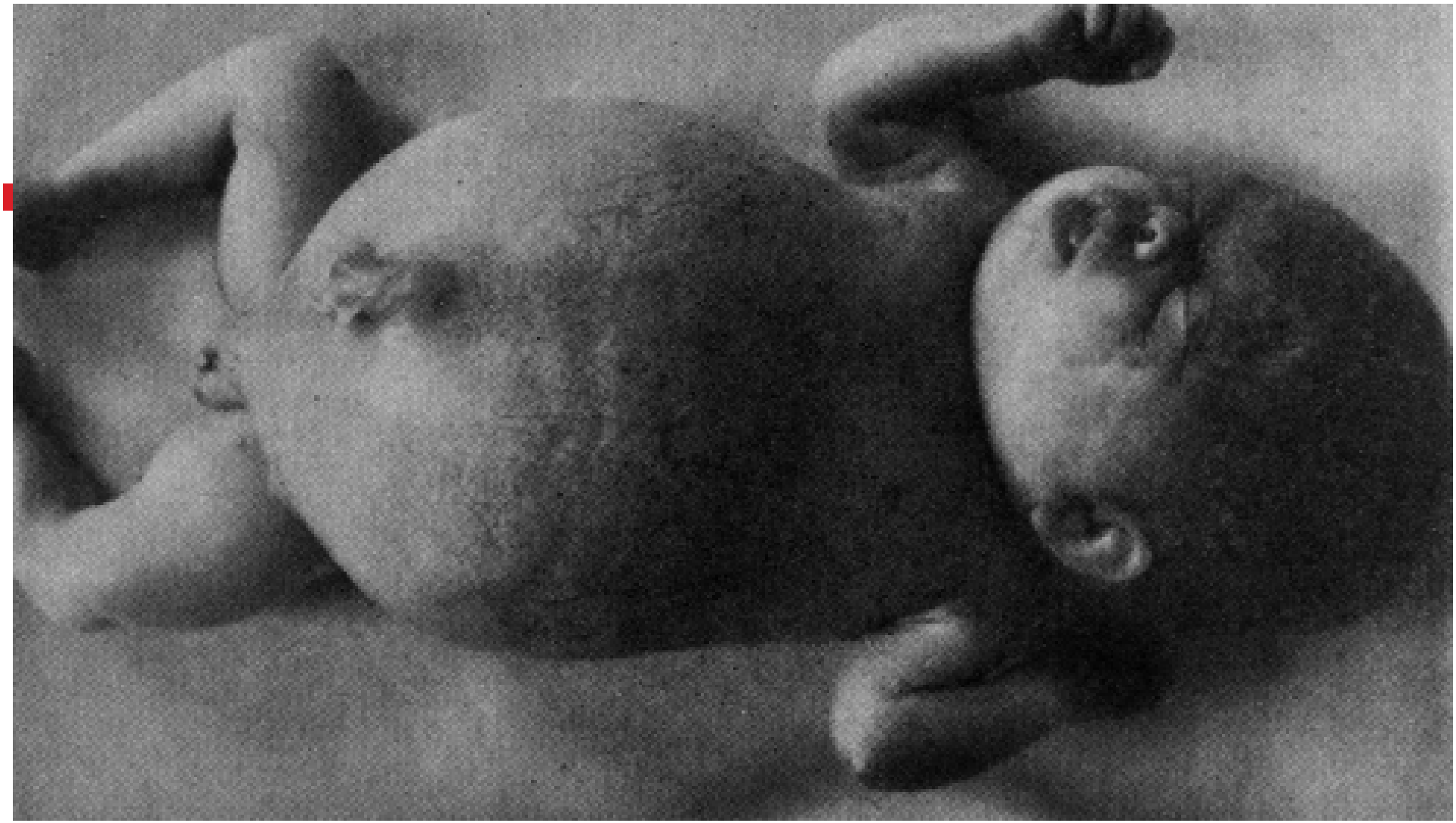
The disease, called **erythroblastosis fetalis** or **hemolytic disease of the newborn**, may be so severe as to kill the fetus or even the newborn infant. It is an example of an antibody-mediated cytotoxicity disorder.

Pathogenesis Of Rh Iso-immunisation

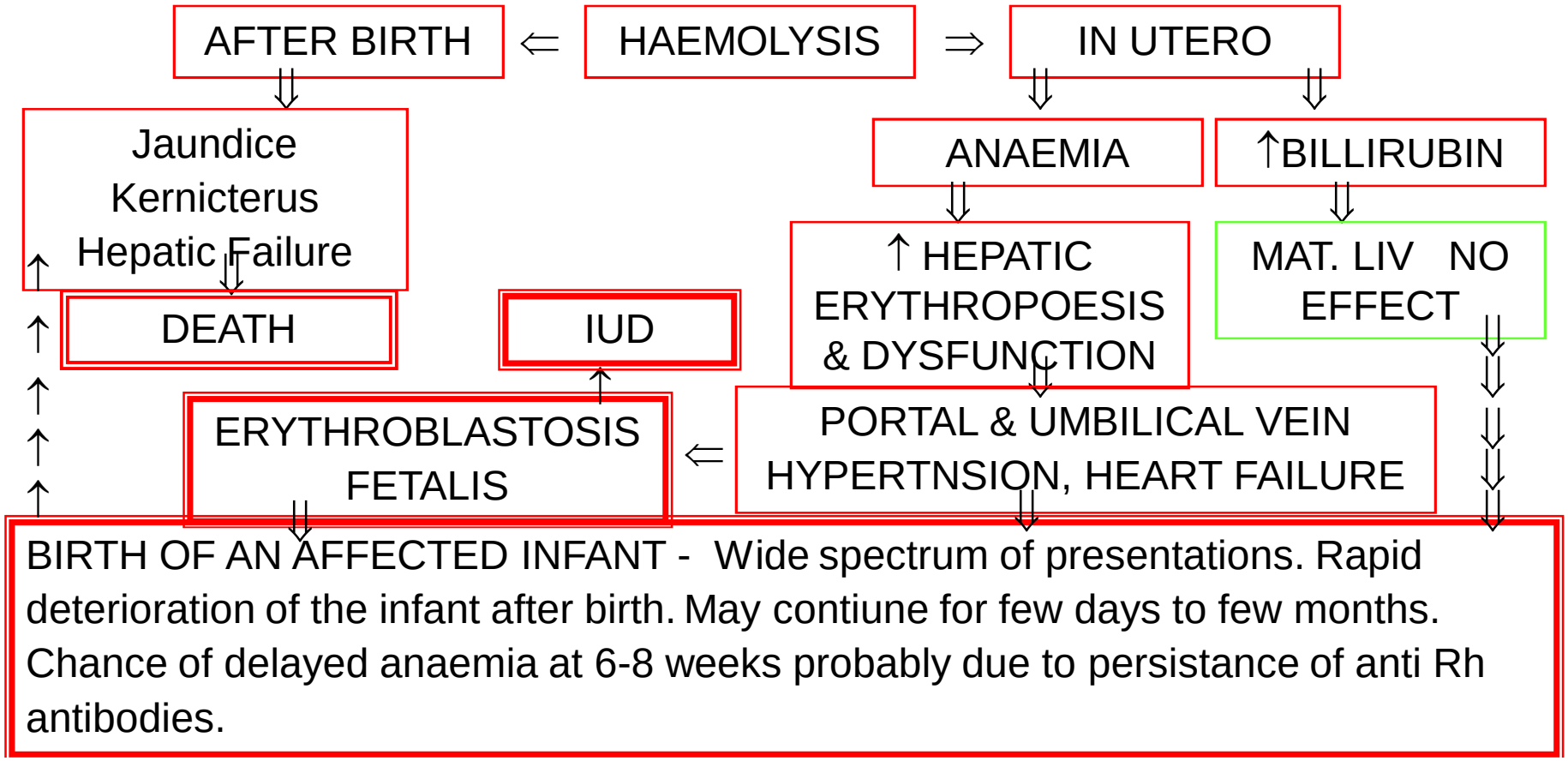


ERYTHROBLASTOSIS FETALIS

- **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.**



Pathology Of Iso-immunisation



Exposure to Antigens: How?

- In case of an Rh-ve woman, if she is married to an Rh+ve man, she can conceive an Rh+ve child.
- In this case, the D antigen present on the erythrocytes of the fetus does not go into the maternal circulation throughout the pregnancy (due to the Feto-Placental barrier)

❑During the delivery of the baby, some blood of the fetus spills over into the maternal circulation.

❑The maternal circulation is exposed to the D antigens from the fetal erythrocytes.

❑The maternal circulation slowly develops Anti D antibodies.

❑The first child is however spared.

Prevention of Rh Incompatibility

- Premarital counseling? Ambitious?
- Proper matching of blood particularly in women before childbearing.
- Blood grouping must for every woman, before 1st pregnancy.
- Rh+ve Blood transfusion- 300mcg Immunoglobulin (minimum).
- Proper management of unsensitised Rh negative pregnancies.

Management of Unsensitised Pregnancy

- In Abortion, Ectopic, CVS-
  - Pregnancy < 12 weeks- 50mcg Anti D
  - Pregnancy >12 weeks- 300mcg Anti D
- APH, IUD, Amniocentesis, Abdominal trauma, Foetal-maternal hemorrhage -300mcg Anti D
- At birth- cord blood for ABO & Rh typing
- Baby Rh negative – Be happy

Hemolytic Disease of the Newborn

- Hemolytic disease of the newborn – Rh^+ antibodies of a sensitized Rh^- mother cross the placenta and attack and destroy the RBCs of an Rh^+ baby
- Rh^- mother becomes sensitized when exposure to Rh^+ blood causes her body to synthesize Rh^+ antibodies

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

- When serum containing anti-A or anti-B agglutinins is added to blood, agglutination will occur between the agglutinin and the corresponding agglutinogens
- Positive reactions indicate agglutination

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**

Plasma Volume Expanders

- Plasma expanders
  - **Have osmotic properties that directly increase fluid volume**
  - **Are used when plasma is not available**
  - **Examples: purified human serum albumin, plasminate, and dextran**
- Isotonic saline can also be used to replace lost blood volume

Diagnostic Blood Tests

- Laboratory examination of blood can assess an individual's state of health
- Microscopic examination:
  - Variations in size and shape of RBCs – predictions of anemias
  - Type and number of WBCs – diagnostic of various diseases
- Chemical analysis can provide a comprehensive picture of one's general health status in relation to normal values

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