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

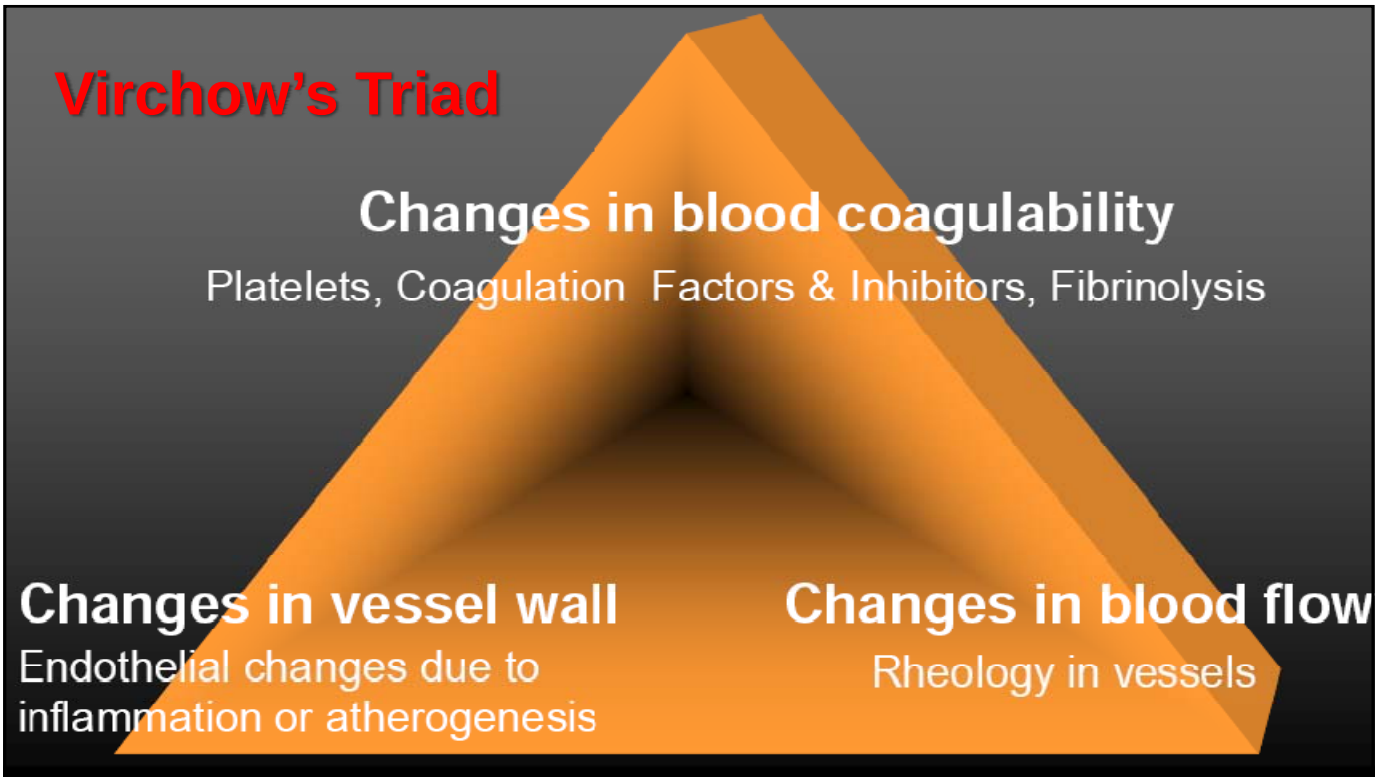
- Primary Hemostasis
  - Platelet Plug Formation
  - Dependent on normal platelet number & function
  - Initial Manifestation of Clot Formation

Secondary Hemostasis

- Activation of Clotting Cascade ☒ Deposition & Stabilization of Fibrin

Tertiary Hemostasis

- Dissolution of Fibrin Clot
- Dependent on Plasminogen Activation



Overview of Hemostasis: Clot Formation & Vessel Repair

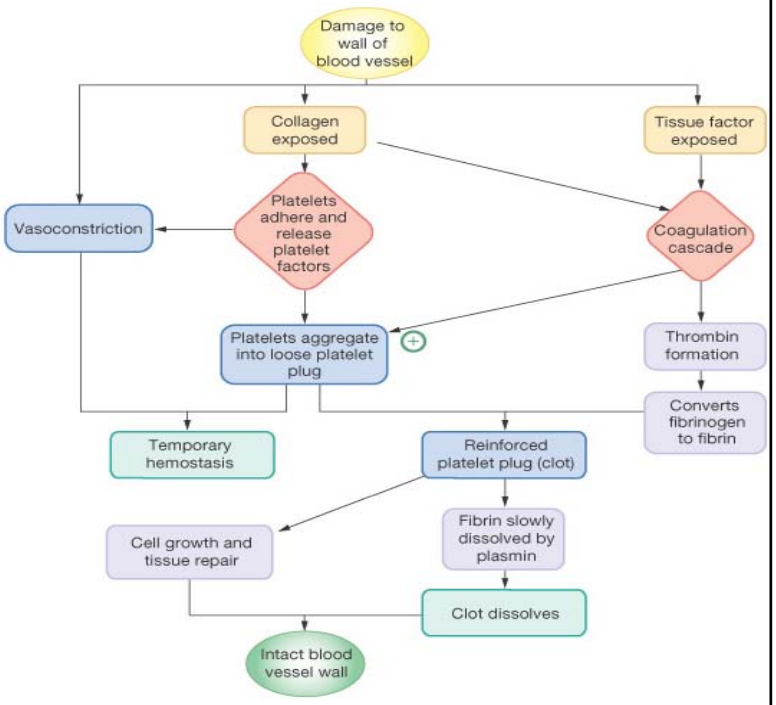
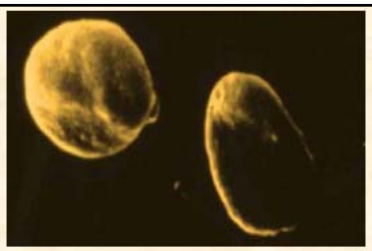


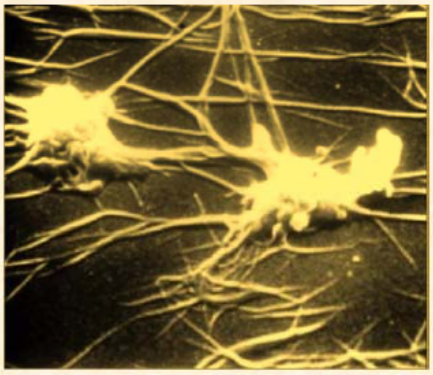
Figure 16-11: Overview of hemostasis and tissue repair

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)**



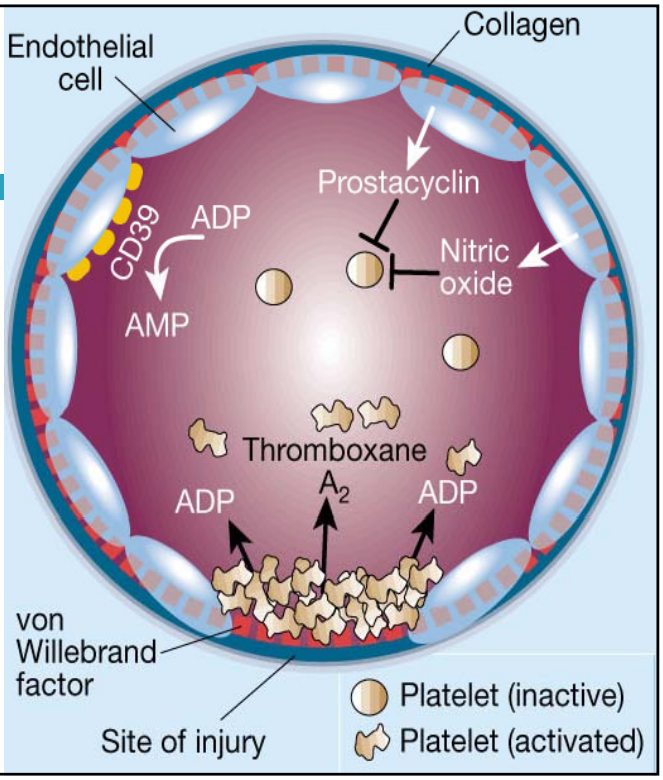
Resting platelets

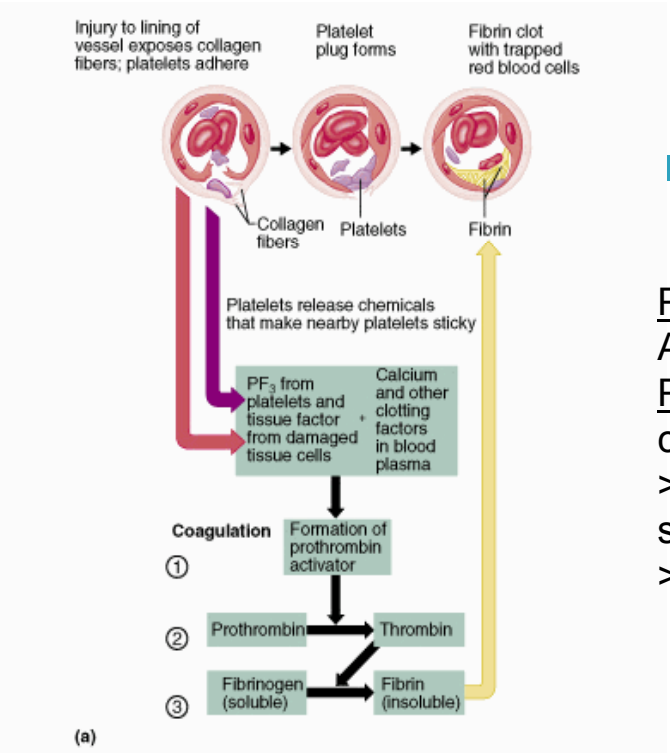


Activated platelets

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**

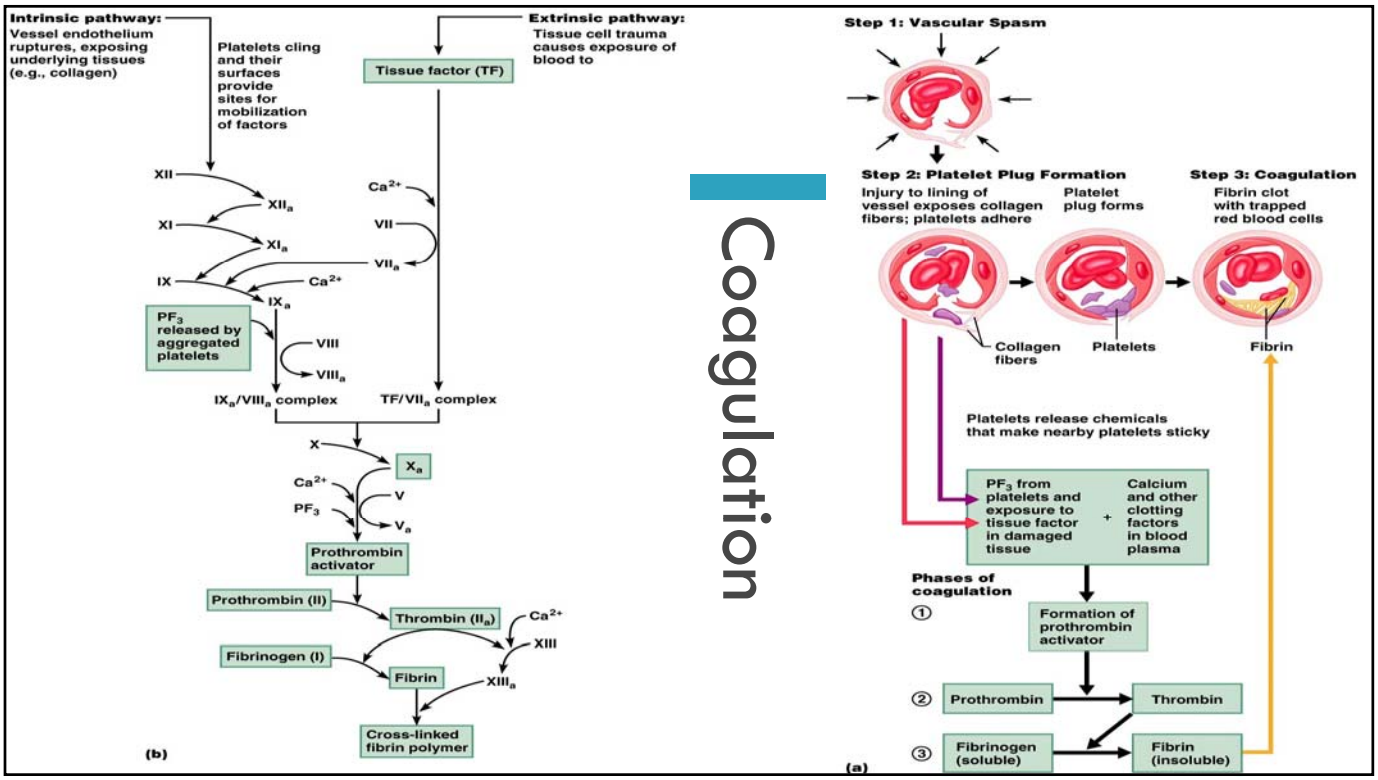




Platelets release > PF3 > tissue factor other clotting factors > Prothrombin activator is formed, > Activator transforms prothrombin > Prothrombin becomes thrombin > catalyzes fibrinogen activates factor XIII > fibrinogen becomes fibrin fibrin stabilizing factor > Fibrin Mesh Forms >Clot Forms

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

CLOT-ED

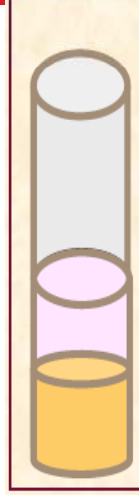
Coagulation Factors

- 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

Prothrombin Time (PT)

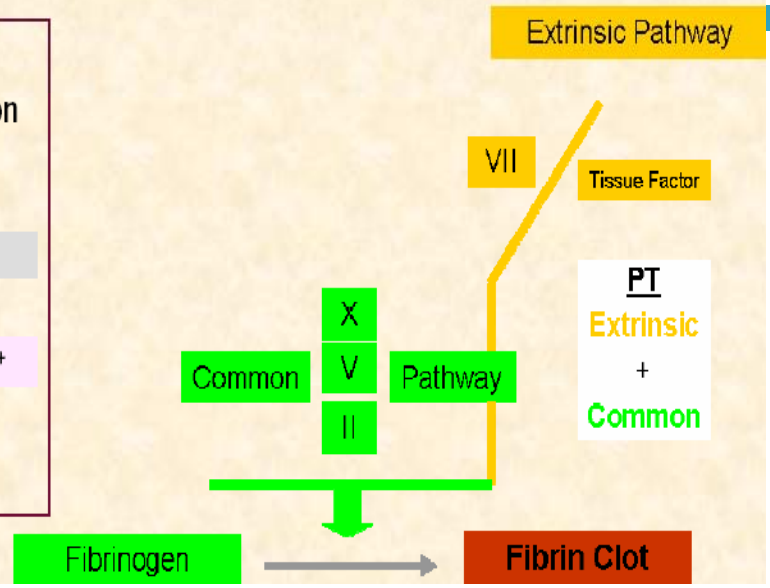


Time for clot formation
~ 12 seconds

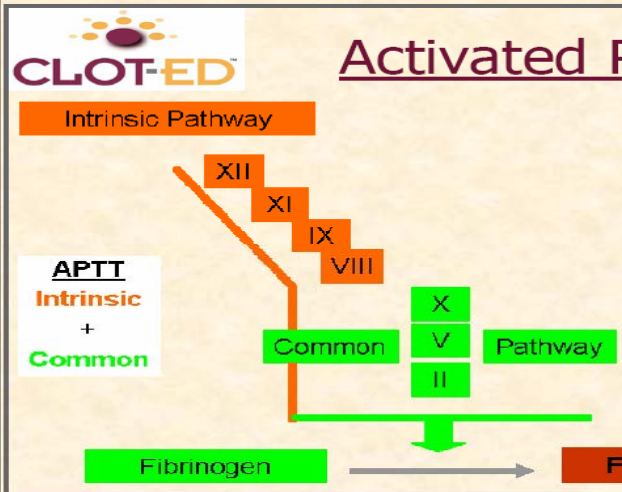
Incubate at 37 °C for ~3 minutes

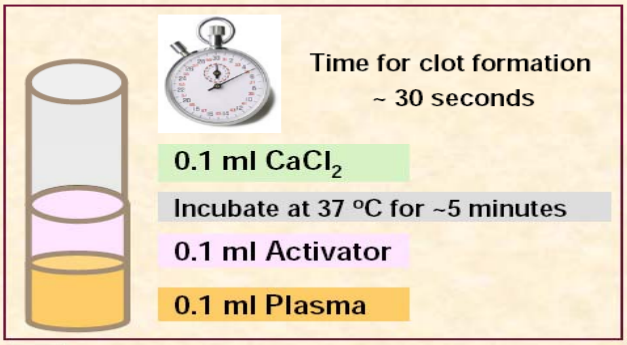
0.1 ml Thromboplastin + Ca^{++}

0.1 ml Plasma



Activated Partial Thromboplastin Time





Time for clot formation
~ 30 seconds

0.1 ml $CaCl_2$

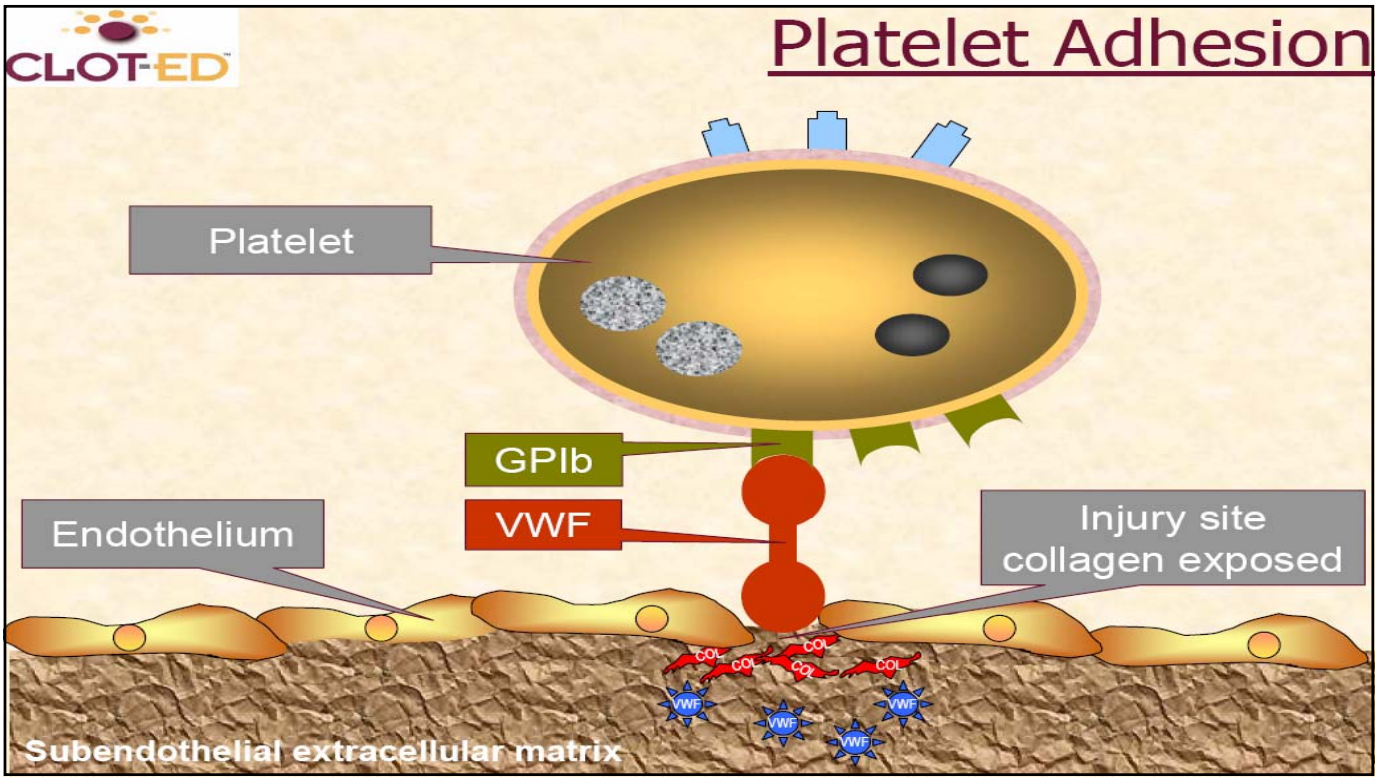
Incubate at 37 °C for ~5 minutes

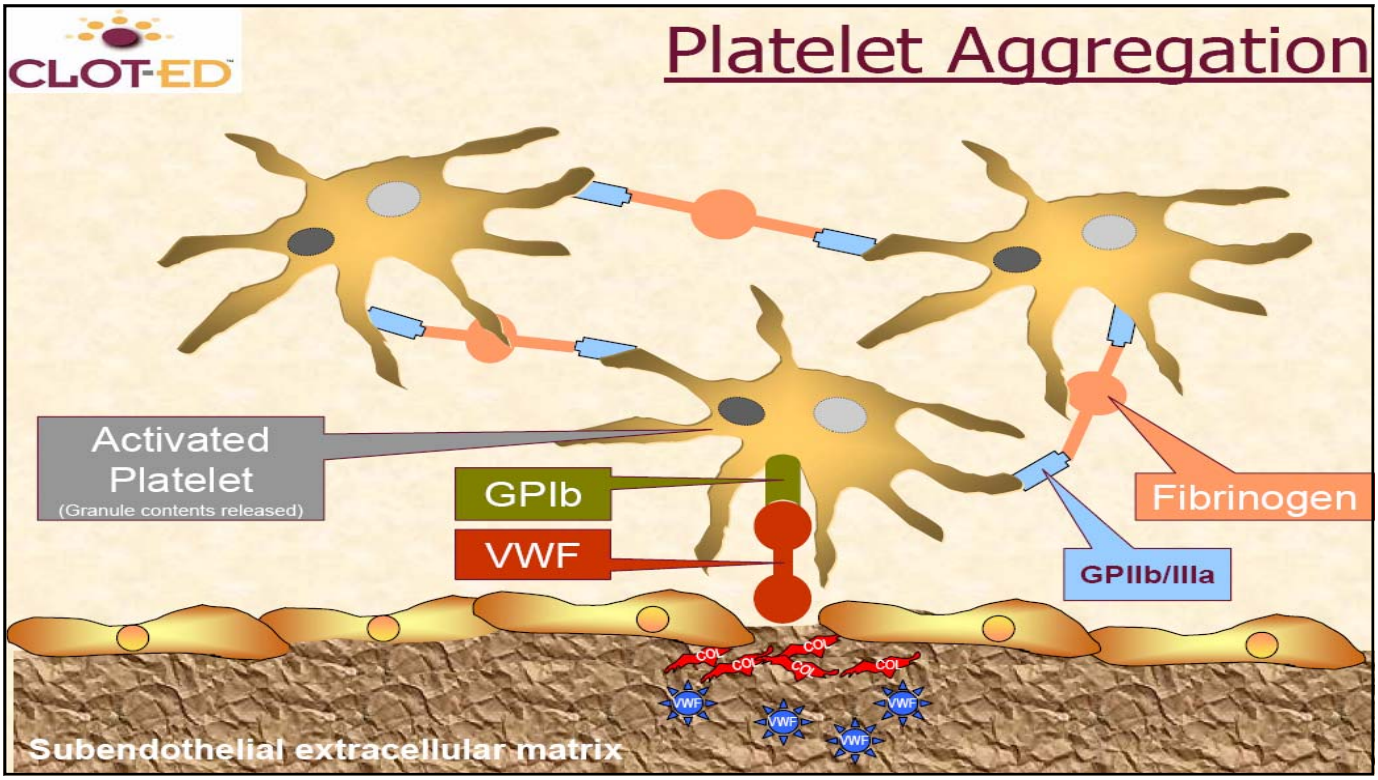
0.1 ml Activator

0.1 ml Plasma

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)


CLOT-ED
Platelet Adhesion
Platelet
GPIb
VWF
Endothelium
Injury site
collagen exposed
COL
VWF
Subendothelial extracellular matrix


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

Hemostasis: Vasoconstriction & Plug Formation

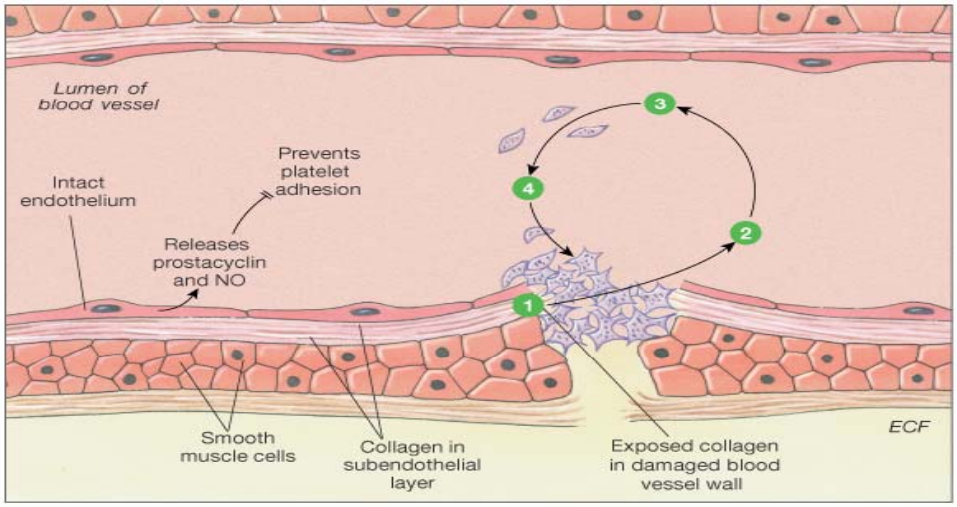


Figure 16-12: Platelet plug formation

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

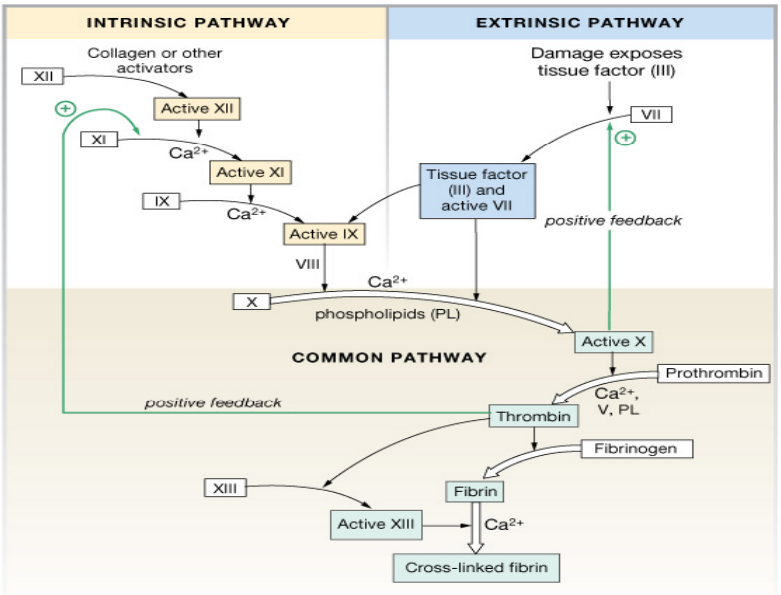


Figure 16-13: The coagulation cascade

Dissolving the Clot and **Anticoagulants**

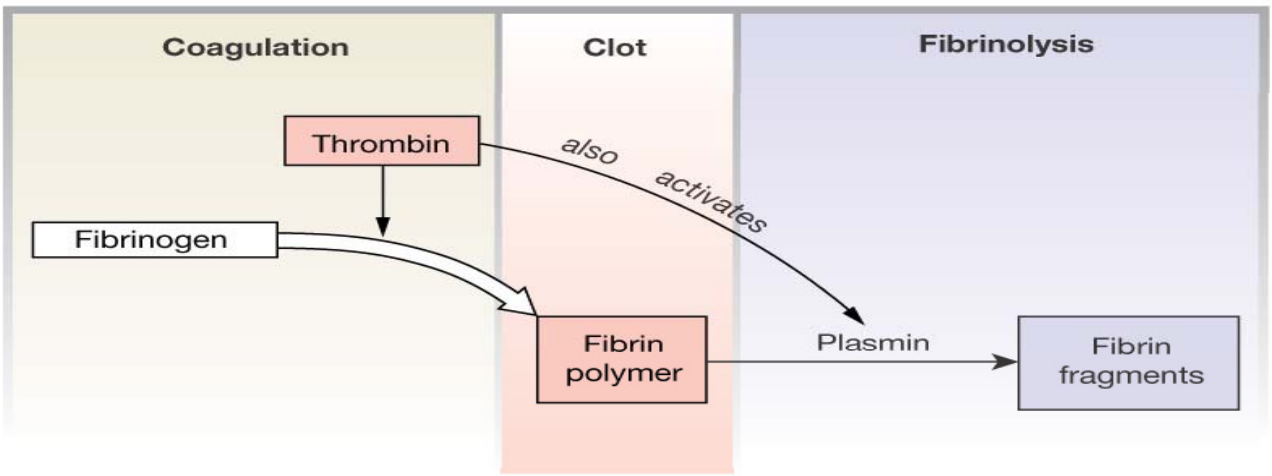


Figure 16-14: Coagulation and fibrinolysis

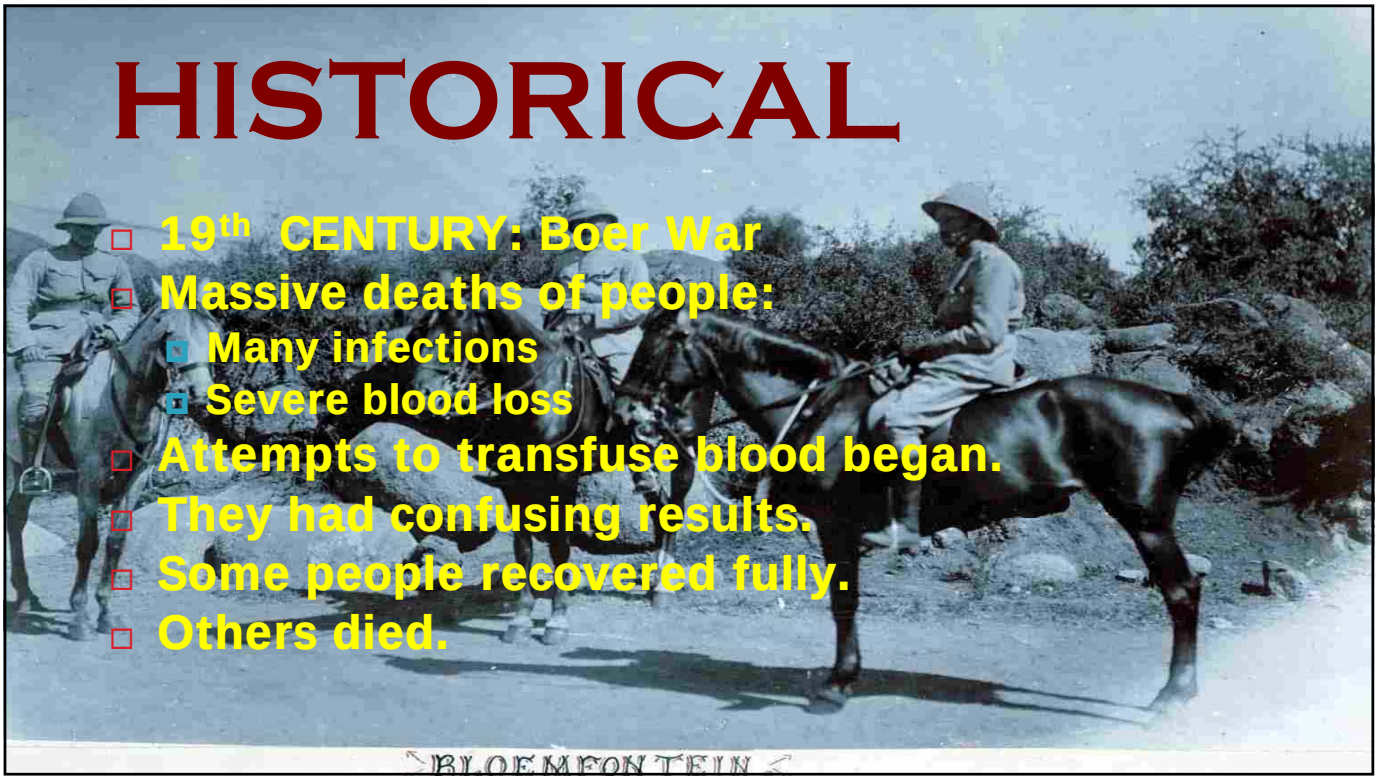
Tests for Primary Hemostasis

- 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

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

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.

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

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- 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.

A AND B, INDIVIDUALS

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- 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*

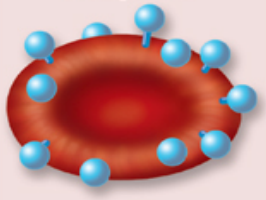
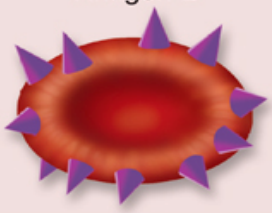
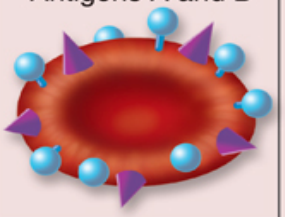
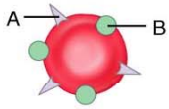
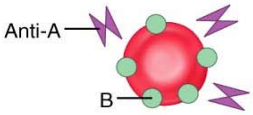
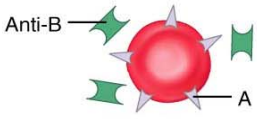
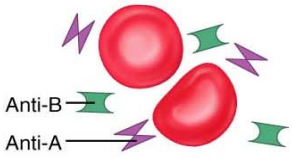
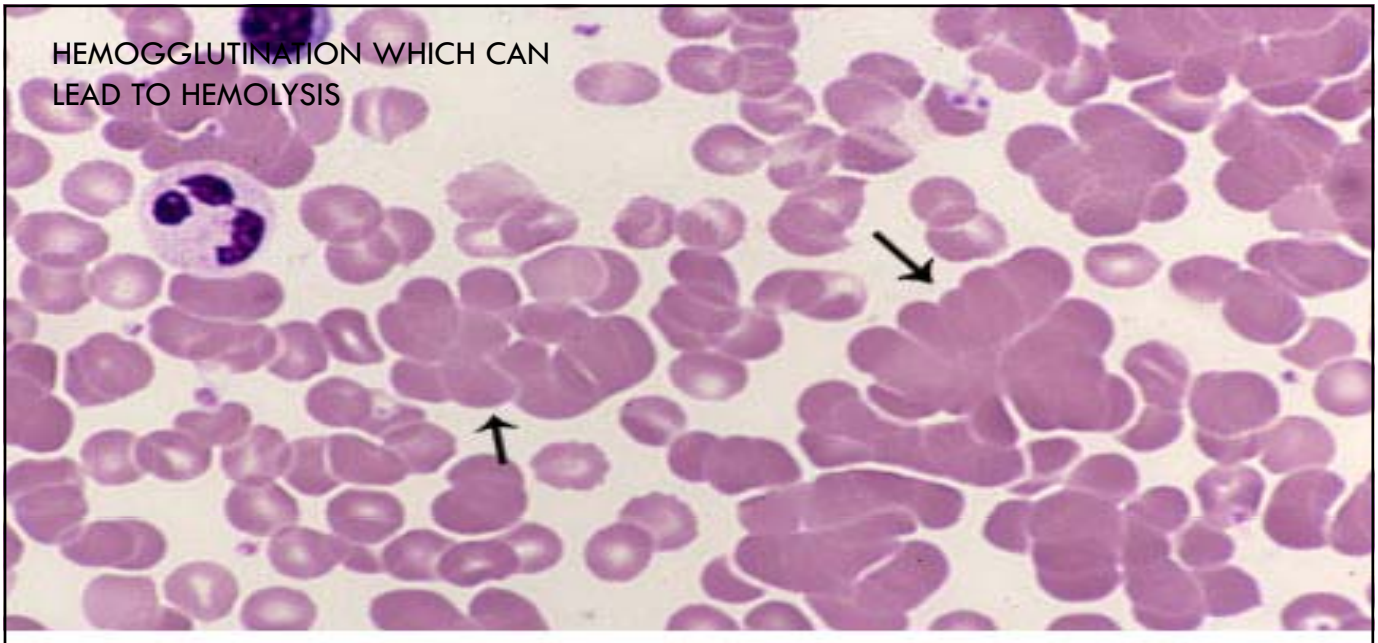
	Antigen A	Antigen B	Antigens A and B	Neither antigen A nor B
Erythrocytes				
Plasma	Anti-B antibodies 	Anti-A antibodies 	Neither anti-A nor anti-B antibodies	Both anti-A and anti-B antibodies 
Blood type	**Type A** Erythrocytes with type A surface antigens and plasma with anti-B antibodies	**Type B**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

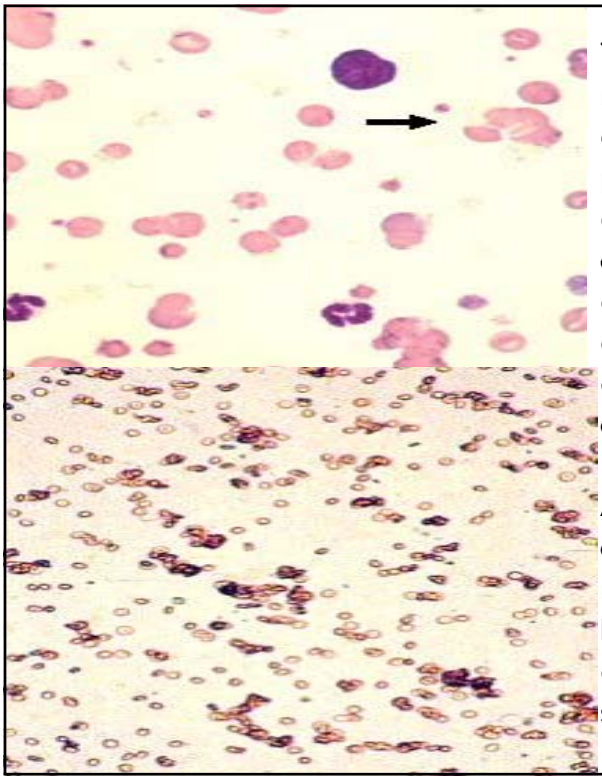
HEMAGGLUTINATION

- **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.**



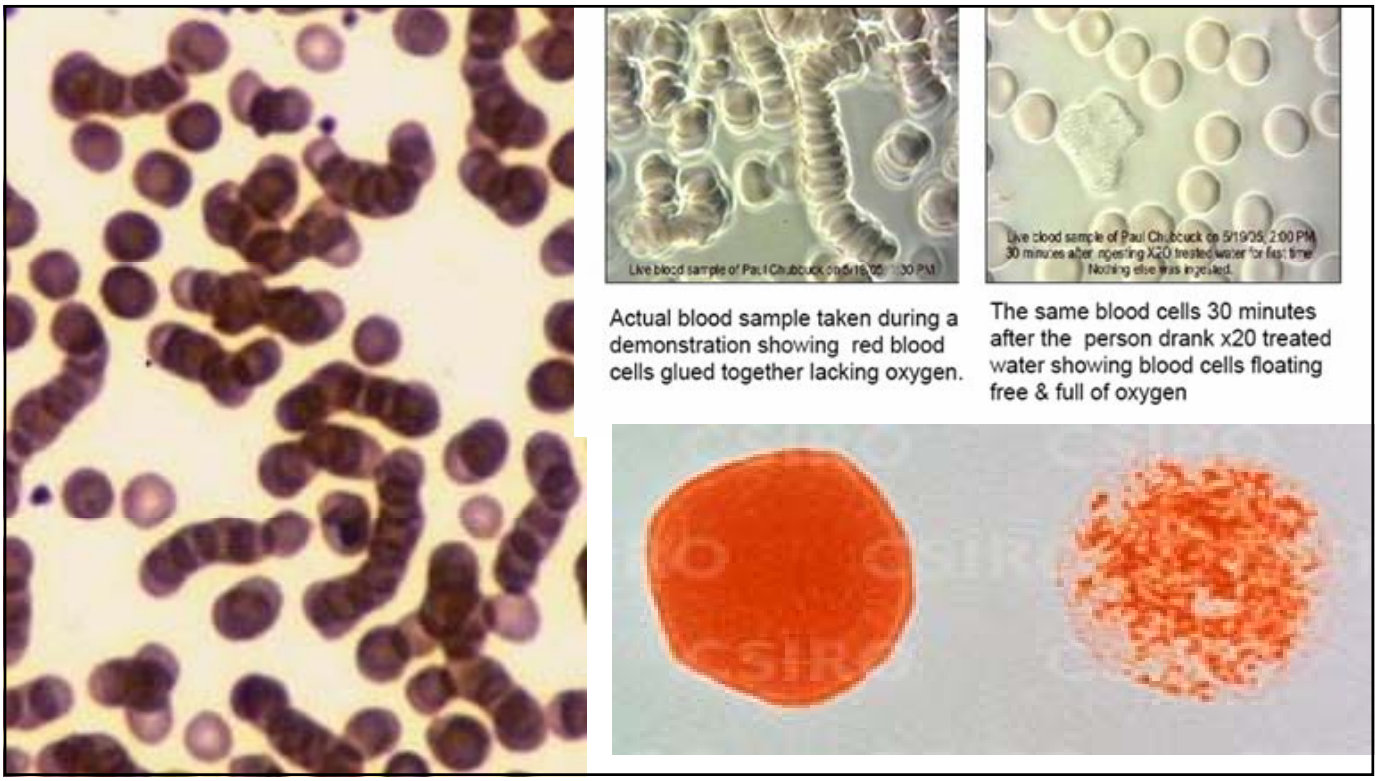
Source: Lichtman MA, Shafer MS, Felgar RE, Wang N:
Lichtman's Atlas of Hematology: http://www.accessmedicine.com

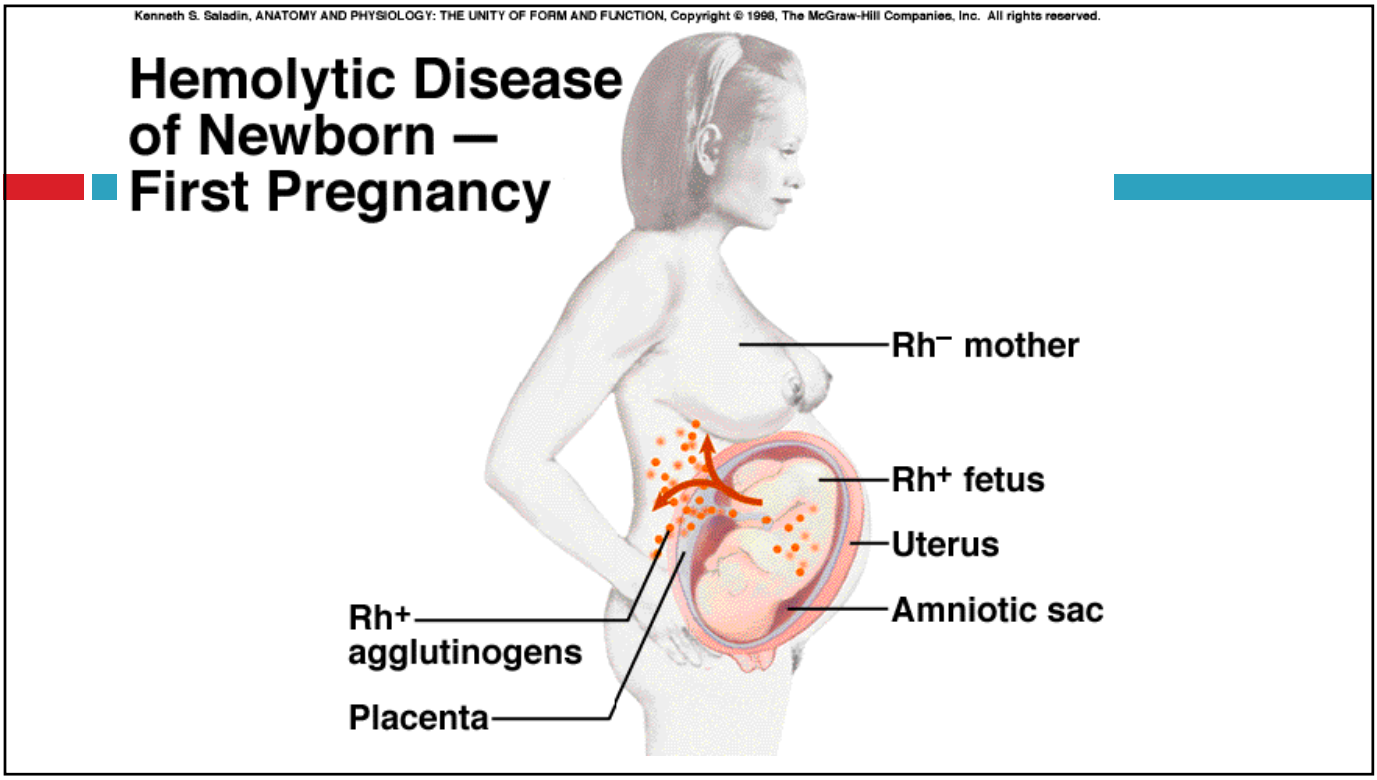
Blood type of cells	Genotype	Antibodies made by body	Reaction to added antibodies	
			Anti-A	Anti-B
A	I^AI^A or I^AI^O	Anti-B		
B	I^BI^B or I^BI^O	Anti-A		
AB	I^AI^B	Neither anti-A nor anti-B		
O	I^OI^O	Both anti-A and anti-B		



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


Kenneth S. Saladin, ANATOMY AND PHYSIOLOGY: THE UNITY OF FORM AND FUNCTION, Copyright © 1998, The McGraw-Hill Companies, Inc. All rights reserved.
Hemolytic Disease of Newborn — First Pregnancy
Rh⁻ mother
Rh⁺ fetus
Uterus
Amniotic sac
Rh⁺ agglutinogens
Placenta

Rh TYPING: INTRODUCTION

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- 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

Rh or D Agglutinins

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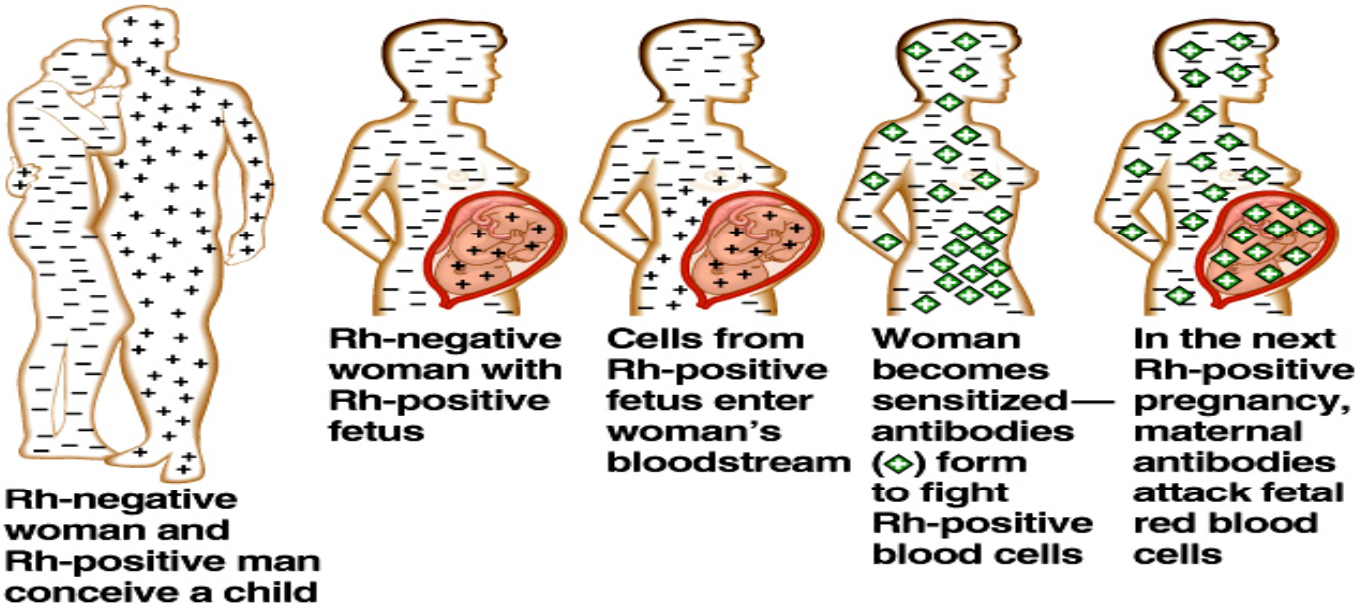
- Anti-D agglutinins or antibodies do not occur naturally.
- They are produced by the Immune systems as and when it is exposed to the D antigens.
- So these Anti D agglutinins are found only in some of the Rh Negative people.
- Those who have been exposed to the Rh or D antigen

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.

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.

ERYTHROBLASTOSIS FETALIS

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- **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.**

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