ABO and Rh Blood Group Systems

Teaching Aim

- Understanding inheritance, synthesis, various antigens and antibodies and their clinical significance in ABO & Rh blood group systems
- Understanding practical aspects of ABO & Rh blood grouping

Human Blood Groups

- Red cell membranes have antigens (protein / glycoprotein) on their external surfaces
- These antigens are
  - unique to the individual
  - recognized as foreign if transfused into another individual
  - promote agglutination of red cells if combine with antibody
  - more than 30 such antigen systems discovered
- Presence or absence of these antigens is used to classify blood groups
- Major blood groups – ABO & Rh
- Minor blood groups – Kell, Kidd, Duffy etc

ABO Blood Groups

- Most well known & clinically important blood group system.
- Discovered by Karl Landsteiner in 1900
- It was the first to be identified and is the most significant for transfusion practice
- It is the **ONLY** system that the reciprocal antibodies are consistently and predictably present in the sera of people who have had no exposure to human red cells
- ABO blood group consist of
  - two antigens (A & B) on the surface of the RBCs
  - two antibodies in the plasma (anti-A & anti-B)

Reciprocal relationship between ABO antigens and antibodies

Antigens on RBCs	Antibody in plasma / serum	Blood group
A	Anti-B	A
B	Anti-A	B
AB	None	AB
None	Anti-A, Anti-B	O

Development at birth

- All the ABH antigens develop as early as day 37 of fetal life but do not increase very much in strength during gestational period
- Red cell of newborn carry 25-50 % of number of antigenic sites found on adult RBC
- Although cord red cells can be ABO grouped, the reactions may be a bit weaker than expected
- A or B antigen expression fully developed at 2-4 yrs of age and remain constant throughout life

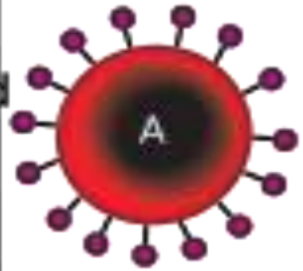
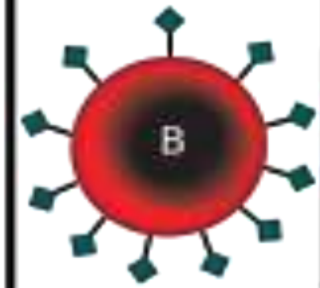
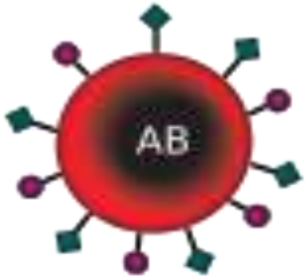
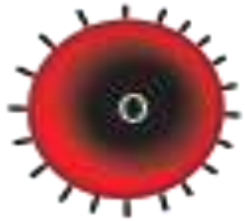
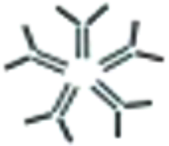
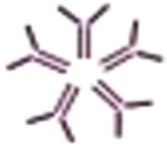
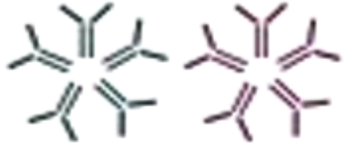
Expression of ABO Antigens

- Although the ABO blood group antigens are regarded as RBC antigens, they are actually expressed on a wide variety of human tissues and are present on most epithelial and endothelial cells
- ABH antigens are not only found in humans, but also in various organisms such as bacteria, plants, and animals
- Present both on red blood cells and in secretions only in humans and some of the apes (chimpanzee, gorilla)
- In all other mammalian species these substances are found only in secretions

Anti-A and anti-B antibodies

- Not present in the newborn, appear in the first years of life (4-6 months usually), reach adult level at 5-10 years of age, decreases in elderly
- Naturally occurring as they do not need any antigenic stimulus
- However, some food & environmental antigens (bacterial, viral or plant antigens) are similar enough to A and B glycoprotein antigens and may stimulate antibody development
- Immunocompetent person react to these antigens by producing antibodies to those absent from their own system
- Usually IgM, which are not able to pass through the placenta to the fetal blood circulation
  - Anti-A titer from group O > Anti-A titer from group B
  - Anti-A titer from group B > Anti-B titer from group A

ABO antigens & corresponding antibodies

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	A antigen	B antigen	 A and B antigens	None

'Landsteiner's law :-
the plasma contains natural antibodies to A or B, if these antigens are absent from the red cells of that person

Inheritance of ABO Blood Groups

- Follows Mendelian principles
- Blood group antigens are “codominant”- if the gene is inherited, it will be expressed.
- There are three allelic genes -A, B & O
- Some aberrant genotypes do occur but they are very rare.
- Understanding of basic inheritance important.

Inheritance of ABO Blood Groups

- Two genes inherited, one from each parent.
- Individual who is A or B may be homozygous or heterozygous for the antigen.
  - Heterozygous: AO or BO
  - Homozygous: AA or BB
- Phenotype is the actual expression of the genotype, ie, group A
- Genotype are the actual inherited genes which can only be determined by family studies, ie, AO.

Example of Determining Genotype

- Mother's phenotype is group A, genotype AO
- Father's phenotype is group B, genotype BO

	B	O
A	AB 25% (Group AB)	AO 25% (Group A)
O	BO 25% (Group B)	OO 25% (Group O)

Other Examples

Mother	Father	Offspring Blood Group
AA	BB	100% AB
BO	OO	50% each of B or O
OO	OO	100% O
OO	AO	50% each of A or O

ABO Antigen Synthesis

- Blood group antigens are actually sugars attached to the red blood cell.
- Antigens are “built” onto the red cell.
- Individuals inherit a gene which codes for specific sugar(s) to be added to the red cell.
- The type of sugar added determines the blood group.

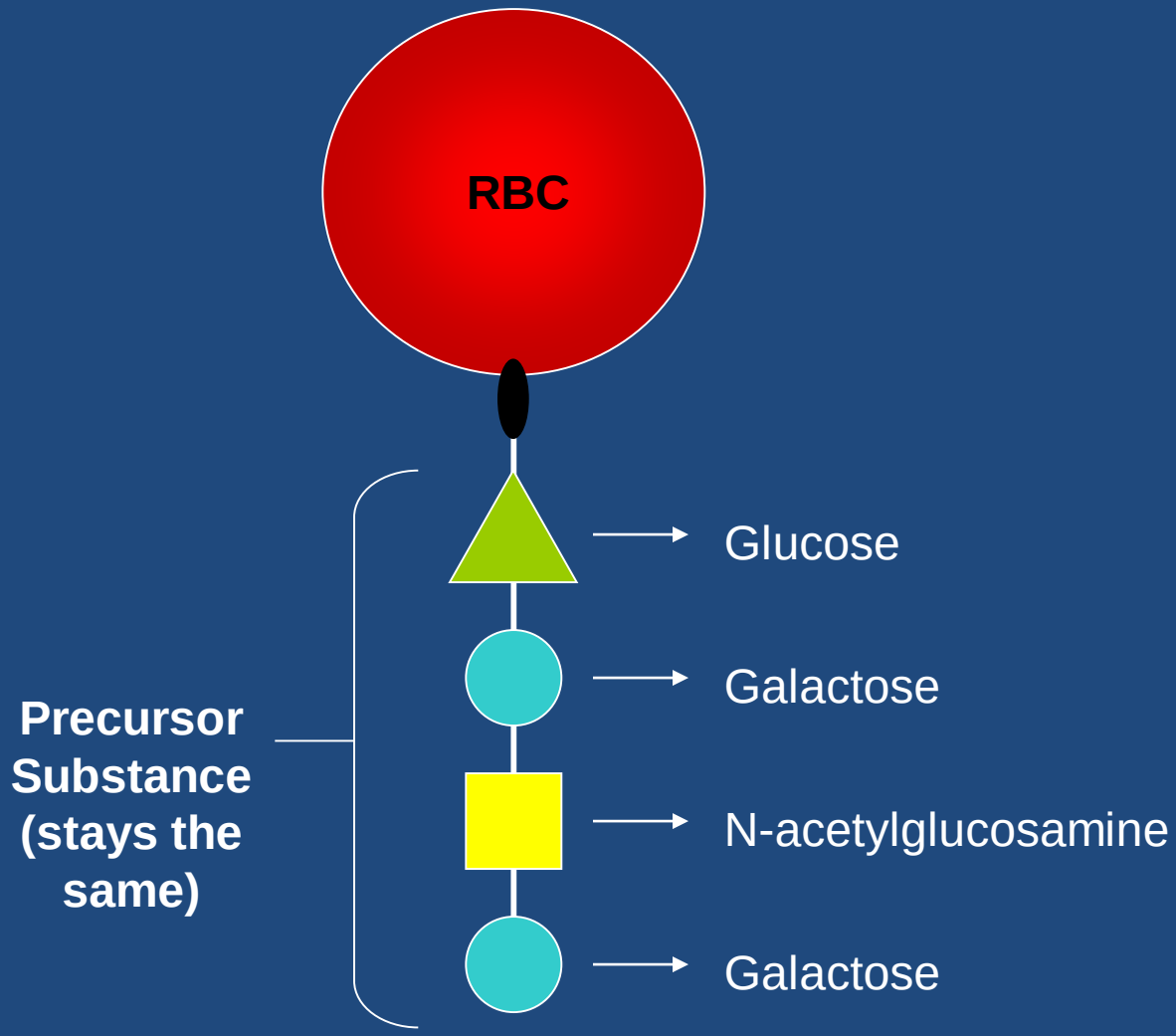
ABO and H Antigen Genetics

- Genes at three separate loci on chromosome number 9 control the occurrence and location of ABO antigens
- Presence or absence of the ABH antigens on the red cell membrane is controlled by the H gene
- Presence or absence of the ABH antigens in secretions is indirectly controlled by the Se gene
  - H gene – H and h alleles (h is an amorph)
  - Se gene – Se and se alleles (se is an amorph)
  - ABO genes – A, B and O alleles

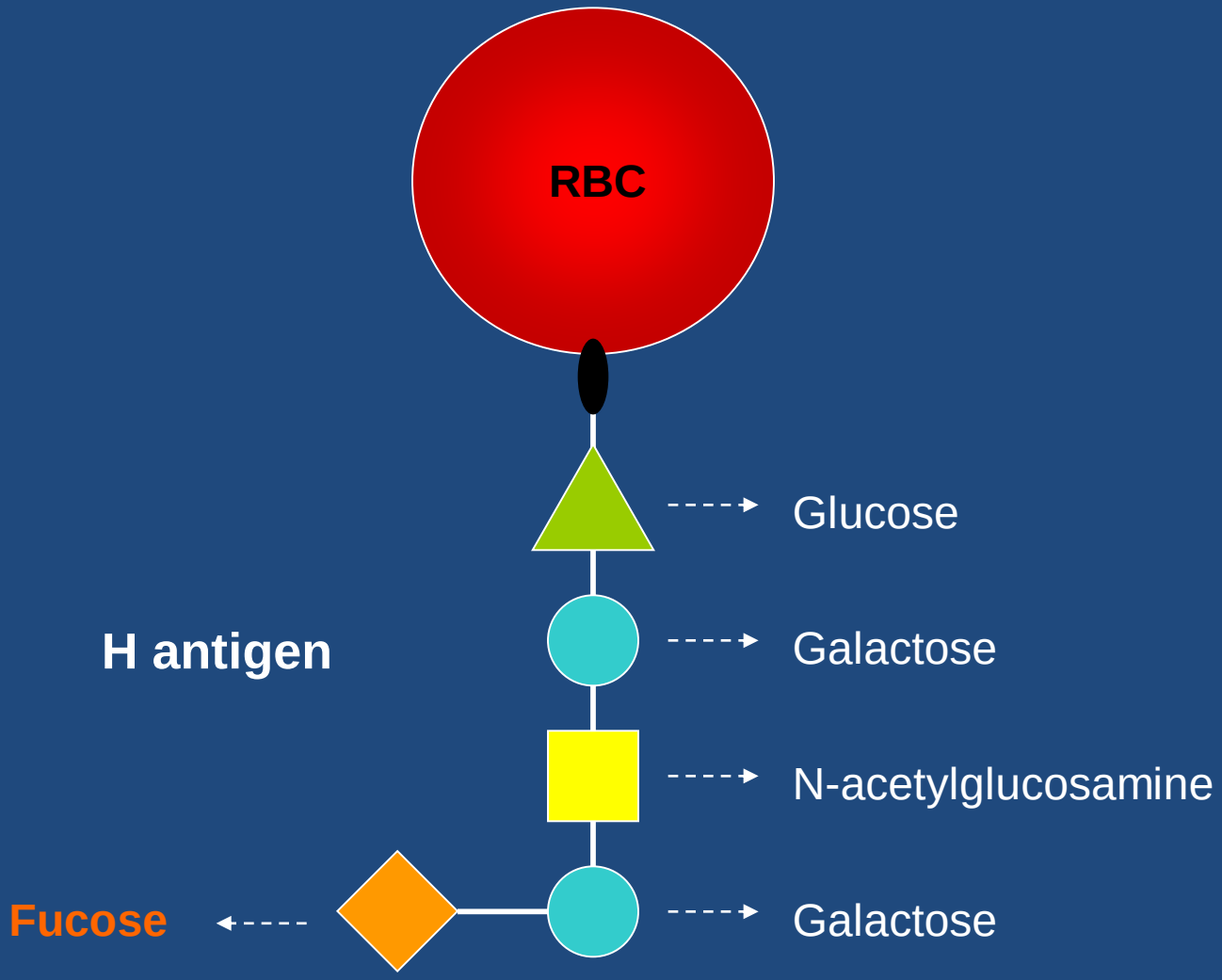
H Antigen

- The H gene codes for an enzyme (fucosyltransferase) that adds the sugar fucose to the terminal sugar of a precursor substance
- The precursor substance (proteins and lipids) is formed on an oligosaccharide chain (the basic structure)
- The H antigen is the foundation upon which A and B antigens are built
- A and B genes code for enzymes that add an immunodominant sugar to the H antigen
  - Immunodominant sugars are present at the terminal ends of the chains and confer the ABO antigen specificity

RBC precursor substance



Formation of the H antigen

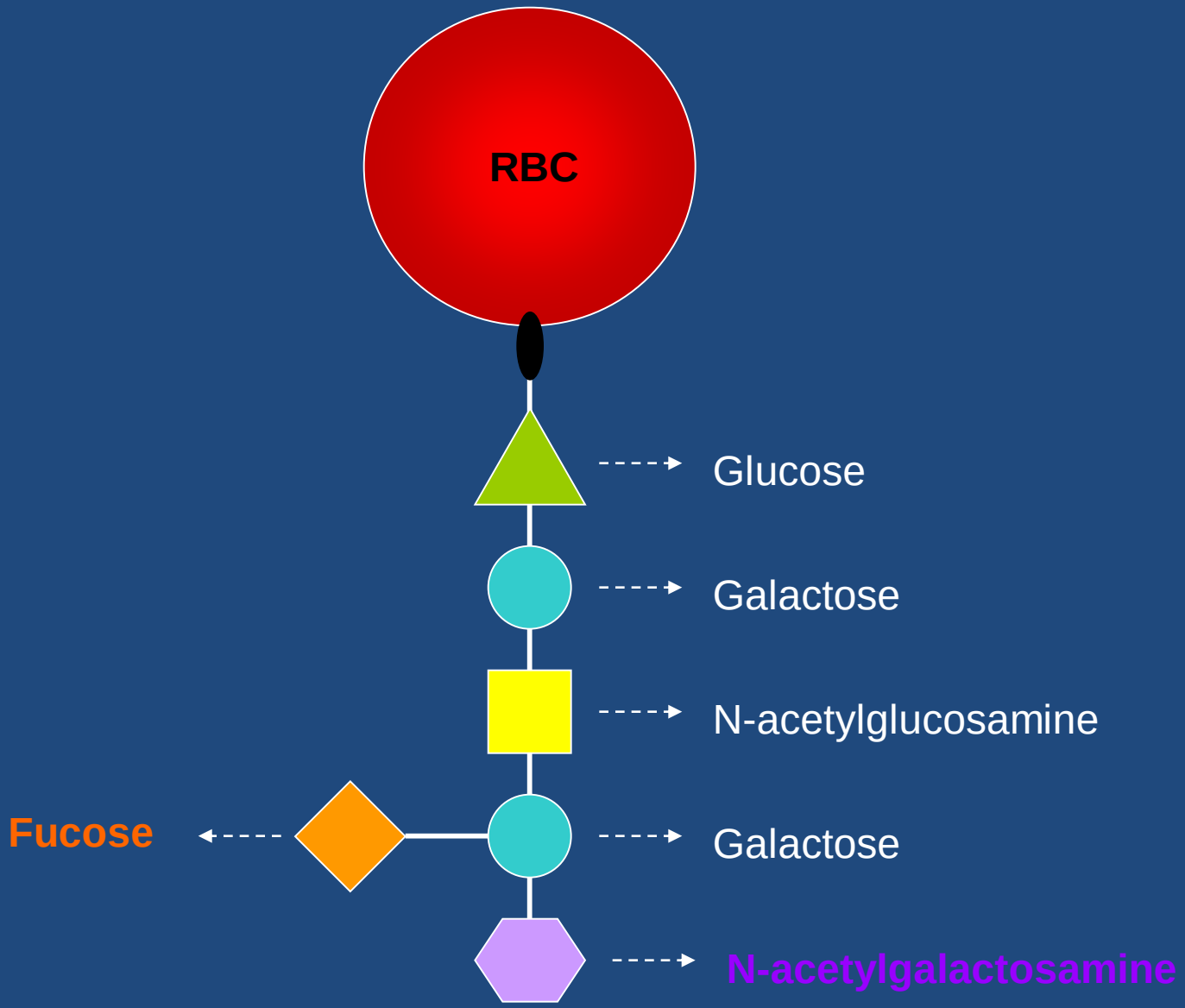


A and B Antigen

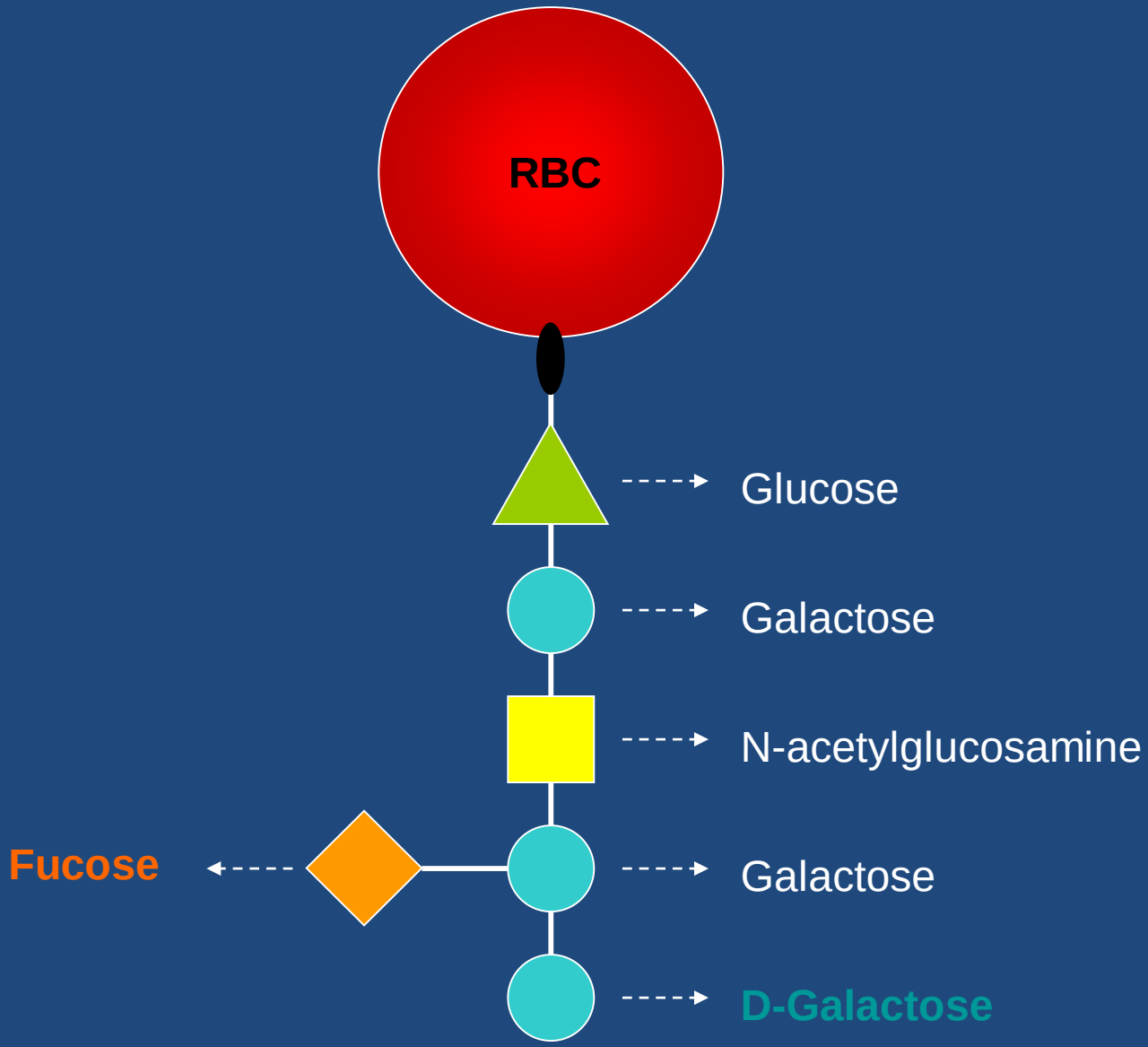
- The "*A*" gene codes for an enzyme (transferase) that adds N-acetylgalactosamine to the terminal sugar of the H antigen
  - N-acetylgalactosaminyltransferase

- The "*B*" gene codes for an enzyme that adds D-galactose to the terminal sugar of the H antigen
  - D-galactosyltransferase

Formation of the A antigen

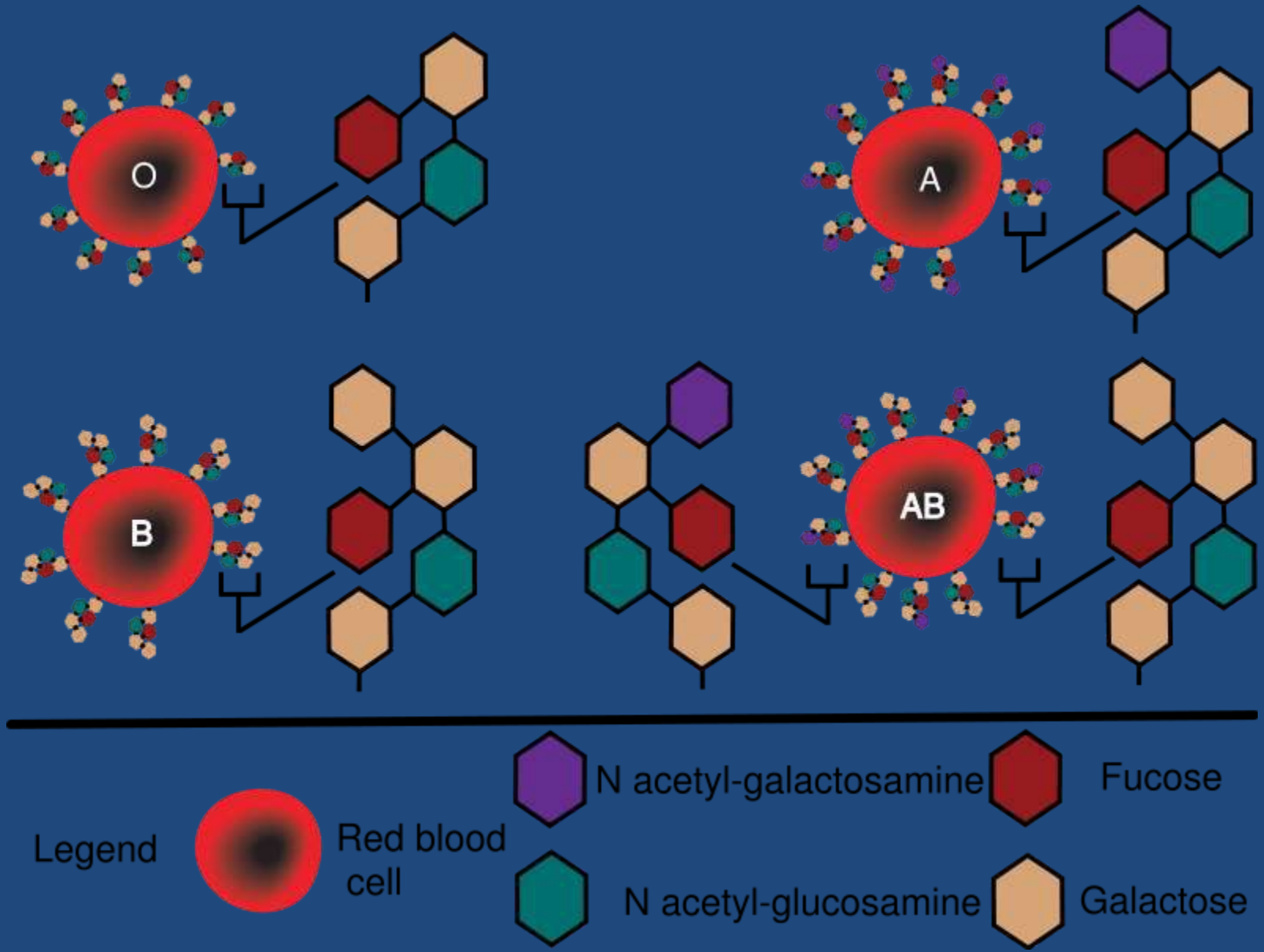


Formation of the B antigen



Immunodominant sugars responsible for antigen specificity

Gene	Glycosyltransferase	Immunodominant sugar	Antigen
H	L-fucosyltransferase	L-fucose	H
A	N-acetylgalactosaminyltransferase	N-acetyl-D-galactosamine	A
B	D-galactosyltransferase	D-galactose	B


O
A
B
AB
Legend
Red blood cell
N acetyl-galactosamine
Fucose
N acetyl-glucosamine
Galactose

Secretor Status

- A, B, H substances are found in all body secretions (except CSF) in 80% of individuals
- Ability to secrete these substances is determined by the presence of secretor gene (Se) in either homozygous (SeSe) or heterozygous (Sese) state.

Blood Group	Substances Secreted
O	H
A	A & H
B	B & H
AB	A, B, & H
Oh	Nil

Characteristics of Bombay Phenotype

- First reported by Bhende et al in Bombay in 1952.
- Frequency estimated to be about 1 in 7600 in Bombay.
- Absence of H, A & B antigens. No agglutination with anti-A, anti-B or anti-H
- Presence of anti-H, anti-A and anti-B in the serum
- No A, B or H substances present in saliva
- Incompatible with any ABO blood groups, compatible with Bombay phenotype only
- A recessive mode of inheritance (identical phenotypes in children but not in parents)

ABO Subgroups

- ABO subgroups differ in the amount of antigen present on the red blood cell membrane
  - Subgroups have less antigen

- Subgroups are the result of less effective enzymes. They are not as efficient in converting H antigens to A or B antigens (fewer antigens are present on the RBC)

- Subgroups of A are more common than subgroups of B

Subgroups of A

- Two principle subgroups of A are: A_1 and A_2
- Both react strongly with reagent anti-A
- To distinguish A_1 from A_2 red cells, the lectin Dolichos biflorus is used (anti-A_1)
- 80% of group A or AB individuals are A_1 and A_1B
- 20% are A_2 and A_2B

A_2 phenotype

- Clinical significance of A_2 phenotype
  - 8% of A_2 and 25% of A_2B individuals may produce anti-A_1 in the serum
  - This may result in discrepancy in blood grouping or incompatibility in cross match
  - However, these anti-A_1 antibodies are cold reacting & therefore may not cause problems routinely.
- Difference between A_1 and A_2
  - It is quantitative
  - The A_2 gene doesn't convert the H to A very well resulting in fewer A_2 antigen sites compared to the many A_1 antigen sites

A_1 and A_2 Phenotypes

	Anti-A	Anti-A_1	Anti-H	Antibody in serum	Antigens / RBC
A_1	4+	4+	0	Anti-B	9×10^5
A_2	4+	0	3+	Anti-B & Anti-A_1	2.5×10^5

Practical aspects of ABO grouping

- Routine ABO grouping must include both cell & serum testing as each test serves as a check on the other
- Test should be done at room temperature or lower; testing at 37^{o}C weakens the reactions
- Tubes, slides should be dry and labeled properly
- Serum should always be added before adding cells
- Results should be recorded immediately after observation
- Hemolysis is interpreted as positive result

Blood Grouping

- There are 2 components to blood typing:
  - Test unknown cells with known antibodies
  - Test unknown serum/plasma with known red cells
- The patterns are compared and the blood group is determined.

Blood Sample for Blood Grouping

Blood sample

- Clearly labeled blood samples in sterile tubes (plain & EDTA)
- Test should be performed on the fresh sample for best results. In case the test can not be performed immediately, sample can be stored at 4^{o}C & should be tested with in 48 hours
- No signs of hemolysis should be there
- If serum is not completely separated, centrifuge tube at 1000-3000 rpm fro 3 min
- Preferably use saline washed red cells and make 2-5% suspension

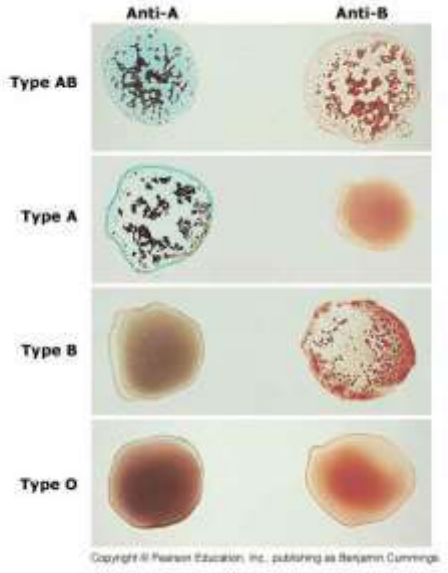
Slide Method for ABO Grouping

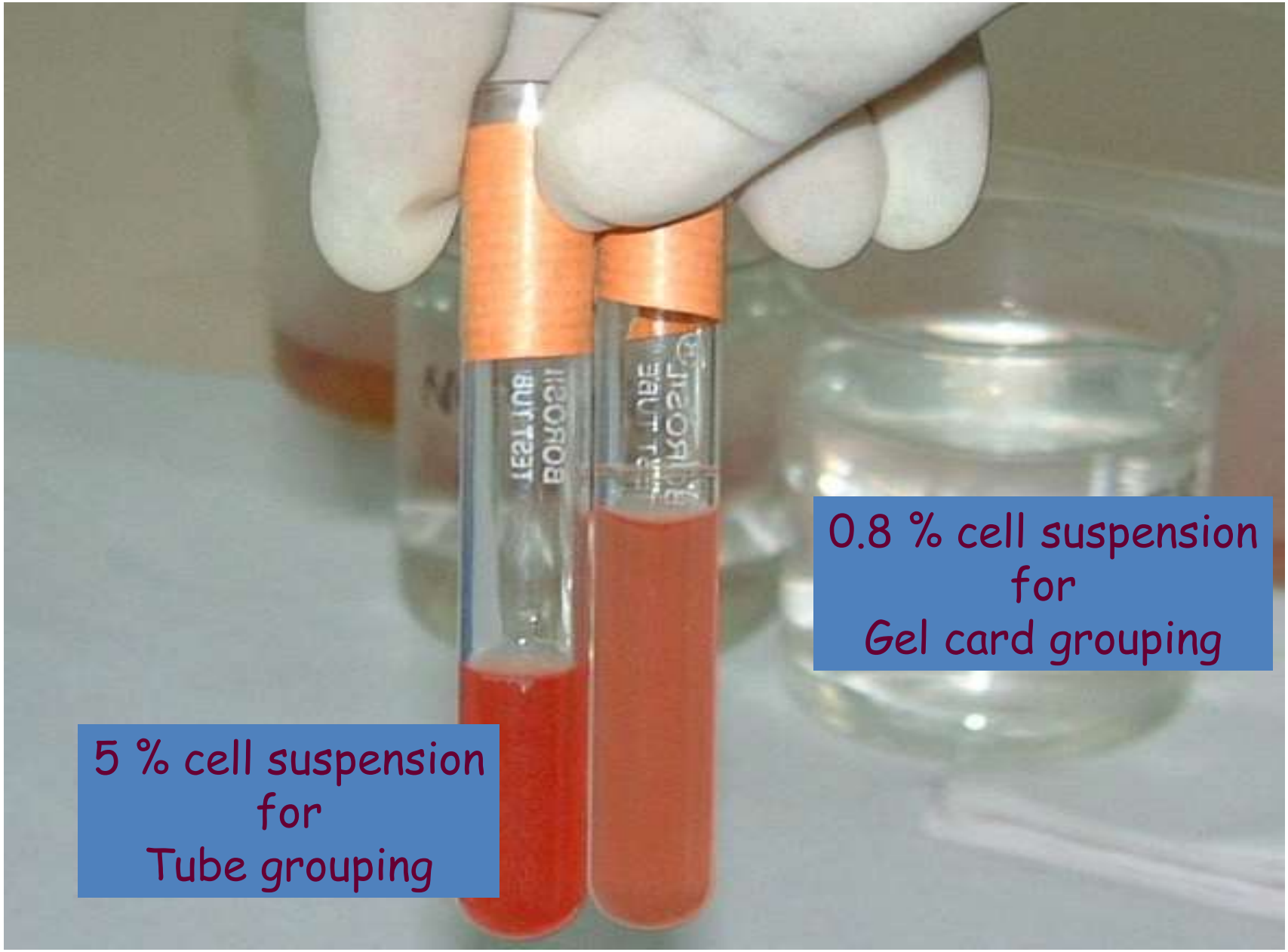
Not recommended as a routine method

- ✓ Very rudimentary method for determining blood groups.
- ✓ CANNOT be used for transfusion purposes as false positives and negatives do occur. Drying of reaction mixture can cause aggregation - **false positive**
- ✓ Less sensitive, not reliable for weakly reactive antigens and antibodies
- ✓ Can only be used for emergency ABO grouping or for selection of plateletpheresis donors

Slide Method for ABO grouping

- Put 1 drop anti-A & anti- B separately on slide
- Add 1 drop of 40-50% suspension of test red cells to each drop of typing antisera
- Mix & spread each mixture evenly on the slide over an area of about 15 mm diameter
- Leave the test for 2 min at room temp (20-24^{o}C)
- Record the results immediately




0.8 % cell suspension
for
Gel card grouping
5 % cell suspension
for
Tube grouping

Test Tube Method of ABO Grouping

Recommended method

- Allows longer incubation of antigen and antibody mixture without drying
- Tubes can be centrifuged to enhance reaction
- Can detect weaker antigen / antibody

Two steps in ABO grouping

Cell grouping (Forward grouping)

- Tests the patients red cells with known Anti-A & Anti-
  B to determine the antigen expressed

Serum grouping (Reverse grouping)

- Test the patients serum with known A & B cells to determine the presence of antibody

Lay Out of Tubes for ABO & Rh grouping

Forward grouping
Cell grouping

Rh grouping

Reverse grouping
Sera grouping

Forward Grouping

2 vol of anti- A / anti-B/ Anti-AB

+

1 vol of 2-5% red cell suspension

Incubate at room temp (20-24°C) for 5 min

↓

Centrifuge at 1000 rpm for 1 min

↓

Check for agglutination against well lighted background

2 vol of test serum/plasma

+

1 vol of 5% suspension of reagent red cells in respective tubes

Reverse Grouping

Shake & leave at room temp (20-24°C) for 5 min

Centrifuge at 1000 rpm for 1 min

Centrifuge & record the results similarly as for cell grouping

Tube Agglutination Grading

Tube Agglutination Grading Chart

Scale			Macroscopically Observed Findings
0-4	0-12		
4	12		One solid agglutinate, no free red cells detected.
3	10		One or two large agglutinates, a strong reaction.
2	8		Medium size agglutinates, clear background.
1	5		Small agglutinates, with a lot of free red cells.
+/–	3		Weak granularity in the red cell suspension.
0	0		No agglutinates, an even red cell suspension.

Recording results of ABO grouping

Reaction of red cells with			Reaction of serum with pooled cells			Interpretation
Anti-A	Anti-B	Anti-AB	Ac	Bc	Oc	
+	+	+	0	0	0	AB
+	0	+	0	+/H	0	A
0	+	+	+/H	0	0	B
0	0	0	+/H	+/H	0	O
0	0	0	+/H	+/H	+	Oh

+ = agglutination, 0 = no agglutination H = hemolysis

Rh Blood Group System

Rh system: Nomenclature

	Genotype			Frequency (%)
	Wiener	Fisher-Race	Rosenfield	(approx., White)
Common genotypes	R^1r	*DCe/dce*	*Rh:1,2,−3,4,5*	33
	R^1R^1	*DCe/DCe*	*Rh:1,2,−3,−4,5*	18
	rr	*dce/dce*	*Rh:−1,−2,−3,4,5*	15
	R^1R^2	*DCe/DcE*	*Rh:1,2,3,4,5*	11
	R^2r	*DcE/dce*	*Rh:1,−2,3,4,5*	9
	R^2R^2	*DcE/DcE*	*Rh:1,−2,3,4,−5*	2
Rarer genotypes	*r'r*	*dCe/dce*	*Rh:−1,2,−3,4,5*	1
	r'r'	*dCe/dCe*	*Rh:−1,2,−3,−4,5*	0.01
	r"r	*dcE/dce*	*Rh:−1,−2,3,4,5*	1
	r"r"	*dcE/dcE*	*Rh:−1,−2,3,4,−5*	0.03
	R^0r	*Dce/dce*	*Rh:1,−2,−3,4,5*	2
	R^0R^0	*Dce/Dce*	*Rh:1,−2,−3,4,5*	0.1
	r'r	*dCE/dce*	*Rh:−1,2,3,4,5*	rare

Rh (D) Antigen

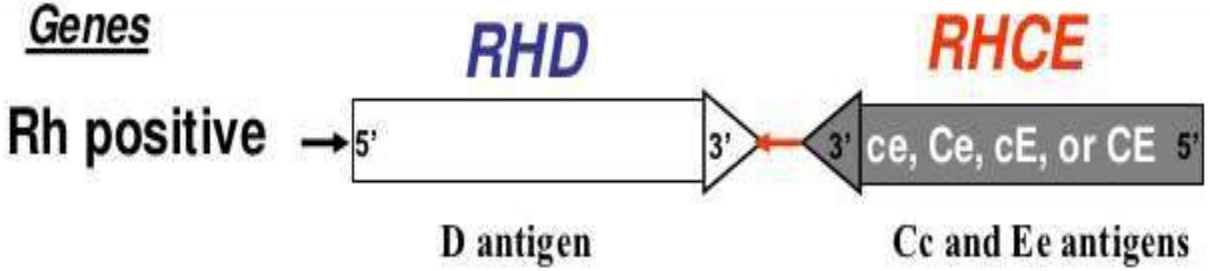
- Of next importance is the Rh type.
  - Rh is a blood group system with many antigens, one of which is D.
- Rh refers to the presence or absence of the D antigen on the red blood cell.
- Unlike the ABO system, individuals who lack the D antigen do not naturally produce anti-D.
- Production of antibody to D requires exposure to the antigen.
- The D antigen is very immunogenic, ie, individuals exposed to it will very likely make an antibody to it.
- For this reason all individuals are typed for D, if negative must receive Rh (D) negative blood.

Rh (D) Antigen (continued)

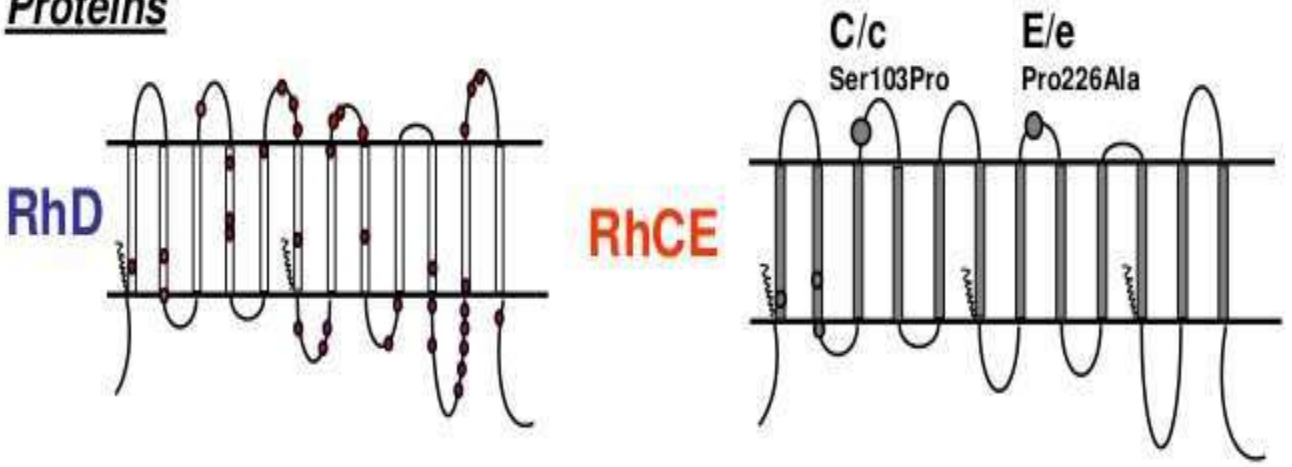
- Rh antigens are an integral part of the red cell membrane.
- They are protein in nature with an active phospholipid component
- Rh antigens do not exist in the soluble form and, therefore are not excreted in body fluids.
- Unlike ABO antigens, Rh antigens are present only on red blood cells. These antigens are not found on other blood cells including platelets and leukocytes

RHD and *RHCE* encode RhD and RhCE proteins

Genes



Proteins



RhD and RhCE differ by 32 to 35 amino acids

Adapted from: Westhoff CM., *Semin.Hematol.* 2007;44:42-50

Rh (D) Antigen (continued)

- A very potent antigen (50% may form antibody to exposure)
- Frequency in UK {Britain) population
  - 85% Rh positive
  - 15% Rh negative
  - ln lraq & Saudi Arabia ; 90 % Rh positive , 10 % Rh negative
- The most important patient population to consider is females of child-bearing age.
- If immunized to Rh (D) antigen the antibody can cross the placenta and destroy Rh (D) positive fetal cells resulting in death.
- This is why Rh negative women are given anti-D (Rhogam) after birth of Rh positive baby.

Rh Antibodies

- All Rh antibodies are immune in nature, developed after immunizing event
- React at 37^{o}C and require anti globulin test to demonstrate the reaction
- Generally do not react at room temperature in saline
- Most are IgG in nature and therefore can cross the placenta
- Generally, do not fix complement and cause extravascular hemolysis
- All are important in HDN and delayed HTR

Rh typing

- Normal typing for Rh antigens only includes typing for Rh (D).
- The result of this typing determines the Rh status of the cells (Rh - positive or Rh - negative).
- Some Rh typing sera is diluted in high protein solutions and may require a negative control.
- It is recommended to use two monoclonal anti-D sera from two different manufacturers labeled as D1 and D2, especially to confirm all Rh negatives

Types of anti- D

1. Polyclonal high protein – obsolete
2. Saline acting
3. Monoclonal antibody – mostly used now

Anti-D reagents

Polyclonal high protein

- Prepared from pooled human sera to which high concentration of protein (20-24% albumin) is added.
- Albumin, being a dipolar molecule, decreases the zeta potential, allowing the red cells to come closer
- Reaction with D positive red cells at IS & weak D at 37°C/AHG test
- Use control reagent & follow manufactures' directions
- It is now almost obsolete

Monoclonal Anti-D

Three types

1. IgM anti-D monoclonal reagent
2. Blend of IgM and IgG monoclonal antibodies reagent
3. Blend of monoclonal IgM and Polyclonal (human) IgG anti-D

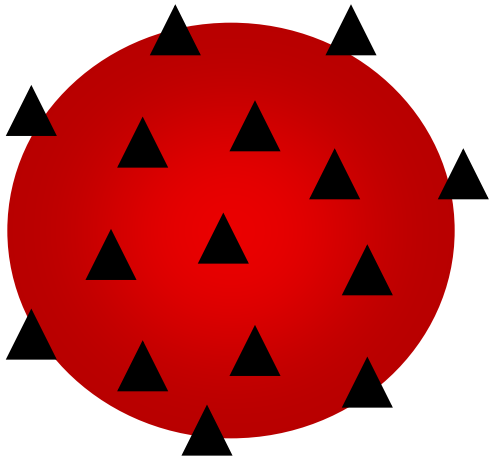
- IgM antibodies are highly specific and saline reacting equally at RT and 37^0 C but unreliable for detection of weak D
- Blended antibodies are now routinely used and can be used for detecting weak D

Tube Technique for Rh Typing

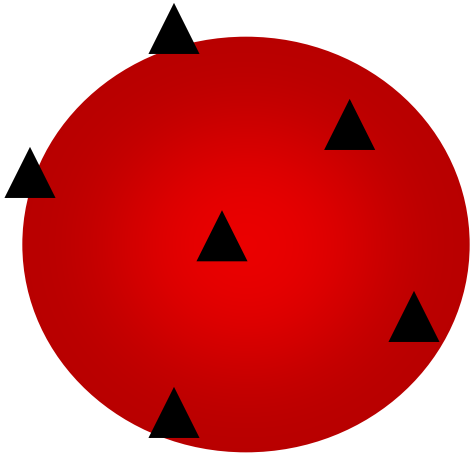
- Prepare 5% washed red cell suspension of test sample.
- Take two clean test tubes and label tubes 1 & 2 as “test” and “control”.
- Place 1 drop of anti-D in tube 1
- Place 1 drop of 22% bovine albumin in tube 2
- Add 1 drop of 5% test cell suspension to each tube.
- Mix well, centrifuge at 1000 rpm for 1 min.
- Resuspend cell button & look for agglutination
- Control tube should show no agglutination
- For all RhD negative test on blood donor, D^u test recommended

Weak D

Inheritance of D genes which result in lowered densities of D Antigens on RBC membranes, gene codes for less D.



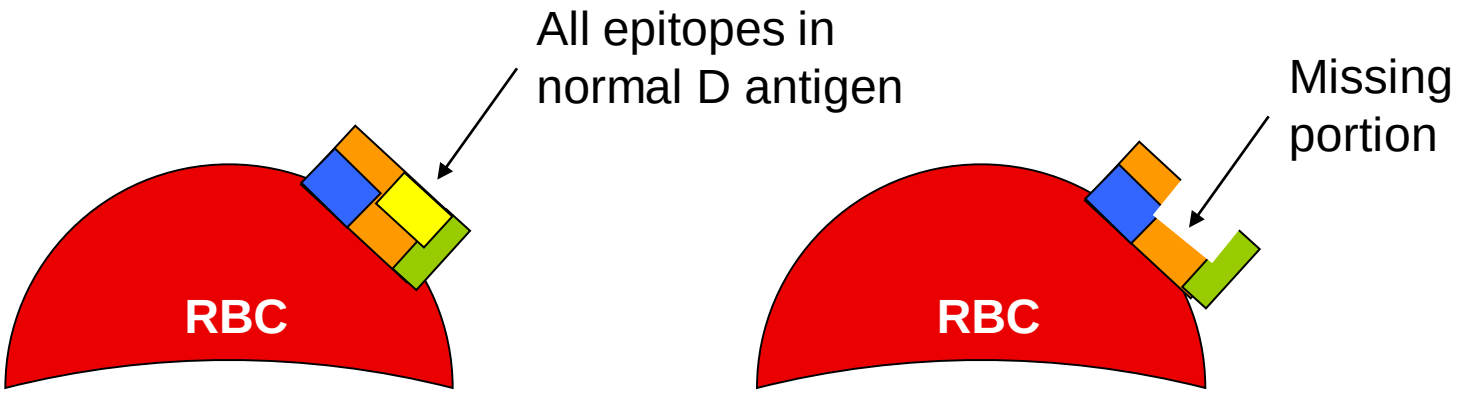
RBC with normal expression of D antigen



Decreased number of D antigens in Du

Partial D

- Absence of a portion of the total material that comprises the D antigen (qualitative defect)
- If the partial D patient is transfused with D positive red cells, they may develop an **anti-D alloantibody** to the part of the antigen (epitope) that is missing



Method for Weak D Testing

- Add 1 drop of 10% suspension of D negative red cells to a test tube and add 2 drops of Anti D (blend of IgG + IgM)
- Incubate at 37°C for 30 minutes.
- Wash three times with normal saline.
- Make dry red cell button and add polyspecific AHG reagent.
- Look for agglutination.

Results:

- If there is agglutination Du Positive.
- If there is no agglutination Du Negative.

Significance of Weak D

Donors

- Weak D testing on donors required.
- Labeled as D positive
- Weak D substantially less immunogenic than normal D
- Weak D has caused severe HTR in patient with anti-D

Patient.

- Weak D testing on patients not required.
- Standard practice to transfuse with D negative

Significance of Weak D (D^u)

- Weak D is much less antigenic in comparison to D, however, such red cells may be destroyed if transfused to a patient already having anti-D. Hence, weak D donor units are labeled as Rh positive.
- The weak D positive recipients are classified as Rh negative and safely transfused with Rh negative blood
- D^u positive infant can suffer from HDN if the mother possess anti-D antibodies
- Rh immunoprophylaxis is recommended for the Rh negative mother if the newborn is D^u positive.

Learning Outcome

- You should now be able to perform ABO & Rh grouping on the donor and recipients sample
- You should be able to resolve discrepancies in the blood grouping
- You should be able to perform weak D testing if required

Blood Groups

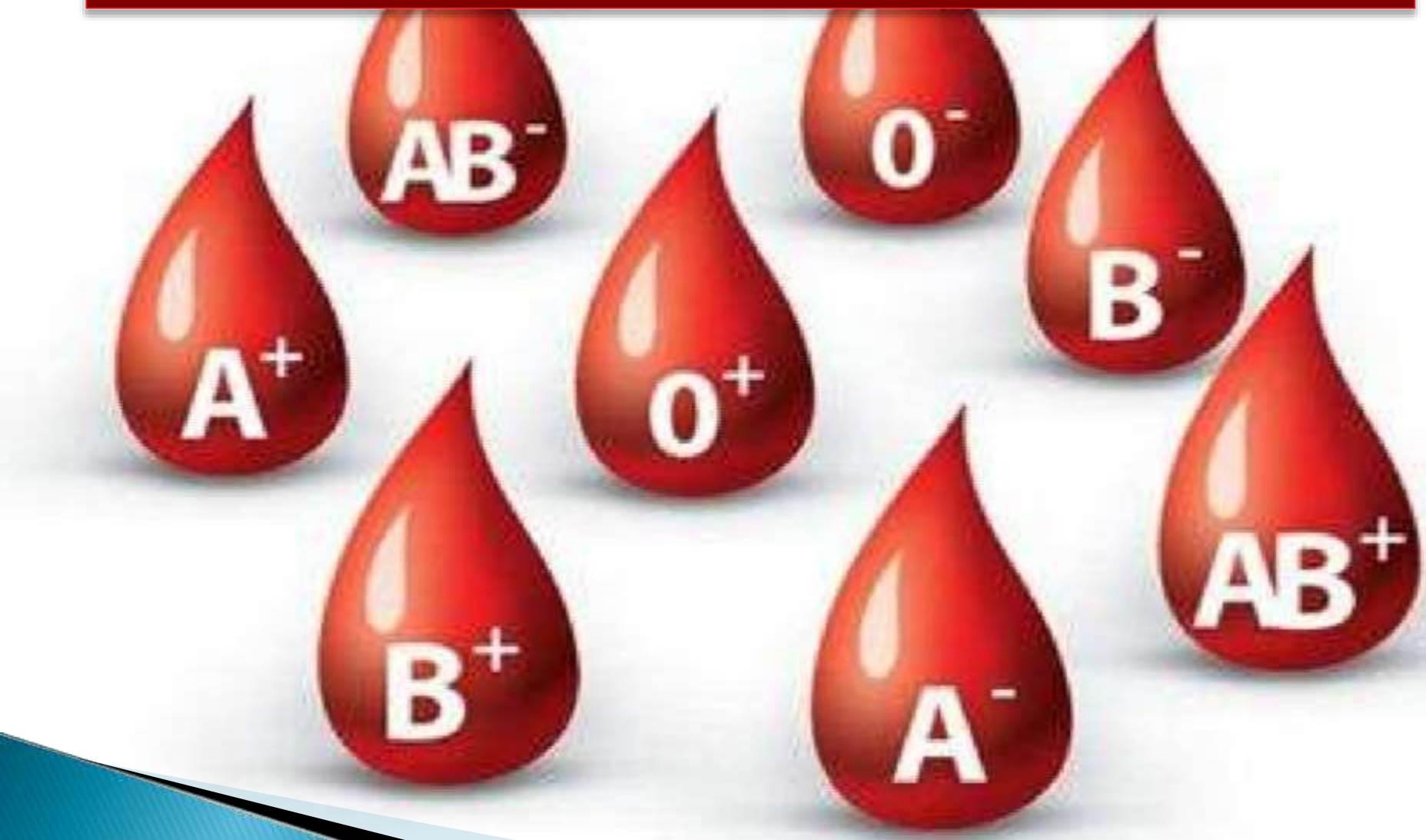
Contents

Introduction

Blood group systems

- ABO blood group system
- Rh blood group system

ABO Blood Group System



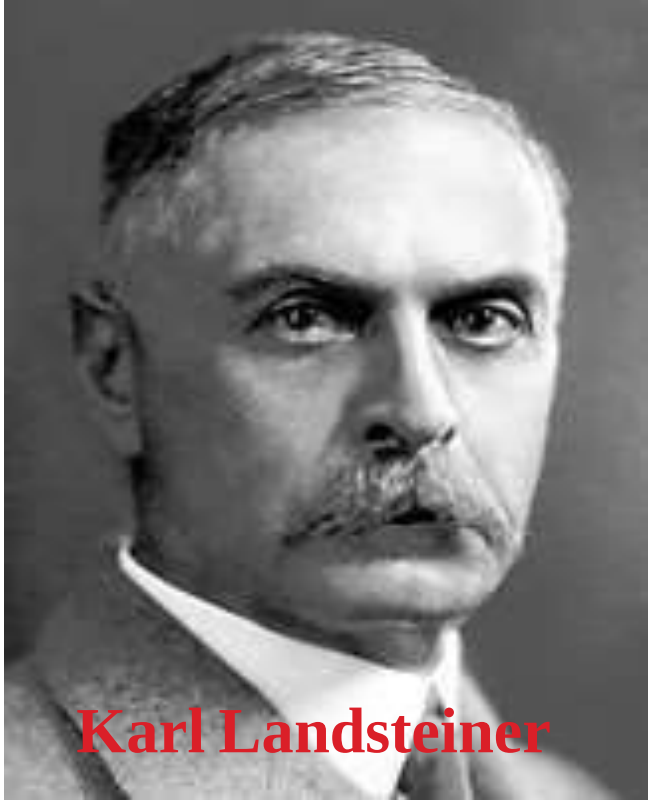
- The **ABO blood group system** is the most important blood type system (or blood group system) in human blood transfusion.

- ABO blood types are also present in some other animals
for example rodents and apes such as chimpanzees, and gorillas.

Determination of ABO blood groups depends upon the immunological reaction between antigen and antibody.

Antigens are also called agglutinogens because of their capacity to cause agglutination of RBCs.

History


Karl Landsteiner

- Karl Landsteiner discovered the ABO Blood Group System in 1901.
- Adriano Sturli and Alfred von Decastello

who were working under Landsteiner discovered type AB a year later in 1902

- Landsteiner was awarded the 1930 Nobel Prize in Physiology or Medicine for his work.

ABO BASICS

- Based on the presence or absence of antigen A and antigen B, blood is divided into four groups: ‘A, B, AB and ‘O’ group.
- Blood having antigen A belongs to ‘A’ group. This blood has β-antibody in the serum.

- Blood with antigen B and α-antibody belongs to ‘B’ group.
- If both the antigens are present, blood group is called ‘AB’ group and serum of this group does not contain any antibody.
- If both antigens are absent, the blood group is called ‘O’ group and both α and β antibodies are present in the serum.

Antigen and Antibody Present in ABO Blood Group

ABO Group	Antigen Present	Antigen Missing	Antibody Present
A	A	B	Anti-B
B	B	A	Anti-A
O	None	A and B	Anti-A&B
AB	A and B	None	None

Principle of Blood Grouping

- Blood grouping is done on the basis of agglutination.
- Agglutination means the collection of separate particles like RBCs into clumps or masses.
- Agglutination occurs if an antigen is mixed with its corresponding antibody which is called *isoagglutinin*, i.e. occurs when A antigen is mixed with anti-A or when B antigen is mixed with anti-B.

IMPORTANCE OF ABO GROUPS IN BLOOD TRANSFUSION

During blood transfusion, only compatible blood must be used.

The one who gives blood is called the '**donor**' and the one who receives the blood is called '**recipient**'.

While transfusing the blood, antigen of the donor and the antibody of the recipient are considered.

The antibody of the donor and antigen of the recipient are ignored mostly.

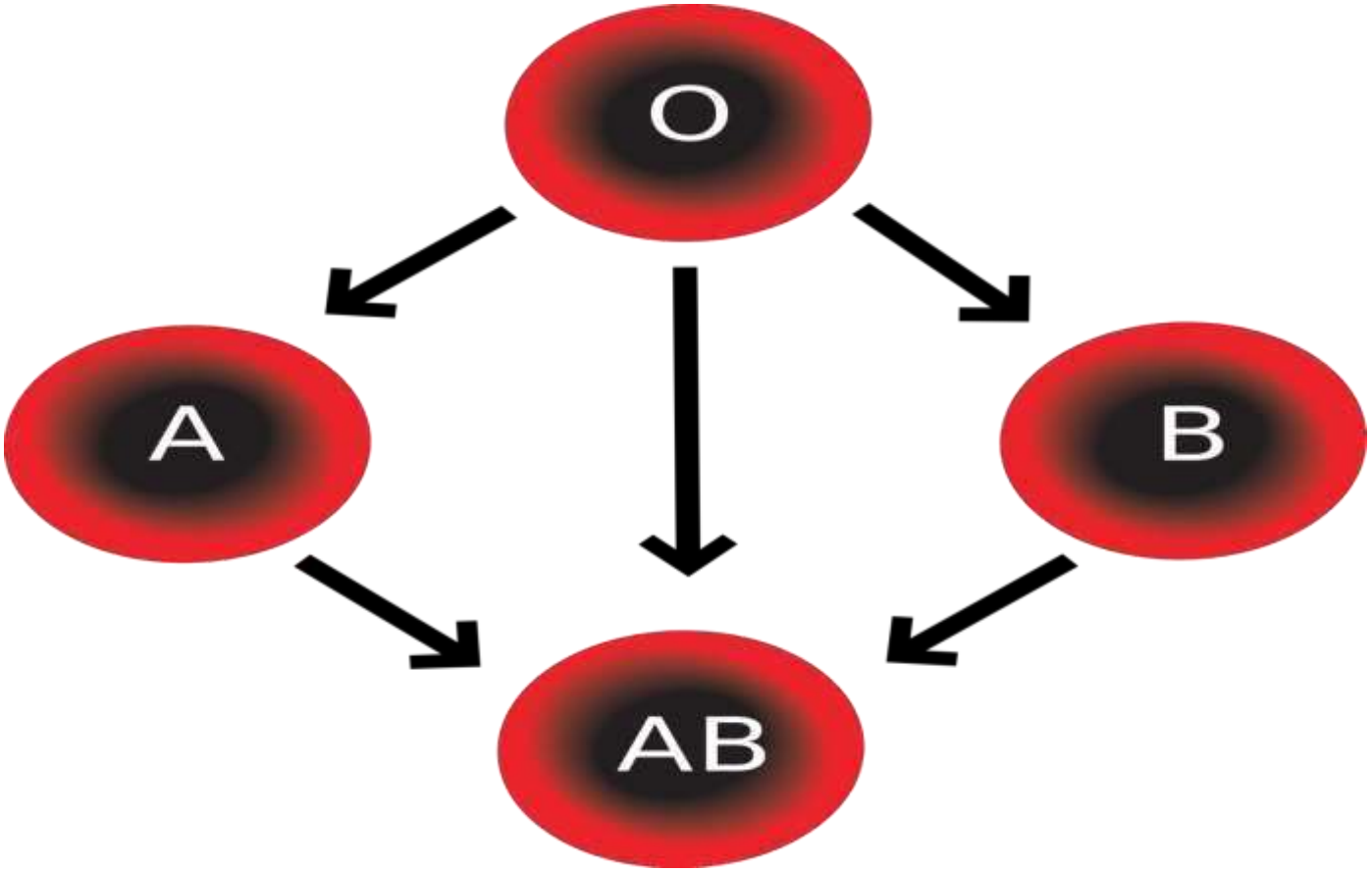
Thus, RBC of ‘O’ group has no antigen and so agglutination does not occur with any other group of blood. So, ‘O’ group blood can be given to any blood group persons and the people with this blood group are called **‘universal donors’.**

Plasma of AB group blood has no antibody. This does not cause agglutination of RBC from any other group of blood.

People with AB group can receive blood from any blood group persons. So, people with this blood group are called **‘universal recipients’.**

In mismatched transfusion,
the transfusion reactions occur between donor’s RBC and recipient’s plasma.
So, if the donor’s plasma contains agglutinins against recipient’s RBC, agglutination does not occur because these antibodies are diluted in the recipient’s blood.

Blood Compatibility



TRANSFUSION REACTIONS DUE TO ABO INCOMPATIBILITY

Transfusion reactions are the adverse reactions in the body, which occur due to transfusion error that involves transfusion of incompatible (mismatched) blood.

The reactions may be mild causing only fever and hives (skin disorder characterized by itching) or may be severe leading to renal failure, shock and death.

ANTICOAGULANTS USED IN HAEMATOLOGY

DEFINITION

- Anticoagulant is an agent that is used to prevent the formation of blood clots. Anticoagulants have various uses.
- Some of them occur naturally in blood-eating animals such as leeches and mosquitoes,
- Some are used for the prevention or treatment of disorders characterized by abnormal blood clots and emboli.

CHARACTERISTICS OF ANTICOAGULANTS

- An anticoagulant selected for use in hematological examination must have the following qualities
- 1. it must not alter the size of the cell
- 2. it must not cause hemolysis
- 3.it must minimize platelet aggregation
- 4.it must minimize disruption of staining and morphology of leukocytes
- 5. it must be readily soluble in water
- 6.it should be soluble in blood
- 8. It must be keep the blood in fluid condition

Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Red 	No additive	•Used for blood bank, some biochemistry Invst. •Collection of serum •10-15 min is required to allow blood to clot before centrifugation
Lavender (purple) 	EDTA	•Collection of whole blood (binds calcium)

Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Green 	Sodium or lithium heparin	•Inhibits thrombin activation. •chemistry studies
Light blue 	Sodium citrate	•Coagulation studies (bind calcium) (PT &PTT) (ESR).

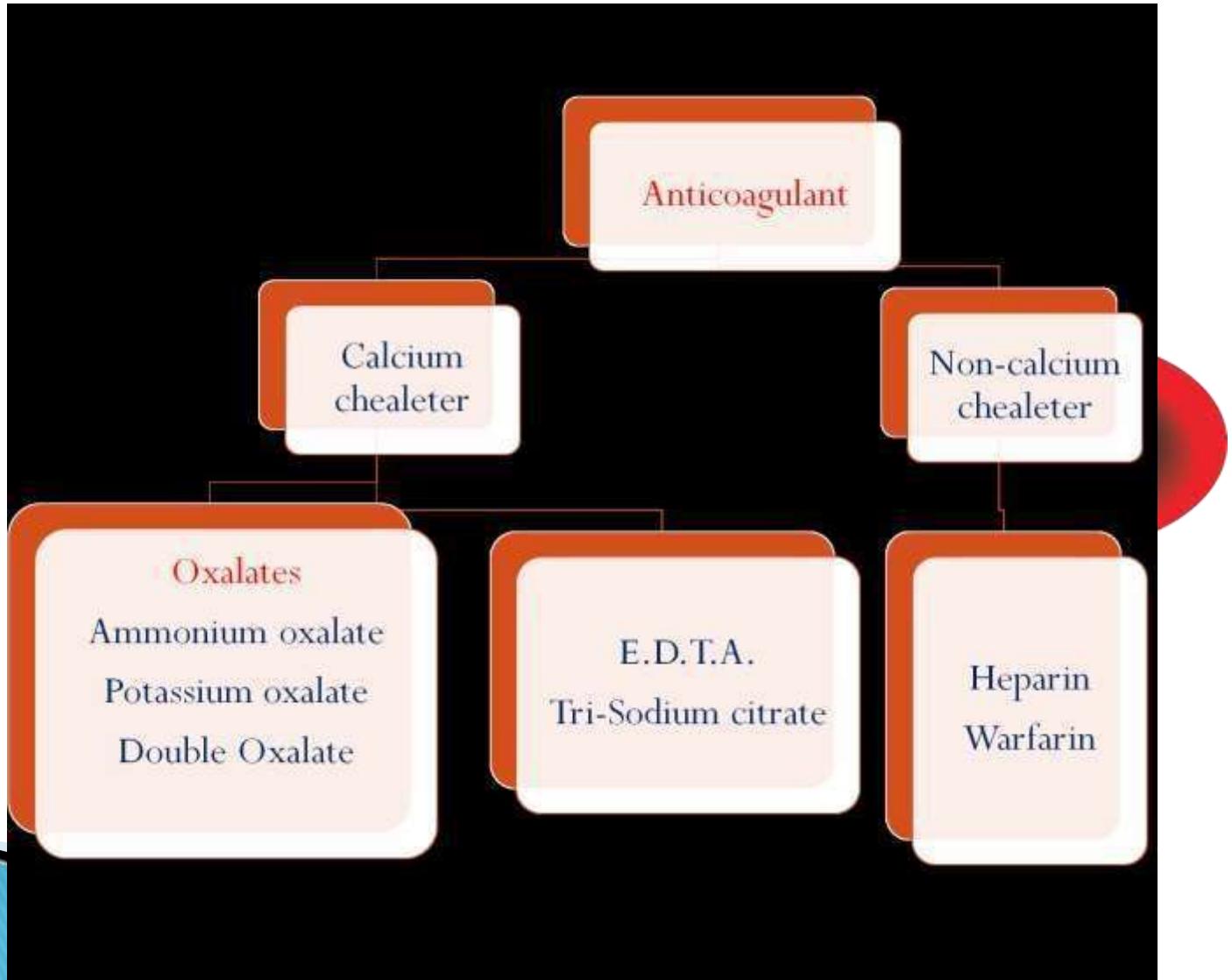
Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Gray 	•**Sodium fluoride & potassium oxalate**: inhibits enolase (phosphopyrovate dehydrogenase) •**Sodium iodoacetate**: inhibits glocose-3-phosphate dehydrogenase	•For glucose determination in chemistry (stabilize glucose in plasma)
	Acid citrate dextrose (ACD)	•For use in blood bank studies, HLA phenotyping, DNA and paternity testing (preserves red cells)

SST (SERUM SEPARATOR TUBE)

- No additives.
- Clotting accelerator and separation gel.
- Uses: Chemistry, Immunology, and Serology.

CLASSIFICATION OF ANTICOAGULANTS



O F ANTICOAGULANTS

- 1. calcium chelaters
- 2.Non- calcium chelaters

calcium chelators

- 1. Ammonium oxalates
- 2.Potassium oxalates
- 3.Double Oxalate
- 4. EDTA
- **Citrates**
- A. Sodium citrate
- B. ACD (Acid Citrate Dextrose)

Non Calcium chelators

- A. Sodium Heparin
- B. Warfarin

COMMONLY USED ANTICOAGULANTS

- 1. EDTA
- 2.OXALATE
- 3.SODIUM HEPARIN
- 4.SODIUM CITRATE
- 5.SODIUM FLUORIDE & POTASSIUM OXALATE

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- EDTA is the most frequently used anticoagulant , also known as sequestrene or Versenate. It is an amino carboxylic acid and a colorless , water-soluble solid.
- **Types/ Forms of EDTA** :
- Routinely used are ….
- 1. Tri potassium salts…EDTA (K3 EDTA)
- 2 Di sodium EDTA (Na2 EDTA)

Example 3

- Causes significant shrinking of the red cells with a decrease of 1–2% in the MCV.
- K_2EDTA in a concentration of 1.5–2.2 mg/ml (4.55 ± 0.8 mmol/ml) as this cause less cellular change[5].

K_3EDTA	K_2EDTA

Reference:
(5) Bachmann F. Molecular aspects of plasminogen, plasminogen activators and plasmin, in Bloom EGD (eds): Haemostasis and Thrombosis. Edinburgh, Churchill Livingstone, 1994, p 575-613

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Mode of Action :**
- It forms insoluble calcium salts by chelation

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Concentration :**
- Eg; 0.5 – 2.0 mg EDTA per/ ml of blood will preserve blood excellently for at least 6 hrs.

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Advantages :**
- Making a blood smear for cell morphology studies.
- used for Tests for CBC, microfilaria,malaria, Coombs test(Direct Coombs), HbA1C (السكر التراكمي)
- EDTA preserves the staining and morphology of Leukocytes

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Disadvantages :**
- Excessive conc% of EDTA will cause shrinkage of RBC's and erroneous PCV, MCV, and MCHC results.
- EDTA interferes with blood chemistry tests as follows Falsely decreases alkaline phosphates by binding Mg^{++}
- Decreases CO_2 combining power of blood.
- Interferes with jaffes reaction for creatinine test
- Decreases or alters Na+. K+, and Ca^{2++}conc % in plasma

OXALATES

- **Mode of Action :**
- These acts by chelating calcium . Calcium oxalate is formed as insoluble precipitate , these are used for blood chemistry and hematocrit.

- Types :
- A. Potassium oxalate
- B. Ammonium oxalate

POTASSIUM OXALATE

- **Concentration :**
- This is used at conc.% of 2 mg/ml of blood . This anticoagulants is most often used for chemical analysis.

POTASSIUM OXALATE

- **Disadvantages :**
- Potassium oxalate shrinks the RBC , about an 8% shrinkage in the PCV and therefore it is not recommended for use with blood for PCV and ESR not recommended.

DOUBLE OXALATES

- Double oxalates used for ESR and HCT
- **Concentration :**
- Potassium oxalate and ammonium oxalate are used together in a ratio 2:3, this is done to counter the swelling effect of ammonium oxalate and shrinkage effect of potassium oxalate on the RBC

DOUBLE OXALATES

- **Advantages :**
- Double oxalates can be used for …
- A. HB
- B. TLC
- C. RBC count
- D. ESR by Wintrobes method

DOUBLE OXALATES

- **Disadvantages:**
- Leukocyte morphology is not well preserved
- The calcium chelated is precipitated in calcium oxalate which is a toxic substance, it is never to be used for blood banking application.

Heparin

- **Uses:**

  -naturally occuring biological anti-coagulant

  -Used in hematological special tests, biochemistry for electrolytes : Osmotic fragility test
  LE cell phenomenon (SLE)

  -For blood gases

  -Transfusions

- **Action:**

  -Inhibition of enzymes involved in coagulation

  eg: Anti thrombin III

  -Inhibits action of thrombin on fibrinogen and formation of thromboplastin

- **Disadvantages:**

  -expensive

  -highly acidic-blue colouration in smear

HEPARIN

- It is a natural anticoagulant in the body, found in the liver, and may also be with in basophils and mast cells, heparin also called anti thromboplastin or antithrombin.
- It is available in a liquid or dry form as sodium, calcium, ammonium and lithium salt, Each of these will interfere with determination of their respective ions in the plasma

HEPARIN

- **Mode of Action :**
- It interferes with the formation and or activity of thrombin and the activity of clotting factors IX, X, XI, XII

HEPARIN

- **Concentration:**
- The optimum con% is 0.1-.2 mg/ml of blood.

HEPARIN

- **Advantages :**
- Heparin is the choice of Anticoagulant for blood pH,and blood gas Analysis. Acid base balance.
- It may be used for special trace elements studies and some cytology .
- Excessive heparin does not alter the RBC volume

HEPARIN

- **Disadvantages :**
- It causes clumping of leukocytes
- It interferes with staining of leukocytes.
- It is the most expensive of the anticoagulant
- Blood clot in 8-12 hrs because clotting is only delayed and not prevented.
- It is not suitable for agglutination tests , and coagulation studies
- It may interfere with some automated biochemical analysis of plasma.

SODIUM CITRATE

- The formal citrate solution (Dacies solution) is used as diluent in the counting of RBCs and PLT’s
- **Concentration :**
- 3.13 grms of Trisodium citrate is dissolved in 100 ml of water, 1 ml of formaldehyde is added to every 99 ml of the solution.

- **Sodium citrate :**
  - (1:9 ratio).
  - Anticoagulant: 3.2%
  - Mechanism: Calcium chelation.
  - Use: Coagulation studies and
  - platelet function.

- **BLACK:**

- Na citrate 1:4.

- 3.8% of sodium citrate

- Action: Remove calcium.

- Uses: Westergren –
  Erythrocyte Sedimentation Rate (ESR).

- **Mode of action :**
- It combines with calcium to form insoluble salt of calcium citrate

- **Advantages** :
- Sodium citrate is the anticoagulant for choice for studies of PLTs function and morphology

- **Concentration**:
- The standard concentration 1 part (3.8%) for 9 parts of blood

- **Disadvantages** :
- It interferes with many chemical tests
- Used alone it preserves blood for only few min.
- It has a tendency to shrink cells. Because of 10% dilution of blood – sodium citrate is generally not used for CBC

ACID CITRATE DEXTROSE (ACD)

- Is prepared from disodium hydrogen citrate and is the anticoagulant of choice for blood transfusion.

Eg; 2 grms of Na2 hydrogen citrate and 3 grms dextrose are added to 120 ml of water autoclaved for 30 min and used the ratio 1 part acid to 4 parts of blood

SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

- **Mode of Action** :
- Sodium fluoride inhibits the glycolytic enzymes responsible for the break down of glucose in the blood.
- (At RT. About 10% glucose is lost per hour from an untreated sample)
- The potassium oxalate is the primary anticoagulant as sodium fluoride has a poor anticoagulant effect.

SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

- **Concentration**:
- The optimum concentration : 1 mg of mixture per 1 ml of blood

- Uses: Glucose determination

SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

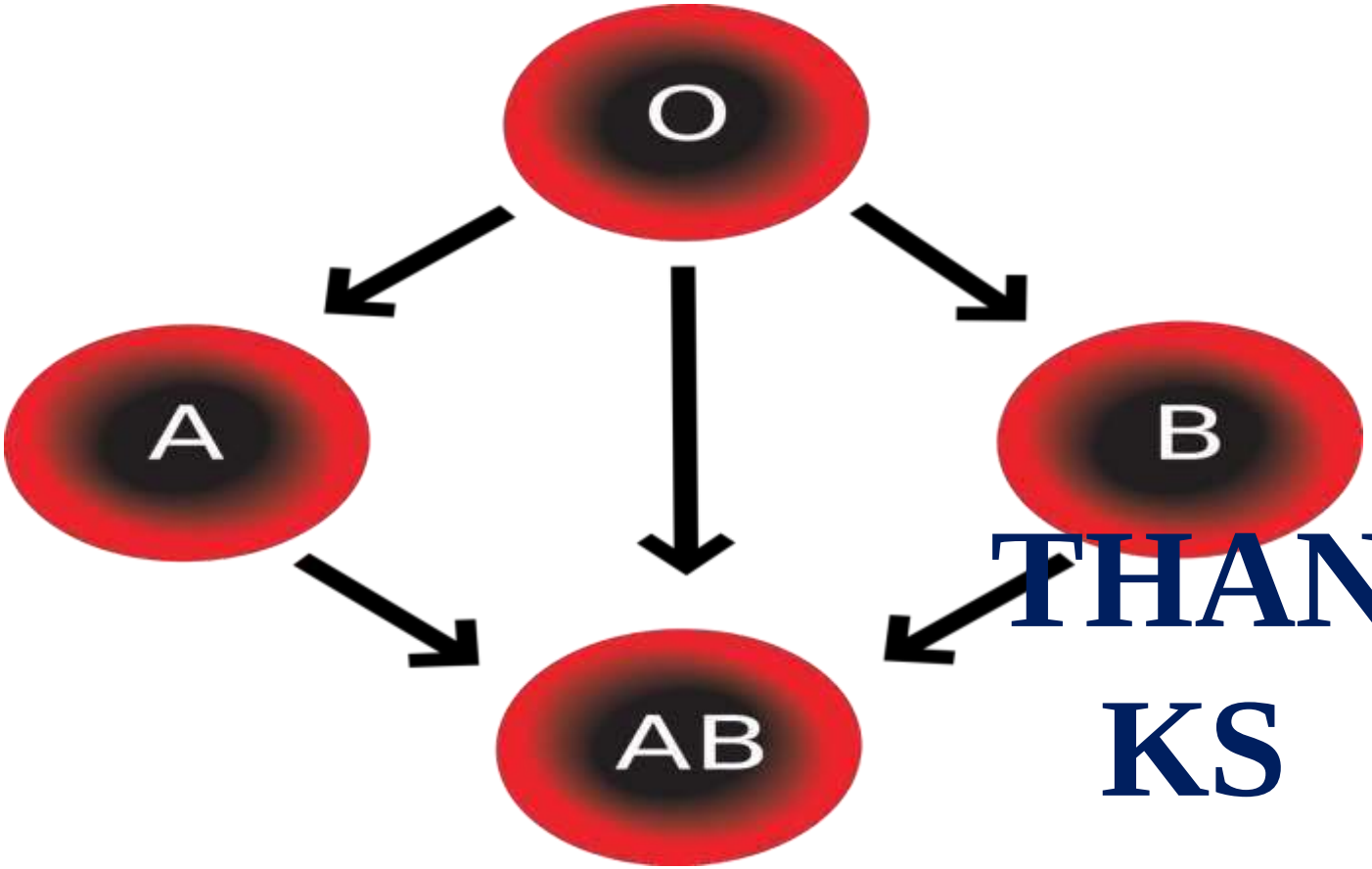
- **Disadvantages** :
- It is poisonous
- It inhibition of urease, and glycolytic enzymes may interfere with urea and glucose determinations that employ enzyme activity
- Alkaline phosphatase, amylase and uric acid cannot be determined in blood containing sodium fluoride

- **GREY:**

- Sodium fluoride +
  Potassium oxalate

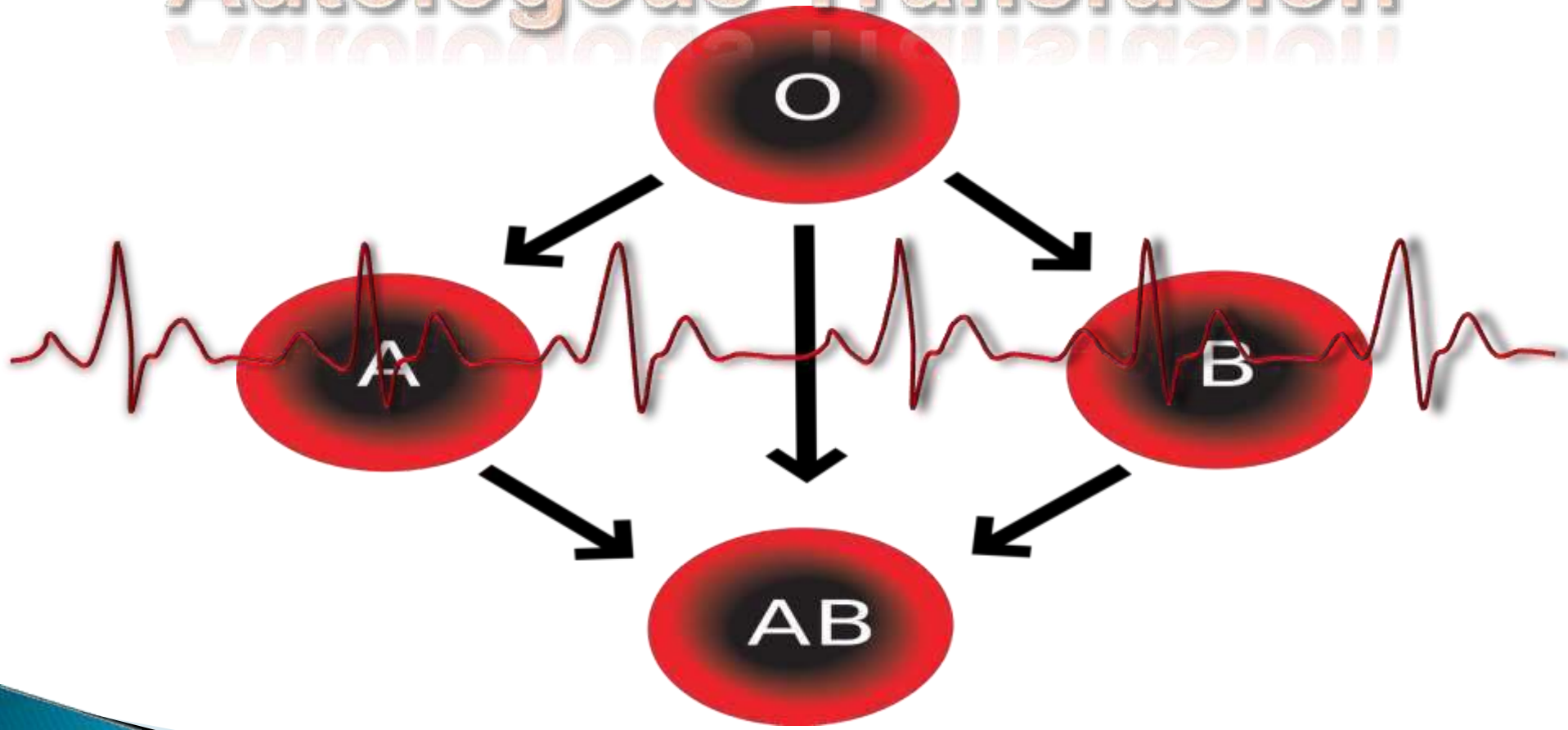
- MOA:
  inhibits red cell glycolytic pathway

- Used: blood glucose



THANKS

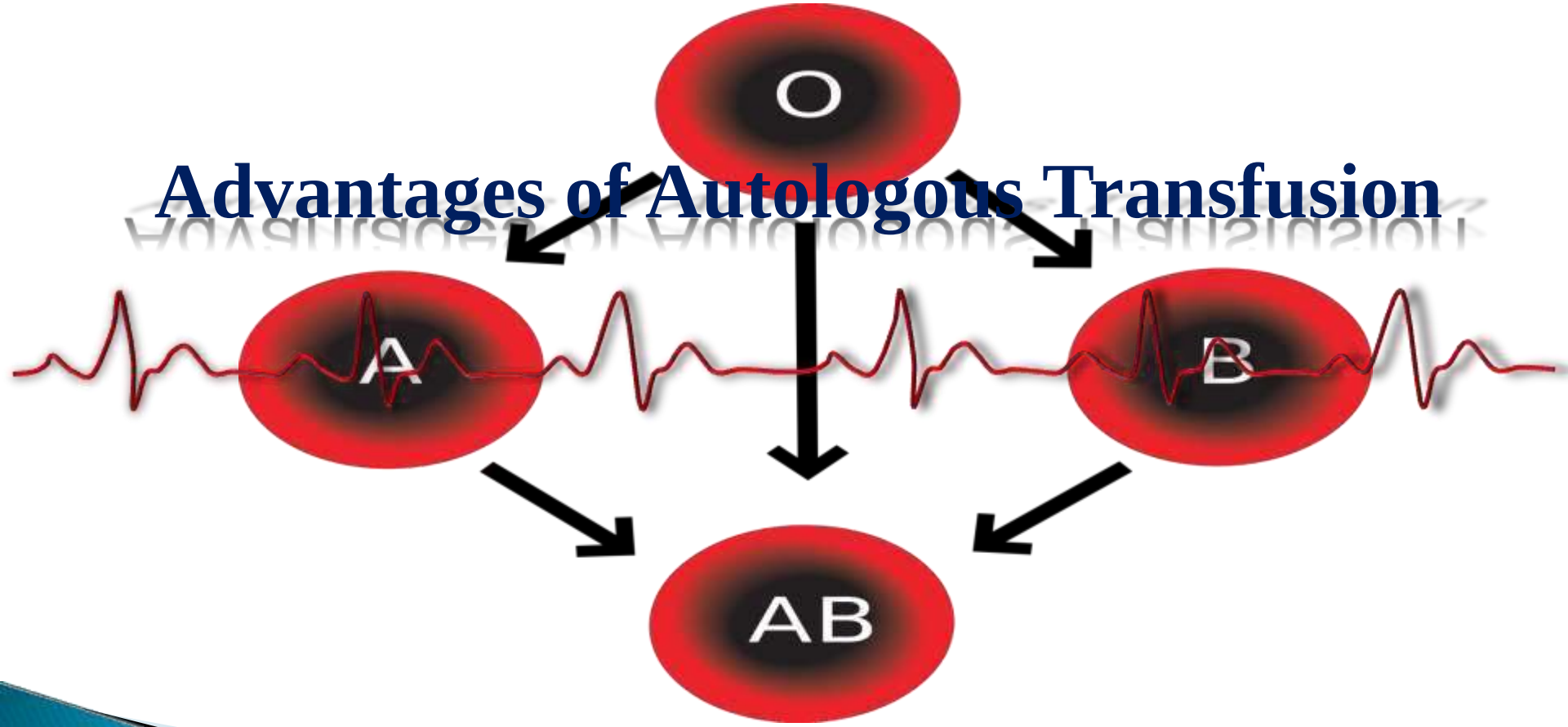
Autologous Transfusion



Introduction

- Autologous Blood Transfusion (ABT) means reinfusion of blood or blood products taken from the same patient

Advantages of Autologous Transfusion



Advantages of ABT

Avoiding many

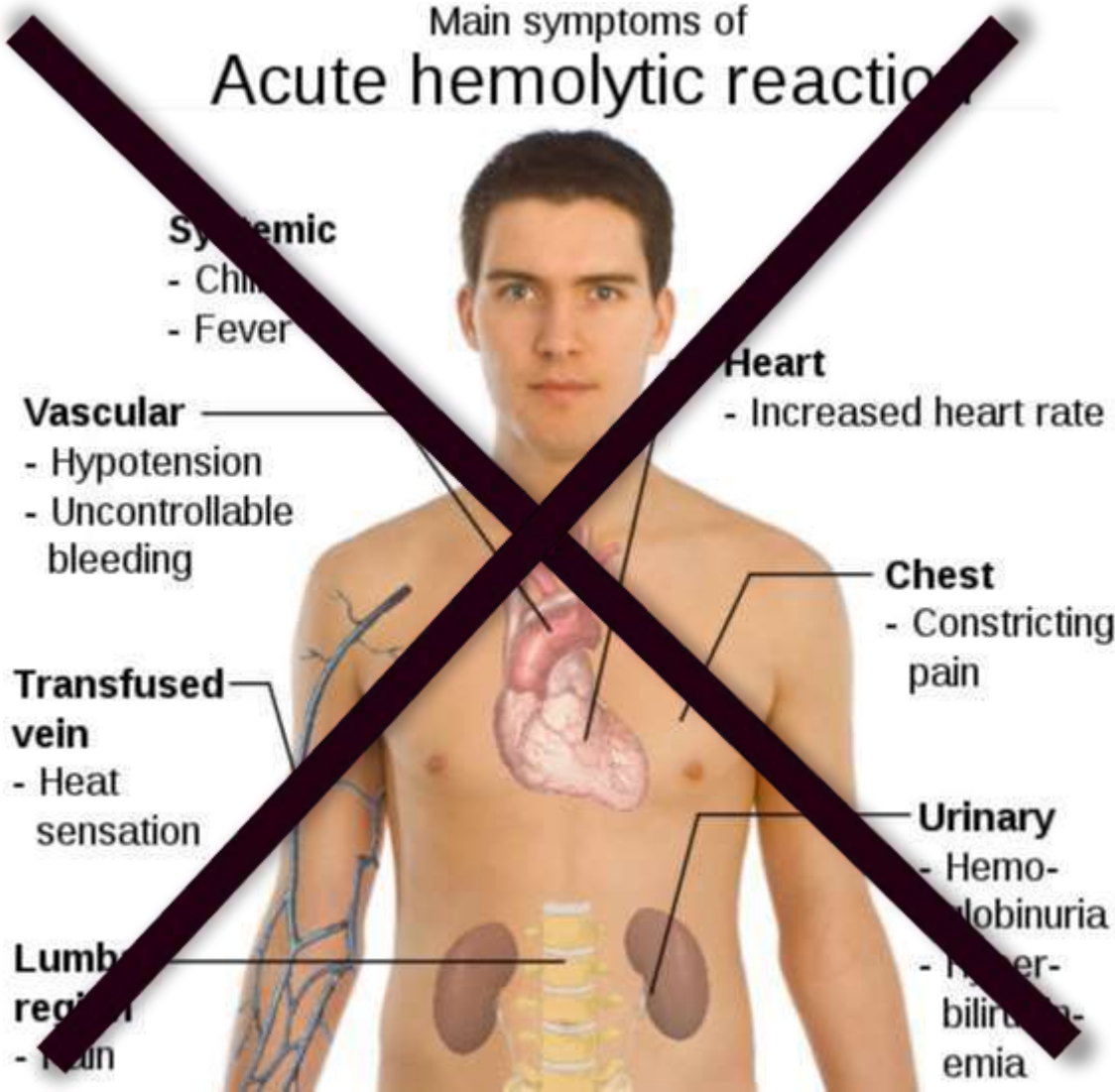
complications

of allogenic blood transfusion

Advantages of ABT

No

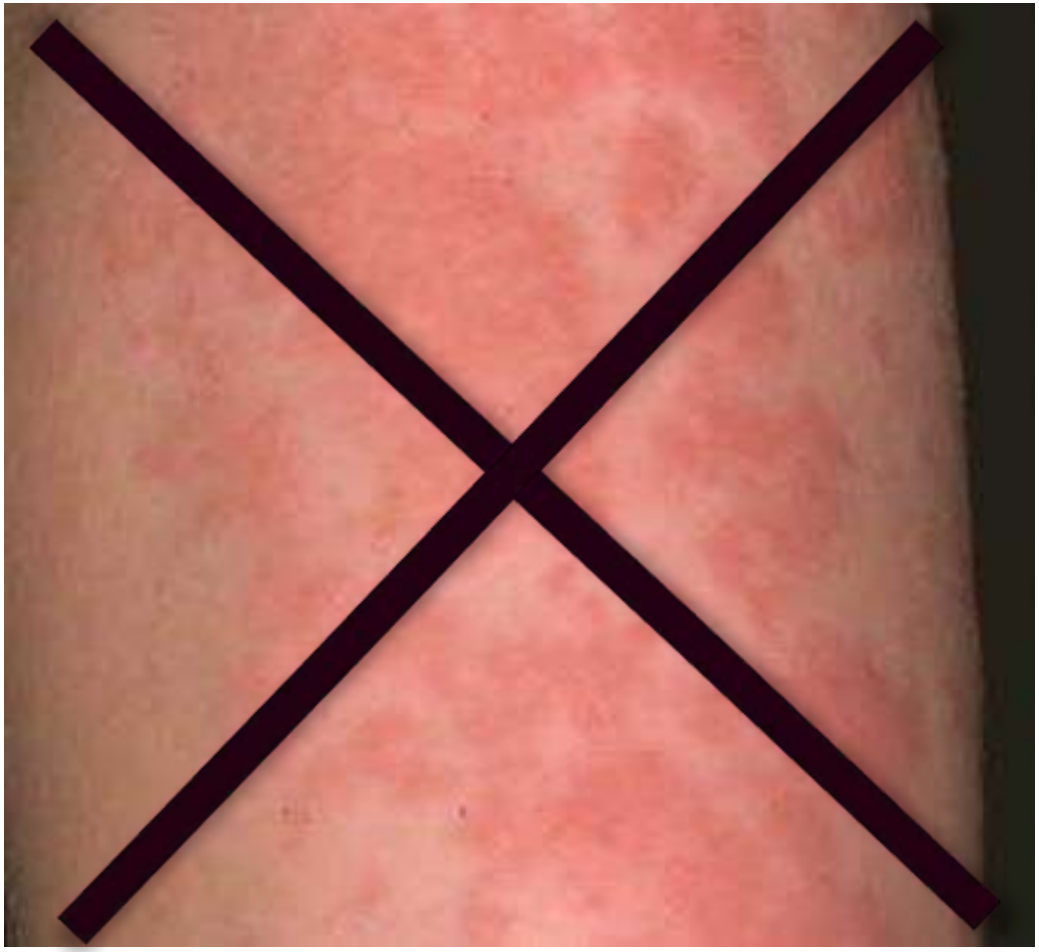
Acute hemolytic reaction



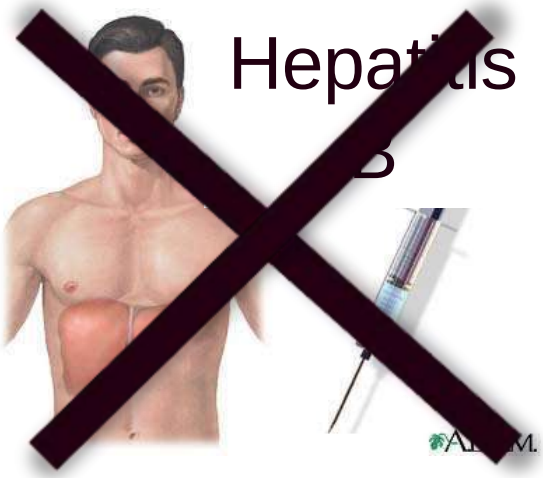
Advantages of ABT

No

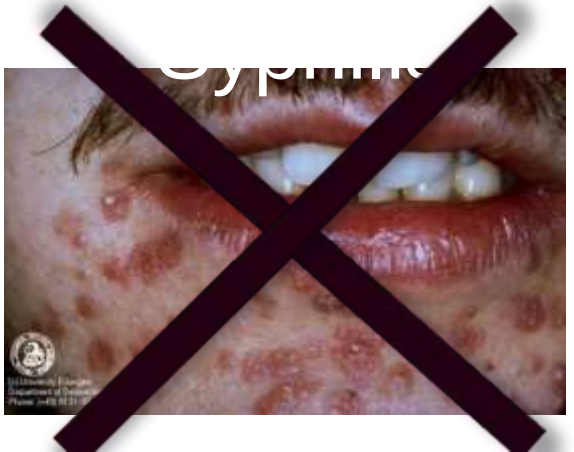
Allergic or Febrile reaction



Advantages of ABT



No Transmission of infections



Advantages of ABT

Avoiding many complications of allogenic blood transfusion

Avoiding Immunosuppression

by allogenic blood transfusion

Advantages of ABT

Avoiding many complications of allogenic blood transfusion

✓Avoiding Immunosuppression by allogenic blood transfusion

Patients with

Rare Blood groups

are benefitted

Advantages of ABT

Avoiding many complications of allogenic blood transfusion

✓Avoiding Immunosuppression by allogenic blood transfusion

✓Patients with Rare Blood groups are benefitted

Conservation

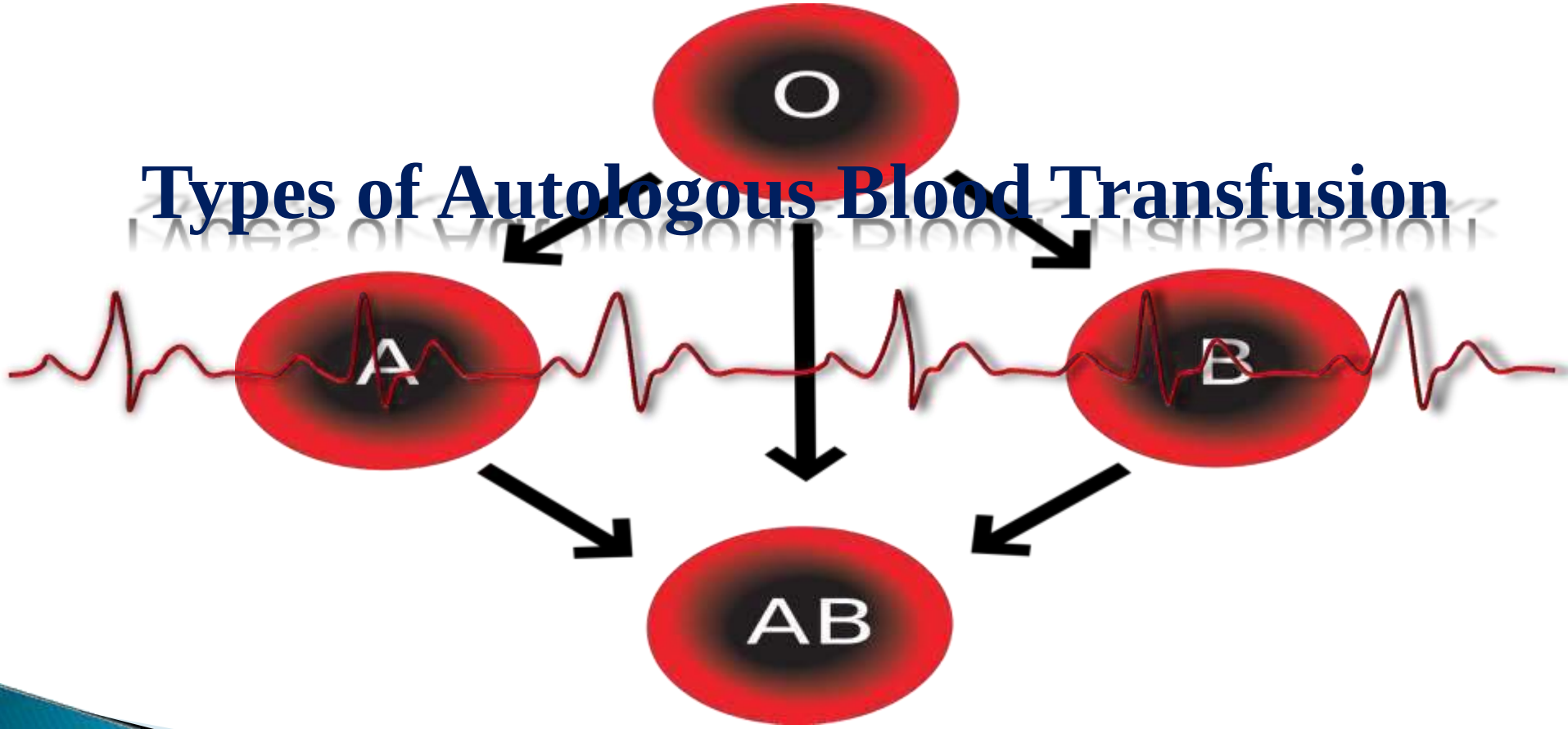
of Blood resources

Advantages of ABT

Avoiding many **complications** of allogenic blood transfusion

- ✓ **Avoiding Immunosuppression** by allogenic blood transfusion
- ✓ Patients with **Rare Blood groups** are benefitted
- ✓ **Conservation** of Blood resources

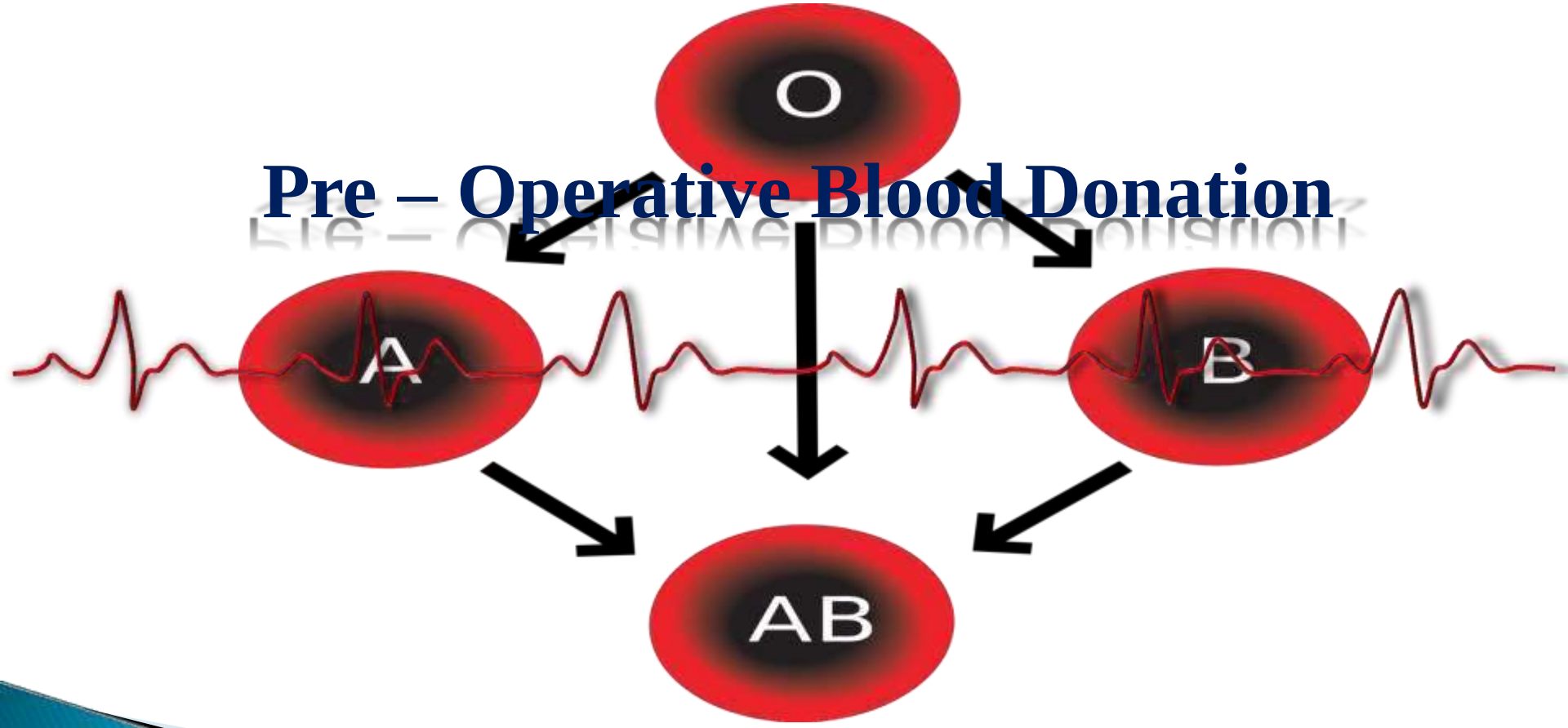
Types of Autologous Blood Transfusion



Types of Autologous Transfusion

- Pre – Operative Blood Donation
- Acute Normo-volemic Hemodilution
- Blood Salvage

Pre – Operative Blood Donation



Pre-Operative Blood Transfusion

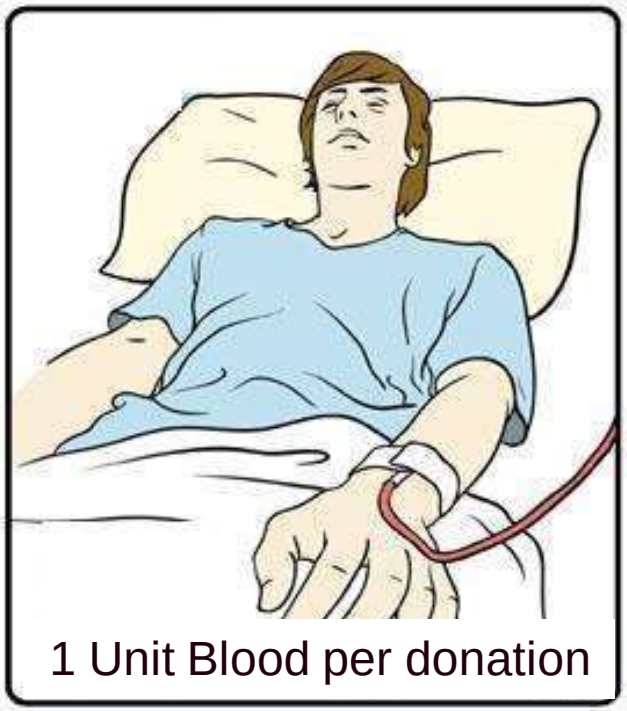
Donating blood weeks before any Elective Surgical procedures where significant blood loss is expected which is transfused back during surgery

- ✓ Person of any age
- ✓ Patient should be : Weighing >50 Kg Hb > 11gm

Hct > 33%

Free of Infections

No Cardiac Disorders



1 Unit Blood per donation

Pre-Operative Blood Transfusion

STEP 1

PATIENT'S CONSENT IS TAKEN

Pre-Operative Blood Transfusion

- STEP 2
- **MEDICAL EXAMINATION & CARDIAC EVALUATION**

Pre-Operative Blood Transfusion

STEP 3

COLLECTION OF BLOOD

✓1 Unit blood per donation

✓1 Donation per week

✓Last donation atleast 4 days before surgery

✓Oral Iron therapy starte

Pre-Operative Blood Transfusion

STEP 4

PROPER LABELLING OF BLOOD COLLECTION PACKS FOR STORAGE

✓Autologous

✓Patient's Name

✓ Id number

Pre-Operative Blood Transfusion

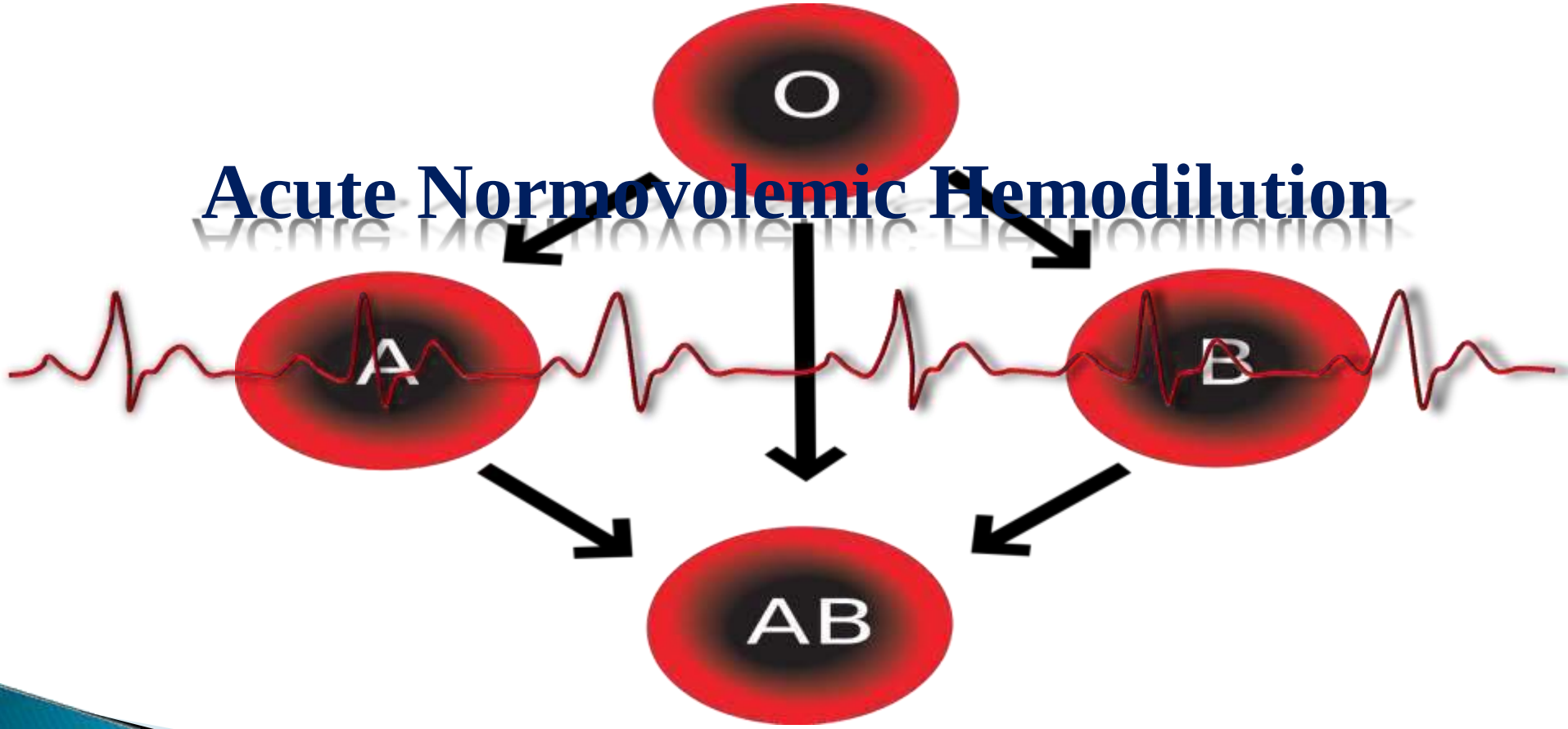
STEP 5

TRANSFUSING BACK THE BLOOD, IF NEEDED, DURING SURGERY

Disadvantages of Pre-operative blood donation

- ✓ Transfusion of Wrong Blood **(Clerical Error)**
- ✓ Higher **cost**
- ✓ Postoperative **anemia**
- ✓ Bacterial **contamination** of unit
- ✓ Not suitable for **Emergency**

Acute Normovolemic Hemodilution



Acute Normovolemic Hemodilution

“Normovolemia” means Maintaining the volume of Blood
“Hemodilution” means ↓ no. of RBCs

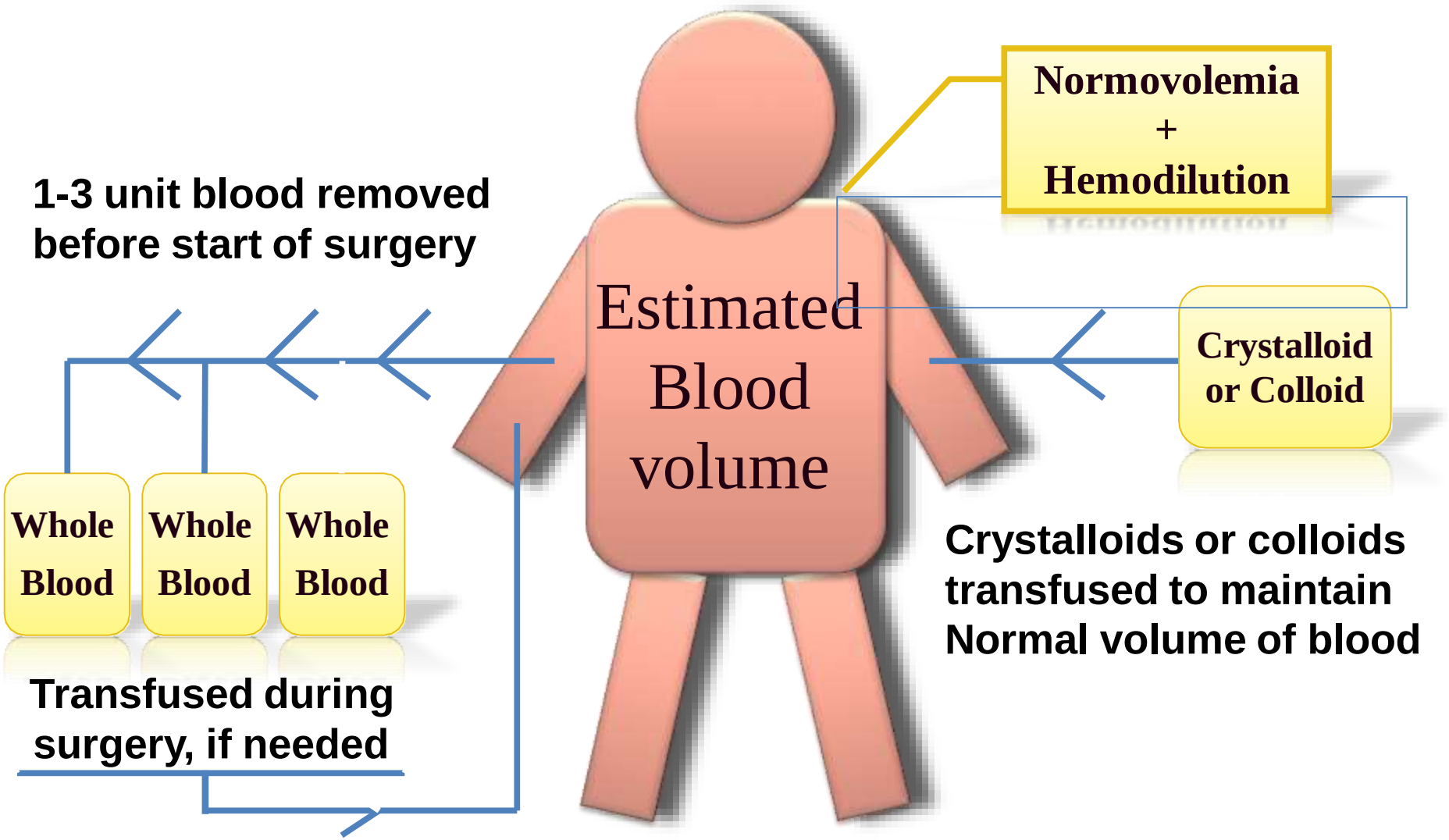
✓Immediately before or after induction of anaesthesia,

1-3 units blood removed Replace with Crystalloid or

colloid

✓Maintains Normovolemia but leads to Hemodilution

Acute Normovolemic Hemodilution



Advantages of Acute Normovolemic Hemodilution

- ✓ **No biochemical alterations** associated with storage of blood
- ✓ **Platelet** function preserved
- ✓ Hemodilution → ↓Blood Viscosity → **Improved Tissue Perfusion**
- ✓ Possible in **Emergency** surgeries
- ✓ **Less Expensive**

Acute Normovolemic Hemodilution

Contraindications

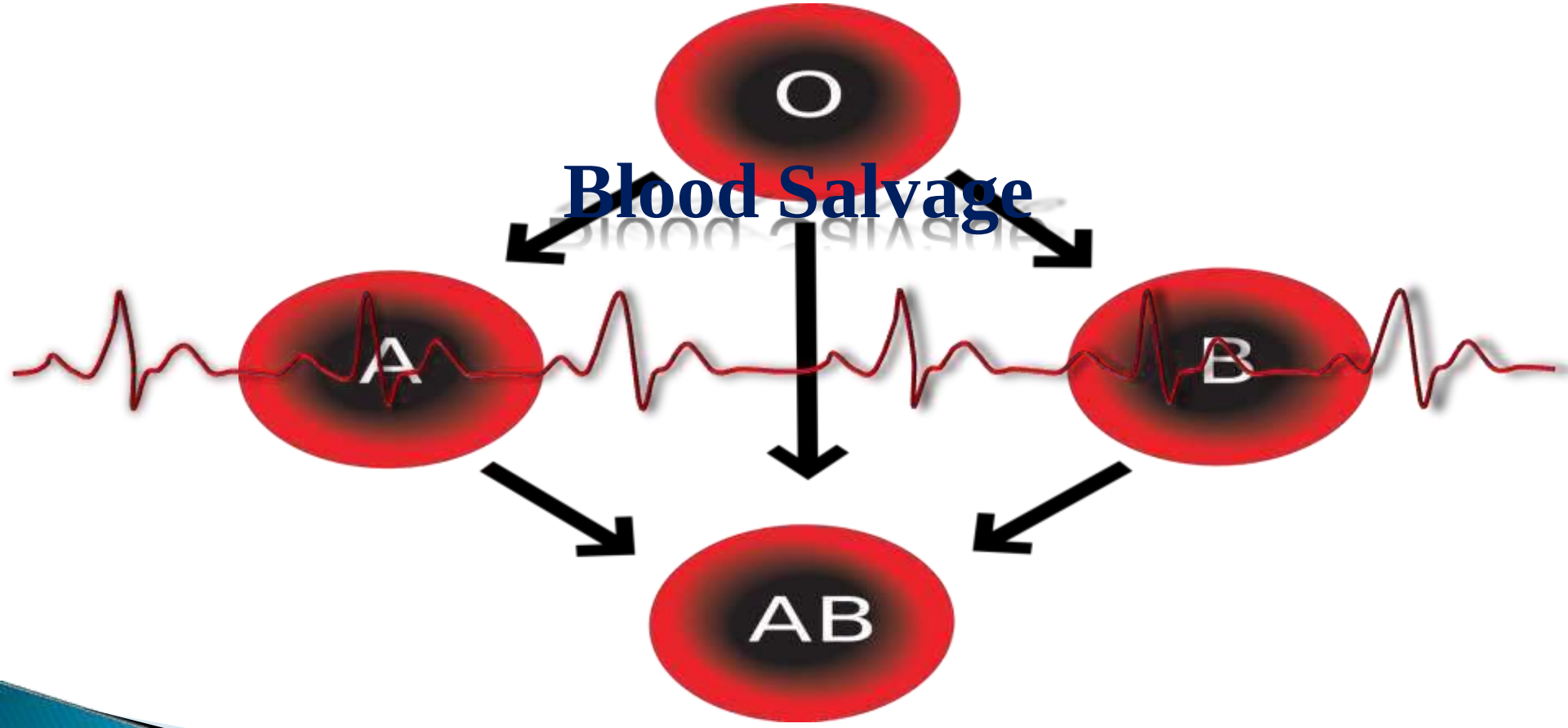
- ✓**Anemia**
- ✓**Renal diseases**
- ✓**Severe CAD, severe pulmonary dysfunction**

Significant Ischemic heart disease

Complications

- ✓**Myocardial ischemia**
- ✓**Cerebral hypoxia**

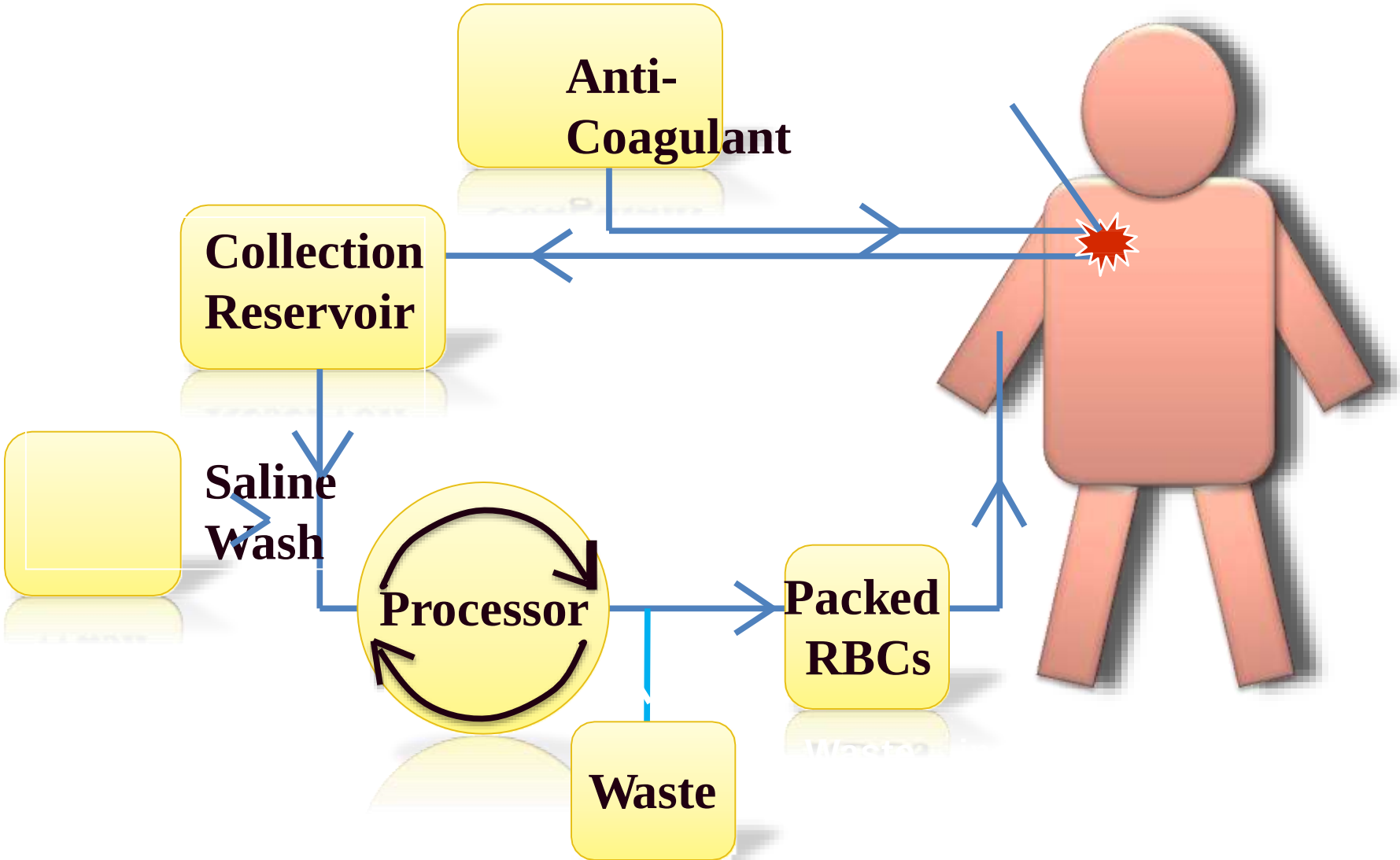
Blood Salvage



Blood Salvage

- ✓ “Salvage” means saving
- ✓ Blood is collected from Operative field and draining site and re-infused into the patient after processing
- ✓ Specialised blood salvage machines are used

Blood Salvage



Blood salvage

Contraindications

- ✓ Gross bacterial contamination in Operative field.
- ✓ Ascitic or Amniotic cavity
- ✓ Free tumour tissue
- ✓ Bowel contents

Blood salvage

Complications

- ✓ Air embolism or Fat embolism
- ✓ Hemolysis
- ✓ Dilutional Coagulopathy

Blood salvage

Applications

- ✓ Cardio-Vascular surgery
- ✓ Liver transplantation
- ✓ Neurosurgery
- ✓ Ortho & Gynaecological operations

Blood Bank

What is Blood Bank..?

A blood bank is a center where blood gathered as a result of blood donation is stored as preserved for later use in blood transfusion. The term ‘Blood Bank’ typically refers to division of a hospital where the storage of blood products occurs and where proper testing is performed.

-Wikipedia-

The policies/ procedures of criteria in selecting blood donors

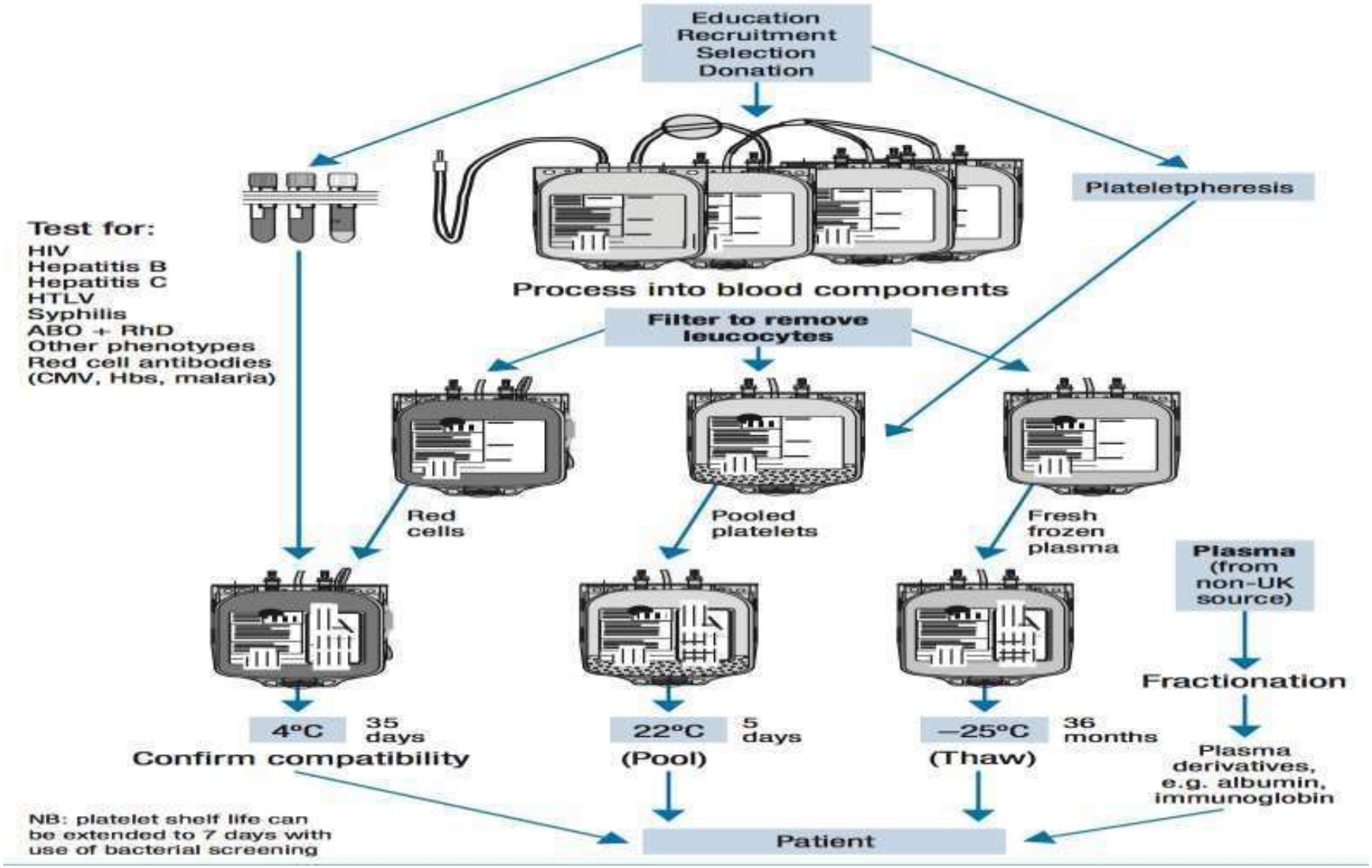
- The person must fulfill several criteria to be accepted as a blood donor. These criteria are set forth to ensure the safety of the donor as well as the quality of donated blood.

Donor Selection Criteria

- Age above 18 years and below 60 years.
- If previously donated, at least 4 months should be elapsed since the date of previous donation.
- Hemoglobin level should be more than 12g/dL. (this blood test is done prior to each blood donation)
- Free from any serious disease condition or pregnancy.

- Should have a valid identity card or any other document to prove the identity.
- Free from "Risk Behaviors".
- Risk Behavior
  - Homosexuals
  - Sex workers and their clients
  - Drug addicts
  - Engaging in sex with any of the above.
  - Having more than one sexual partner

Various blood and blood products in a blood bank

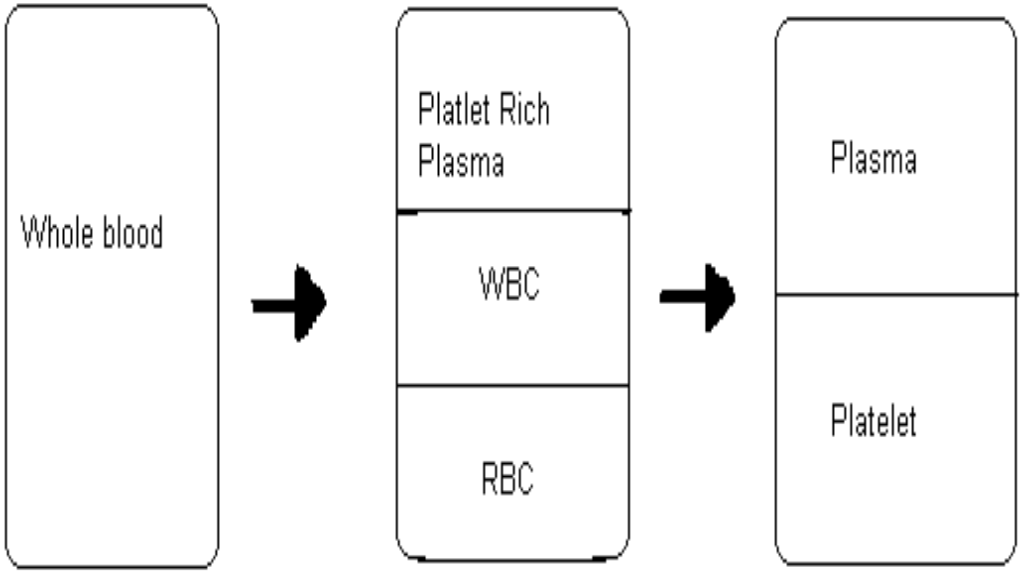


Various blood and blood products in a blood bank (Cont.)

Whole blood

- Blood collected in to CPDA-1 anticoagulant containing bags
- Contains 450ml (+/-10%) of donor blood (blood cells and plasma)
- 63ml of and anticoagulant such as CPD (Citrate, Phosphate, Dextrose)
- Hct 35-45%
- Stored at 2-6 ^{0}C
- Shelf life – with CPD 21 days, with CPDA-1 (Adenine) - 35days

- **From a unit of whole blood,the centrifuged product settle out into RBC,WBC & platelet-rich plasma(PRP).**
- **After separating PRP fr the bag,PRP again being centrifuge for a longer time & harder spin.**
- **Plt is heavier than plasma & will settled at the bottom of the bag.**



RED CONCENTRATE / PACKED RBC

- Red cells with 1/3 of the original plasma
- Saline solution containing added adenine, glucose and manitol, adsol or optimal additive solution
- 45g of hemoglobin per unit
- Stored at 2-6 0C
- 21-35 days or up to 42 days with added above solutions
- Volume - 250ml
- Hct 55-75%
- Contain RBC, WBC and small amount of plasma

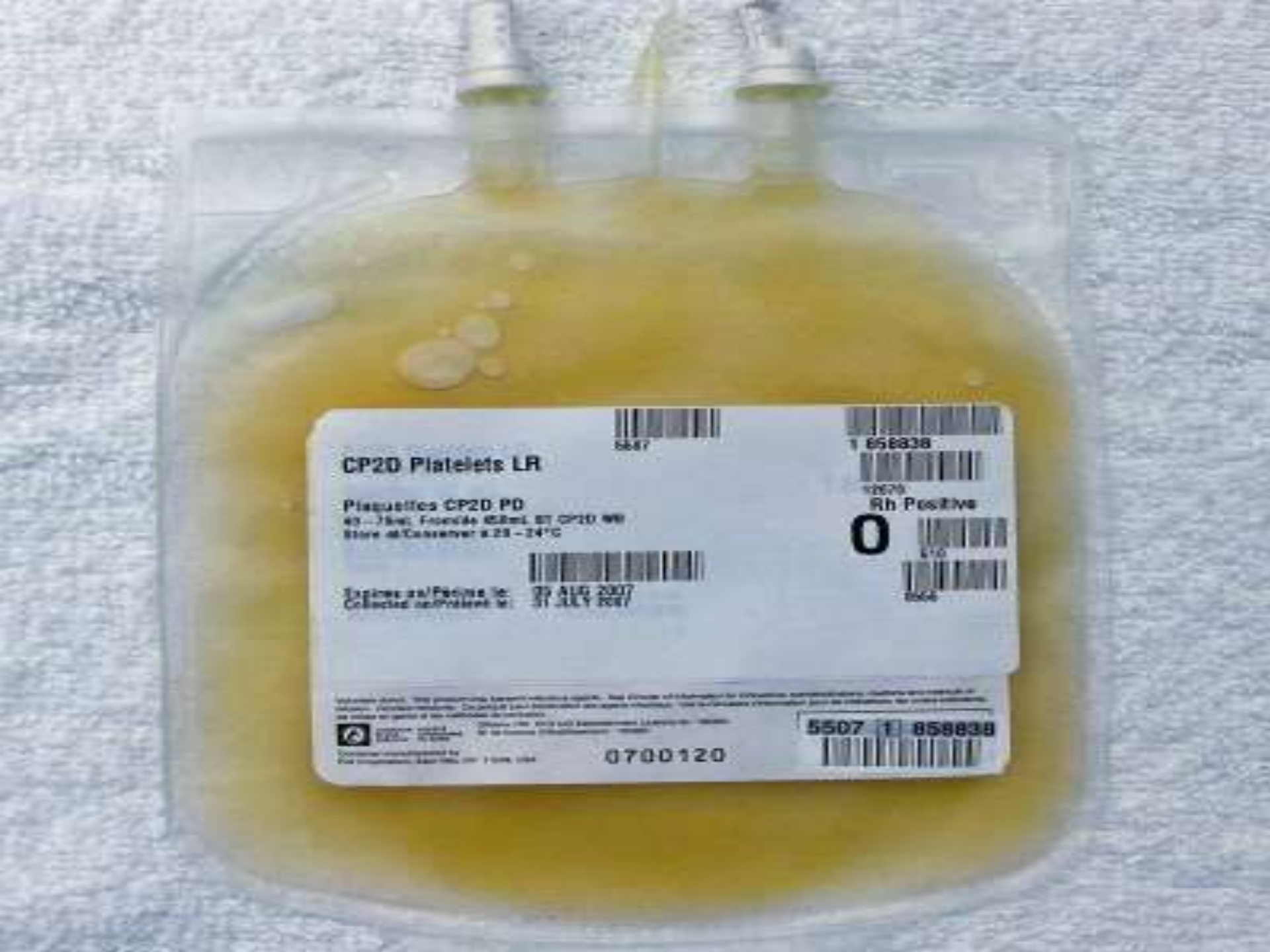

RAD-SURE®
15 Gy INDICATOR
IRRADIATED
ISP TECHNOLOGIES INC.
AS-3 Red Blood Cells LR, Irradiated
Anti-CMV Neg
Culot globulaire AS-3 PD, irradié
Rh Positive
B
Expires on/Périme le: 31 JULY 2007
Collected on/Prélevé le: 19 JUNE 2007
727-92C
0700074
5507 B 851255

Platelet Rich Plasma

- Gentle centrifugation of whole blood
- Supernatant transferred to the 2nd bag

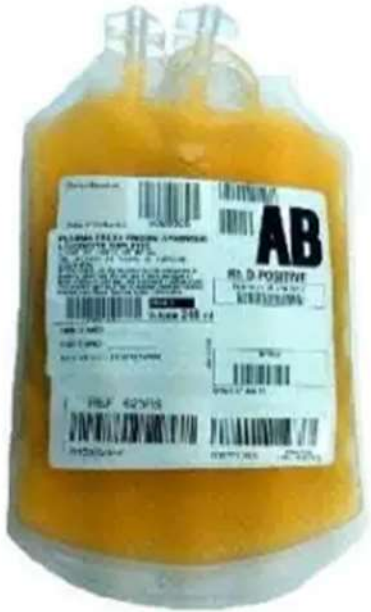
Platelet concentrate

- Prepared from PRP by 2nd centrifugation
- Removal of all but 50ml of plasma
- Contain approximately ≥55╳109platelets
- 60-80% platelets present in whole blood unit
- Volume - 300ml
- Stored at 20-24 0C
- Shelf life - 5 days


CP2D Platelets LR
Plaquettes CP2D PD
Rh Positive
O
1 658838
5507 1 658838
0700120

Fresh Frozen Plasma (FFP)

- Plasma removed from RBC within 6-8 hrs of collection is rapidly frozen to bellow -30^{0}C temperature. Before transfusion is necessary to thaw at 37^{0}C
- Once thawed, there is rapid deterioration of clotting factor, therefore it is very important to use the immediately after thawing
- Dose – 10-12ml by weight
- Shelf life – 12 months
- Stored at < -30^{0}C



- Indications:

1. As a replacement for isolated coagulation fx def.
2. The reversal of warfarin Tx.
3. In the case of massive blood transfusion.
4. Antithrombin III def.Tx.
5. Correction of coagulopathy a/w liver disease.
6. Thrombotic thrombocytopenic purpura.

- **Cryoprecipitate**
- **Description**
- Cryoprecipitate, also called cryo for short, is a frozen blood product prepared from blood plasma. To create cryoprecipitate, fresh frozen plasma thawed to 1–6 °C is then centrifuged and the precipitate is collected. The precipitate is resuspended in a small amount of residual plasma and is then re-frozen for storage.

CRYOPRECIPITATE

- The cold-insoluble portion of plasma that remains after FFP has been thawed at 1-6C.
- Contains of:

1. Factor VIII:C
2. Factor VIII:vWF
3. Factor XIII
4. Fibrinogen
5. About 10-15ml of plasma

- Stored at –18C & below.

- Indications:

1. von Willebrand’s disease
2. Hemophillia A
3. Factor XIII def.
4. Cong./acquired fibrinogen def.

Cryoprecipitate

- Volume - 15ml bags, usual dose of 4-6bags
- Stored at < -25^{0}C
- Shelf life - Up to 1 year

Other products

- Immunoglobulin
- Albumin
- Coagulation factor concentrate

Thank you

BLOOD & BLOOD PRODUCTS

- BLOOD

1. Whole Blood
2. Packed Cell
3. Granulocytes

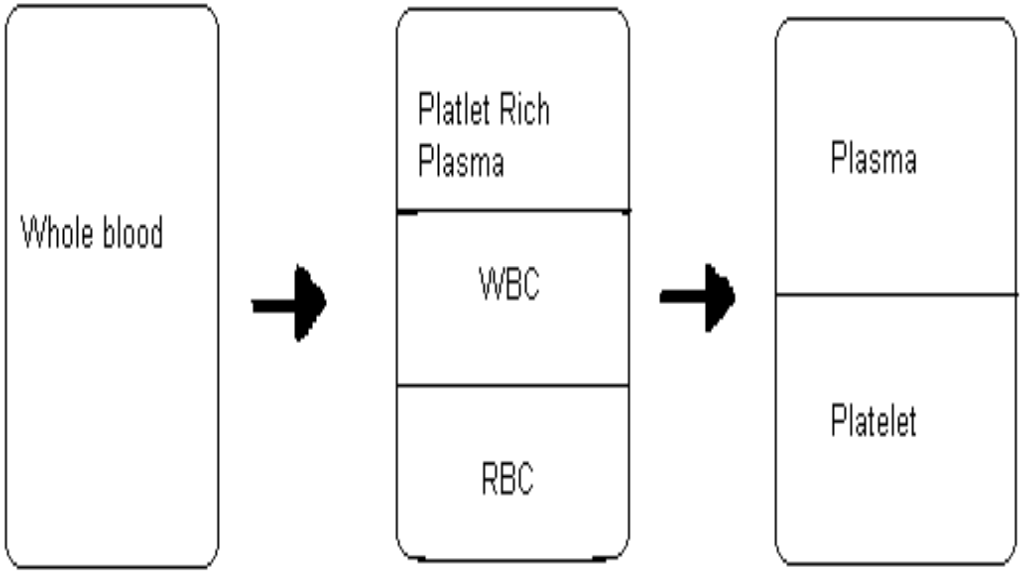
- BLOOD PRODUCTS

1. F.F.P.
2. Cryoprecipitate
3. Platelete

Blood Components Preparation

- Based on different specific gravities
  - RBC : 1.08-1.09
  - Platelet : 1.03-1.04
- By using differential centrifugation,blood components separated into layers

- **From a unit of whole blood,the centrifuged product settle out into RBC,WBC & platelet-rich plasma(PRP).**
- **After separating PRP fr the bag,PRP again being centrifuge for a longer time & harder spin.**
- **Plt is heavier than plasma & will settled at the bottom of the bag.**



WHOLE BLOOD

- Source of product for all blood components
- 400-500 ml
- Storage temperature :1-6 C
- Ind.:to maintain blood volume & O2 carrying capacity in acute,massive blood loss.
  - Actively bleeding pt>20% of body blood volume.

PACKED CELL

- Prepared by removing 200-250ml of plasma from a unit of W.B.
- 200-250 ml
- Do not contain functional platelets or granulocytes
- Have the same O2 carrying capacity with W.B
- Ind.:to increase the O2 carrying capacity in anaemic pt who require an increase in their red cell mass w/out increase in their blood volume.
- 1 unit: increase Hb level about 1g/dL (10g/L)& Hct by 3%.

GRANULOCYTES

- Prepared by leukoparesis tech.
- Contain of

1. Large number of granulocytes
2. Other leucocytes
3. 20-50ml of RBC

- Ind.:

1. Supportive tx for pt with severe neutropenia with documented sepsis unresponsive to a/biotic tx.
2. Neonatal sepsis.

PLATELET

- Prepared by cytapheresis/by seperating PRP fr a unit of W.B w/in 8H of collection & recentrifuge to remove plasma.
- Stored at 20-24C.
- Each unit of plt expected to increase 5000-10000 plt.

- Indications:

1. Prophylaxis.
2. Dilutional thrombocytopenia
3. Active bleeding d/t thrombocytopenia/thrombocytopathy.

FRESH FROZEN PLASMA

- Prepared by removing plasma fr W.B w/in 8H of collection.
- Stored at –18C or below.
- Contains of:

1. Water,carbohydrates,fats,minerals
2. Proteins(all labile & stable clotting fx).

- 200-225ml
- Each unit of FFP=increase the level of each clotting fx by 2-3% in adults.
- Therapeutic dose : 10-15ml/kg.

- Indications:

1. As a replacement for isolated coagulation fx def.
2. The reversal of warfarin Tx.
3. In the case of massive blood transfusion.
4. Antithrombin III def.Tx.
5. Correction of coagulopathy a/w liver disease.
6. Thrombotic thrombocytopenic purpura.

CRYOPRECIPITATE

- The cold-insoluble portion of plasma that remains after FFP has been thawed at 1-6C.
- Contains of:

1. Factor VIII:C
2. Factor VIII:vWF
3. Factor XIII
4. Fibrinogen
5. About 10-15ml of plasma

- Stored at –18C & below.

- Indications:

1. von Willebrand’s disease
2. Hemophillia A
3. Factor XIII def.
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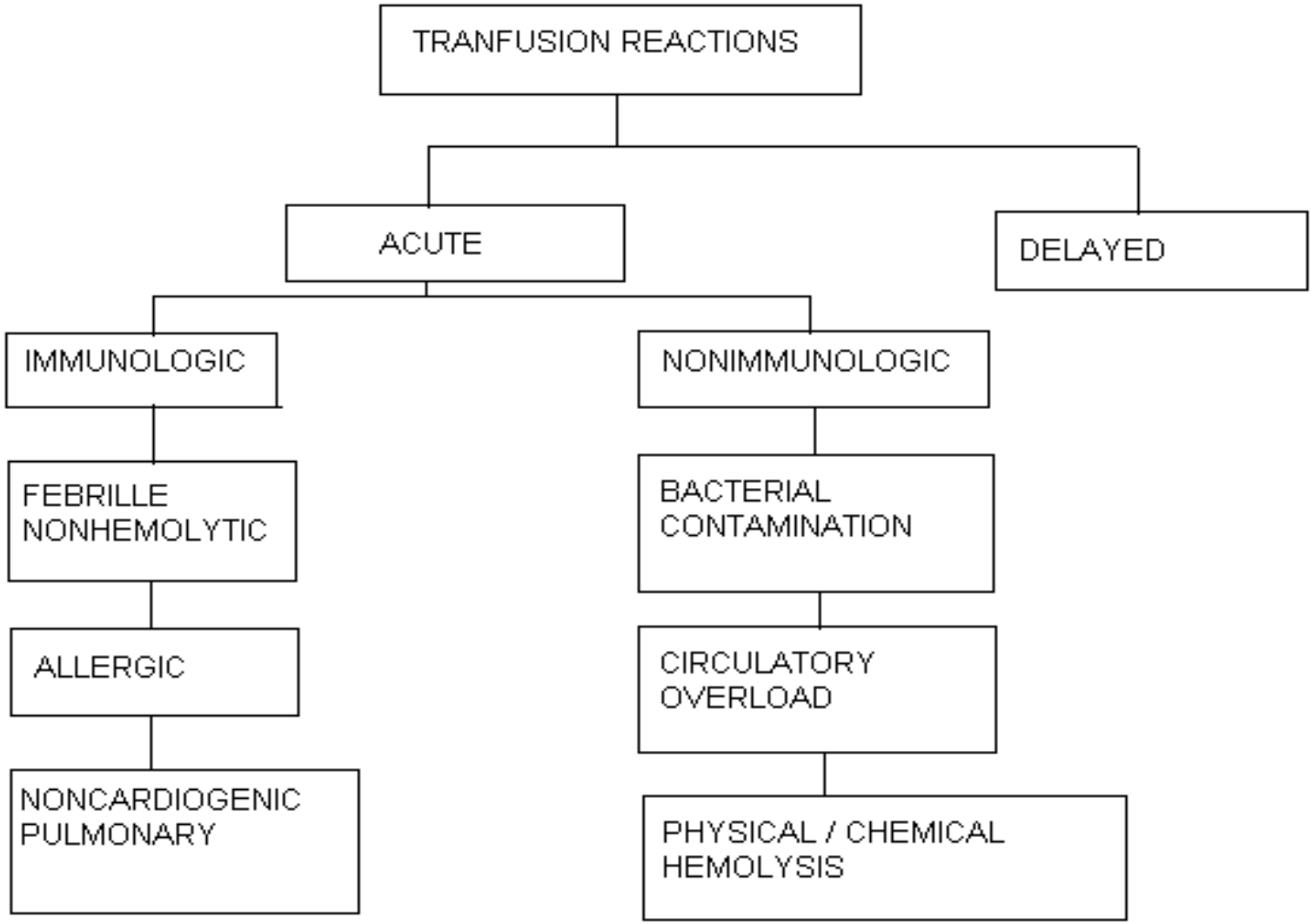
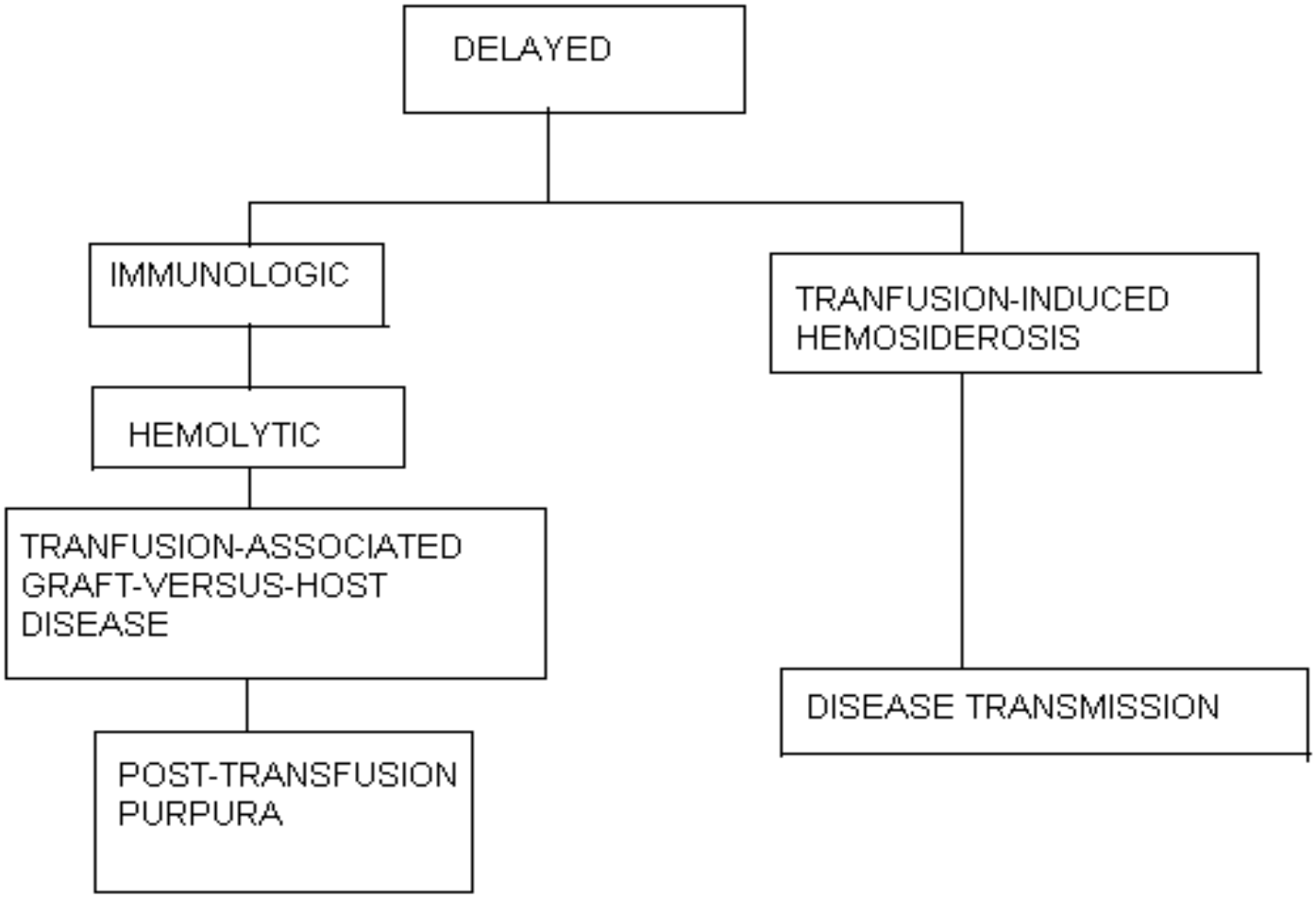
COMPLICATIONS OF BLOOD TRANSFUSION



COMPLICATIONS OF BLOOD TRANSFUSION



ACUTE IMMUNOLOGIC EFFECTS

1}Hemolytic transfusion reactions

- Mediators:IgM A/b (usuallyABO), complement.
- Sx/sn:fever,chill,hemoglobinemia, hemoglobinuria,hypotension,dyspnea.
- Mx/px:decrease opportunities for error,treat ARF & DIC.

2}Nonhemolytic febrile transfusion reactions

- Mediators:A/b to HLA Class I Ag.
- Sx/sn:fever,chill.
- Mx/px:antipyretics,leukocyte depletion.

3}Allergic transfusion reactions.

- Mediators:plasma proteins(mild reactions),
  A/b to IgA(anaphylactic reactions).
- Sx/sn:urticaria,erythema,itching,
  anaphylaxis.
- Mx/px:antihistamines;treat sx,transfuse IgA-deficient components.

4}Noncardiogenic pulmonary transfusion reactions

- Mediators:donor/recipient WBC A/b.
- Sx/sn:ARD,fever,chill,cyanosis,hypotension ,noncardiogenic pulmonary edema.
- Mx/px: vigorous respiratory support, steroids.

ACUTE NONIMMUNOLOGIC EFFECTS

1}Bacterial contamination

- Md :endotoxins produced by GN bact.
- Sx/sn:fever,shock,hemoglobinuria.
- Mx/px:IV a/biotics;treat hypotension & DIC.

2}Circulatory overload

- Md: fluid volume.
- Sx/sn:coughing,cyanosis,orthopnea,severe headache,peripheral edema,diff breathing.
- Mx/px:administer subsequent Tx slowly & in a small volume.

3}Hemolysis d/t physical/chemical means

- Md:exogenous destruction of RBC.
- Sx/sn:hemoglobinuria.
- Mx/px:document & rule out hemolysis d/t other causes;treat DIC.

DELAYED IMMUNOLOGIC EFFECTS

1}Hemolytic transfusion reactions.

- Md:IgG A/b.
- Sx/sn:shortened RBC survival,decreased Hb,fever,jaundice,hemoglobinuria.
- Mx/px:Ag-negative blood for further transfusions.

2}Transfusion associated Graft-versus-host disease

- Md:viable donor lymphocytes.
- Sx/sn:fever,skin rash,desquamation,anorexia,nausea, vomiting,diarrhea,hepatitis,pancytopenia
- Mx/px:gamma irradiation of cellular components.

3}Post-transfusion purpura

- Md:platelet specific A/b.
- Sx/sn:thrombocytopenia,clinical bleeding.
- Mx/px:IV Ig,plasma exchange, corticosteroids.

DELAYED NONIMMUNOLOGIC EFFECTS

Transfusion-Induced Hemosiderosis.

- Md:Iron overload.
- Sx/sn: subclinical to death.
- Mx/px:decrease fq of transfusion,neocytes, iron chelation therapy.

STEPS TO BE FOLLOWED

1. Discontinue the transfusion.
2. Keep the IV line open with N/saline.
3. Check all labels,forms & pt identification.
4. Report to Blood Bank personnel.
5. Send requested blood samples.

THANK
YOU

COMPLICATIONS OF BLOOD TRANSFUSION

Introduction

- Adverse reactions to blood components occurs despite multiple tests, checkups, & inspections.
- Many reactions are not life threatening & serious reactions present with mild symptoms and signs.

Classification

- Classified based on etiology.

1. Immune mediated reactions.
2. Non immune mediated reactions.
3. Infectious complications.

Immune mediated reactions

1. Hemolytic transfusion reactions.
2. Febrile non-hemolytic transfusion reactions.
3. Allergic reactions.
4. Anaphylactic reactions.
5. Graft versus host disease.
6. TRALI.
7. Post transfusion purpura.
8. Alloimmunization.

Hemolytic transfusion reactions

- Occurs-preformed antibodies ~~x~~ donor RBCs.
- Major-ABO incompatibility.
- Minor-Rh, K, jk, Fy incompatibility.
- CF: hypotension, tachypnea, tachycardia, fever, chills, hemoglobinuria, hemoglobinemia, flank pain.
- Immune complexes→complement cascade→hemolysis.
- Immune complexes→renal tubules→acute tubular necrosis→AKI[Acute Kidney Injury]

Cont...

What to do--transfusion is stopped immediately, intravenous access maintained.

- Diuresis-furosemide 20-40mg.IV or inj.Mannitol 20% 100ml IV.
- Monitor urine output, Hb in urine.
- Blood bank-recheck, repeat crossmatching.
- Investigations-LDH,indirect bilirubin, haptoglobin, PT, aPTT, fibrinogen, platelet count, DAT.

Febrile non-hemolytic transfusion reactions.

- Cellular blood components.
- Mild nature.
- CF: chills rigors, >=1 degree C increase of temp.
- Fever-other causes ruled out.
- Antibodies against Donor WBCs&HLA antigens.
- Multiple transfused pt.& multiparous women. Prevention: leukoreduction before storage.

Allergic reactions

- Utricarial, mild nature—plasma proteins.
- Temporary stop, symptomatic Rx.
- Rx: diphenhydramine 50mg orally or IM.

Anaphylactic reaction

- Severe, few milliliters.
- CF: dyspnea, cough, nausea, vomiting, hypotension, bronchospasm, loss of consicousness, respiratory arrest, shock.
- Rx: stop transfusion immediately, epinephrine 0.5-1 ml, 1:1000 dilution S/C, glucocorticoids[if severe]
- IgA deficienct individuals-IgA deficient plasma, washed cellular blood components.

GVHD

- Allogenic stem cell transplantation.
- TAGVHD-donor lymphocytes recognise host HLA antigens as foreign→immune response.
- CF: fever, diarrhea, cutaneous eruption, liver function abnormalities.
- Marrow aplasia, pancytopenia.
- Rx: highly resistant to immunospressive therapies, glucocorticoids, cyclosporine, anti thymocyte globulin, allogenic bone marrow transplantation.

TRALI
(Transfusion Related Acute Lung Injury)

- Presents as acute respiratory distress either during or with in 6hrs. of transfusion.
- CF: respiratory difficulty, non cardiogenic pulmonary edema.
- CXR: bilateral interstitial infiltrates.
- Anti HLA antibodies against recepient leukocytes.
- Dx: testing donor plasma for anti-HLA antibodies.
- Rx: supportive, recovery without sequele.

Posttransfusion purpura:

- Thrombocytopenia- 7 to 10 days after transfusion.
- Platelet specific antibodies-recepient serum.
- Antigen-HPA-1a on platelet glycoprotein 3a receptor.
- Rx: IV immunoglobulin, plasma pheresis.

Alloimmunization

- Women of child bearing age group who are sensitized to RBC antigens[D, E, kell or duffy].
- HDNB.
- Dx: matching for D antigen is the only pre transfusion selection test to prevent RBC alloimmunization.
- Rx: leukoreduction of cellular components.

Non Immunologic Reactions

- **Fluid overload:** blood components are excellent volume overloaders.
- CF: cough, chest pain,. Frothy sputum.
- Rx:vasodilators, diuretics.
- **Hypothermia:** rapid infusion of refrigerated/frozen components.
- Cardiac arrythmias.
- In-line warmer→prevention.


37.3°C

Electrolyte toxicity:

- **hyperkalemia**→RBC leakage during storage.
- Neonates&pt. with renal failure→risk.
- Prevention: washed RBCs.
- **Hypocalcemia**: circumoral numbness, tingling sensation→citrate chelates calcium thereby inhibiting coagulation cascade.
- **Metabolic alkalosis**.

Iron overload:

- Each RBC unit contains 200-250 mg iron.
- After 100 units of transfusion, CF of iron overload as endocrinological, hepatic, cardiac are common.
- Prevention: erythropoietin as alternate therapy or chelating agent desferoxamine

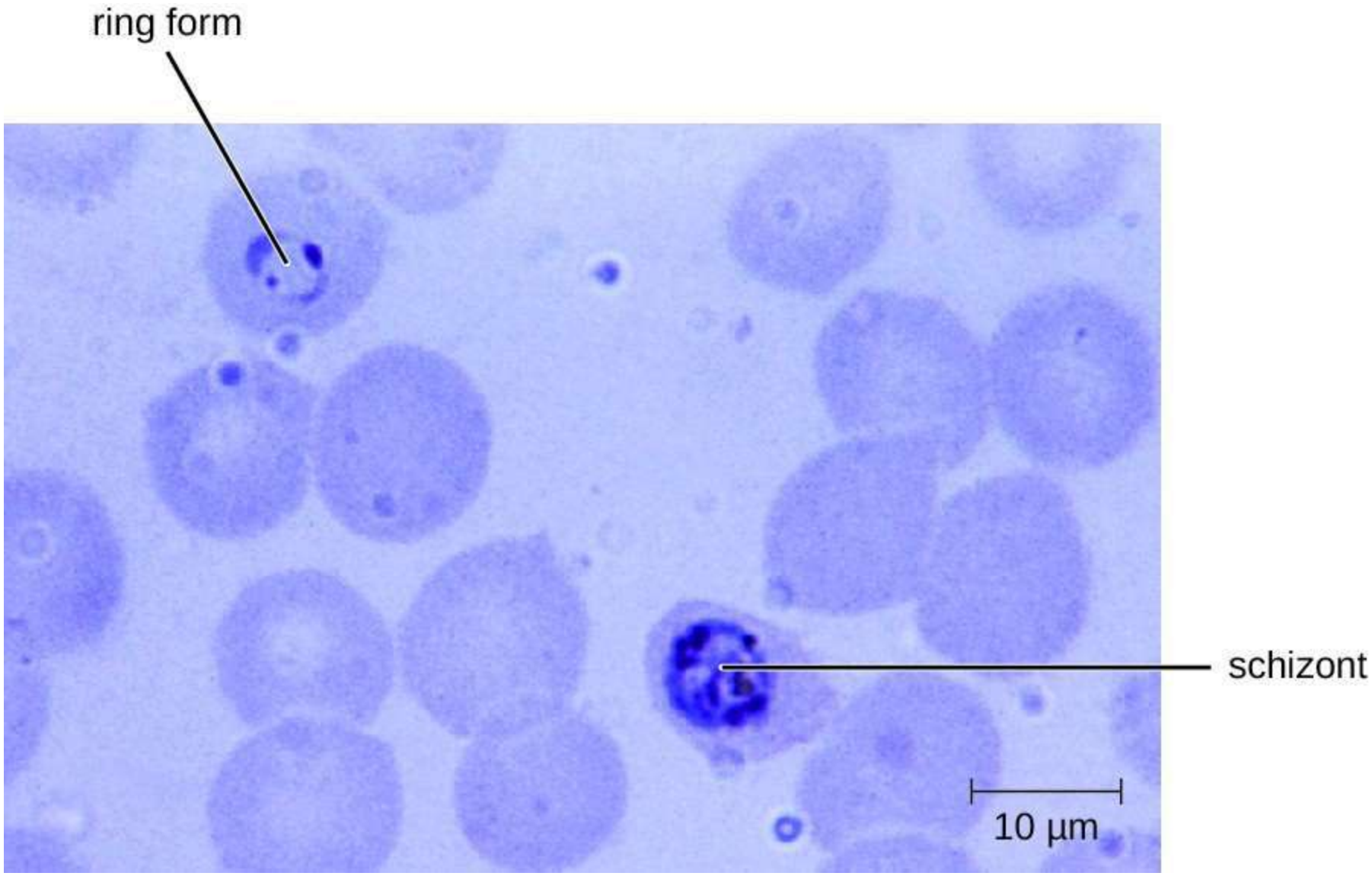
THROMBOPHLEBITIS

- Air embolisim

Infectious complications

- **Viral**: **HCV** – common, asymptomatic to chronic active hepatitis.
- **HIV-1**— p24 antigen ;actual HIV virus particles in blood (p24 is a capsid structural protein which makes up a protein 'shell' on the surface of the HIV virus).
- **HBV**—risk of transmission is more than HCV.
- Vaccination-for long term transfusion therapy.
- **West nile virus**-asymptomatic to fatal.
- **HTLV-1**→T- cell leukemia, lymphoma.

- **Bacterial**: relative risk is more than viral.
- Yersinia(plague الطاعون) pseudomonas,escherichia→can grow in cold temperatures.
- CF:fever, chills, progress to septic shock&DIC.
- Endotoxins
- Rx: stop, reversing shock, broad spectrum antibiotics.
- Other infectiousagent(**Parasitic**)malaria, chagas disease(type of sleeping sickness), babesiosis,dengue(type of haemorrhagic fever),toxoplasmosis.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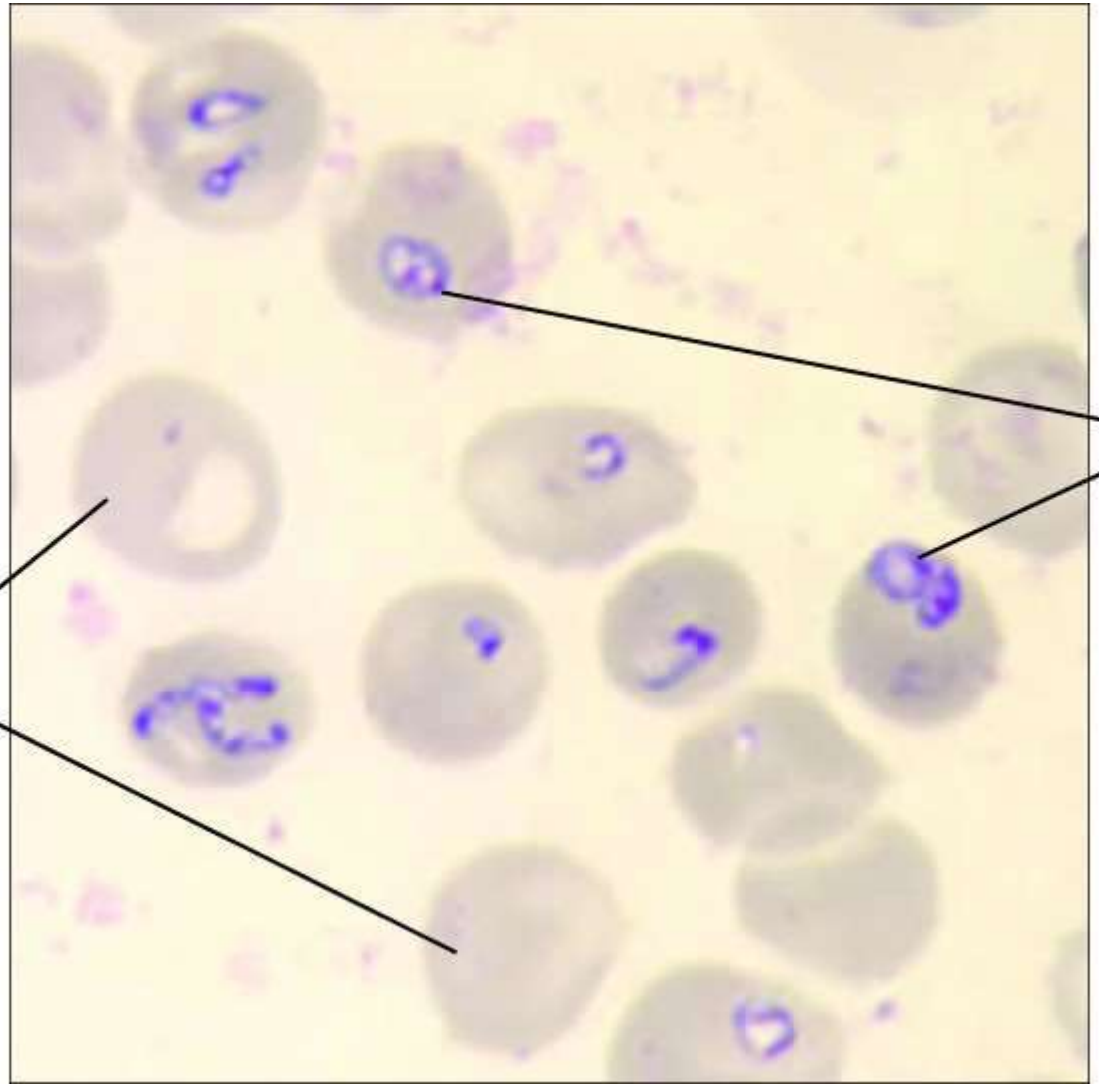
Babesia
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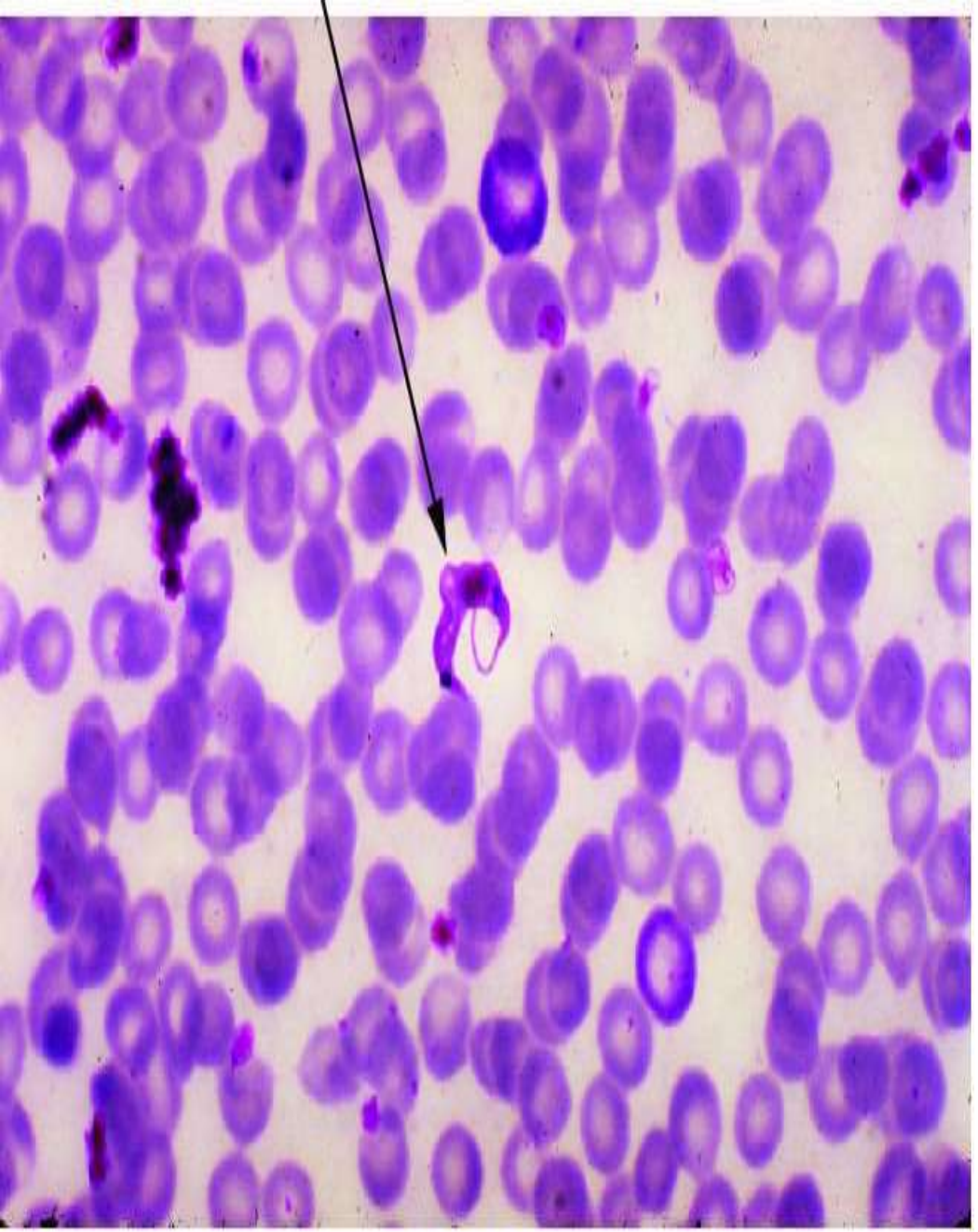
erythrocytes



(a)

(b)

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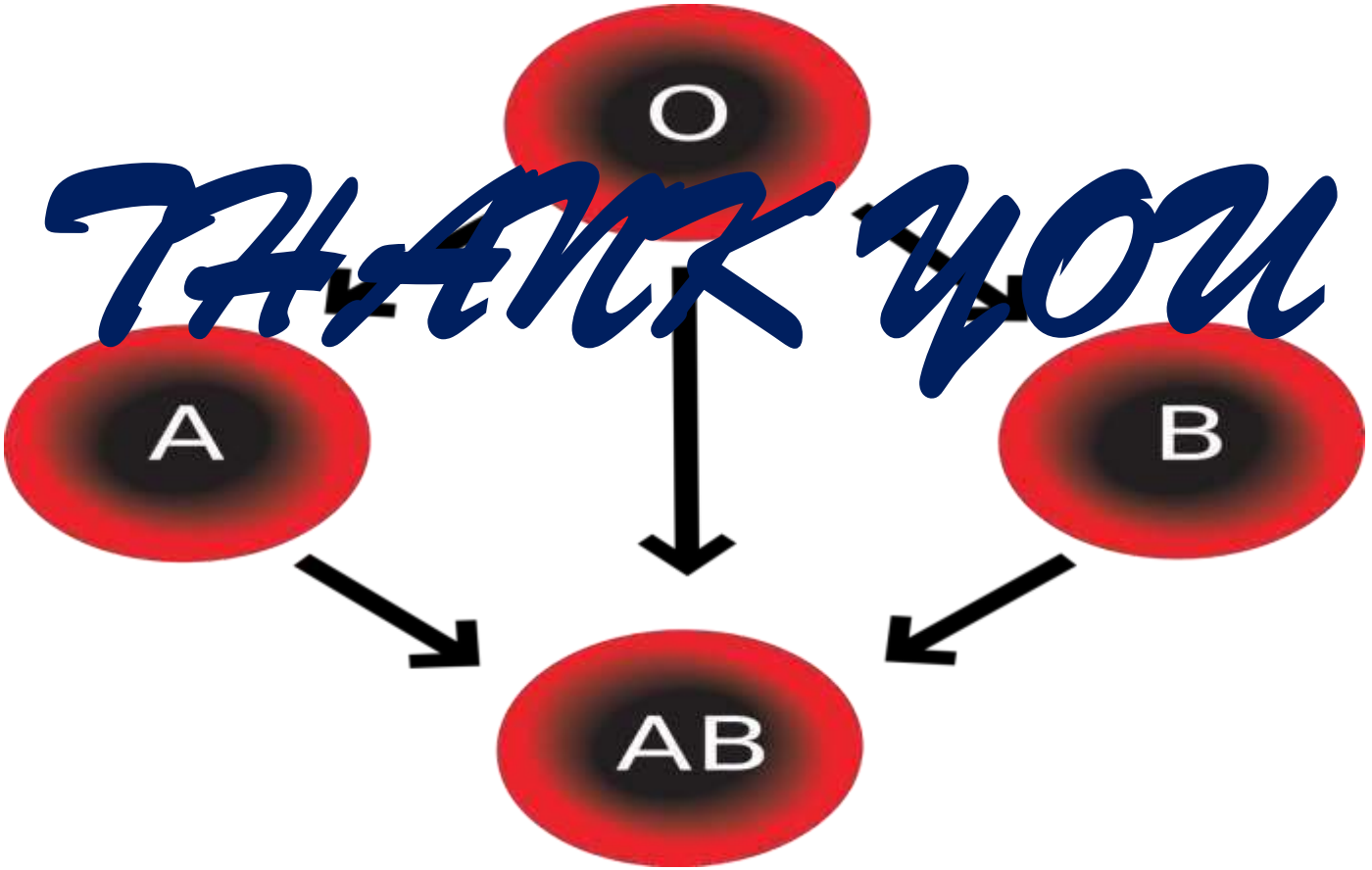


COOMBS TEST

The Antiglobulin Test

principles and practice

The Antiglobulin Test

- Antiglobulin serum

- **Antiglobulin serum** (Coombs'Serum) was discovered by Coombs etal in 1945.
- The antiglobulin test can be used to detect red cells sensitized with **IgG alloantibodies**, IgG autoantibodies or complement components.
- Sensitization of red cells can occur in vivo or vitro. The use of AHG serum to detect sensitization of red cells in vitro is a two stage technique known as indirect antiglobulin test (IAT).

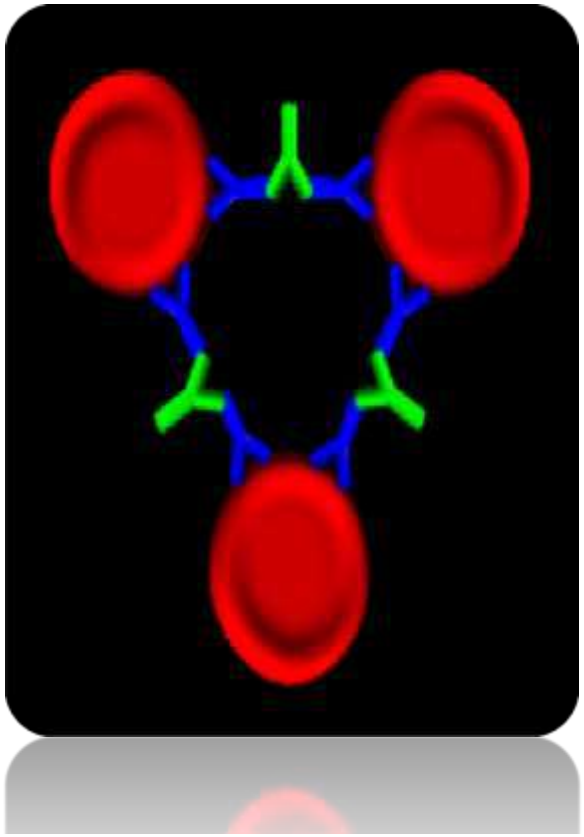
The sensitization of red cells in vivo is detected by one stage technique the direct antiglobulin test (DAT).

Principle of Antiglobulin Test

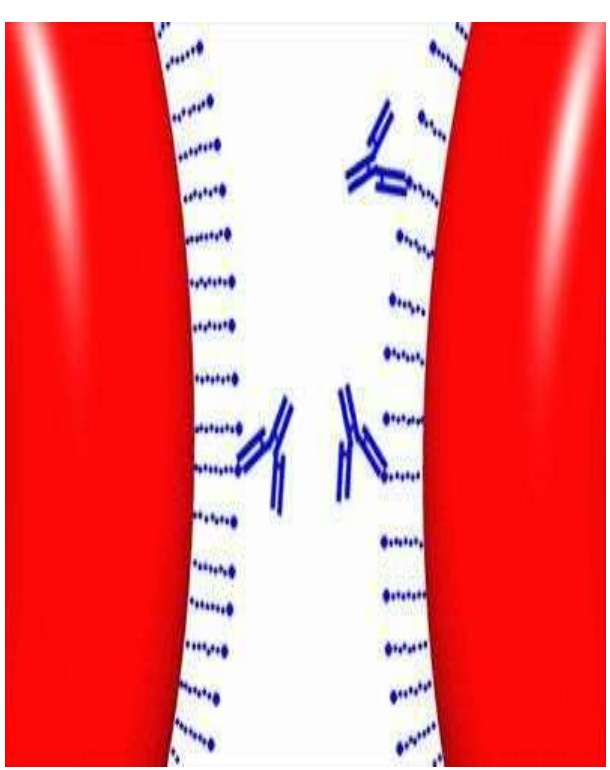
- The incomplete antibodies (IgG) attach to red cell membrane by the Fab portion of the immunoglobulin molecule (IgG).
- The IgG molecules attached to the red cells are unable to bridge the gap between sensitized red cells which are separated from each other by the negative charge on their surface and the sensitized red cells do not agglutinate

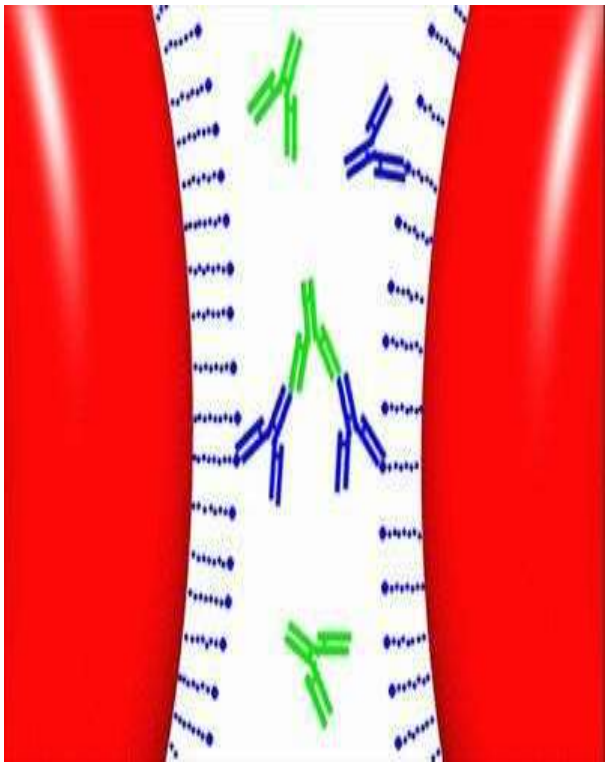
What is Coombs' Serum

- Serum from a rabbit or other animal previously immunized with purified human globulin to prepare antibodies directed against IgG and complement, used in the direct and indirect Coombs' tests. Also called *antihuman globulin*.



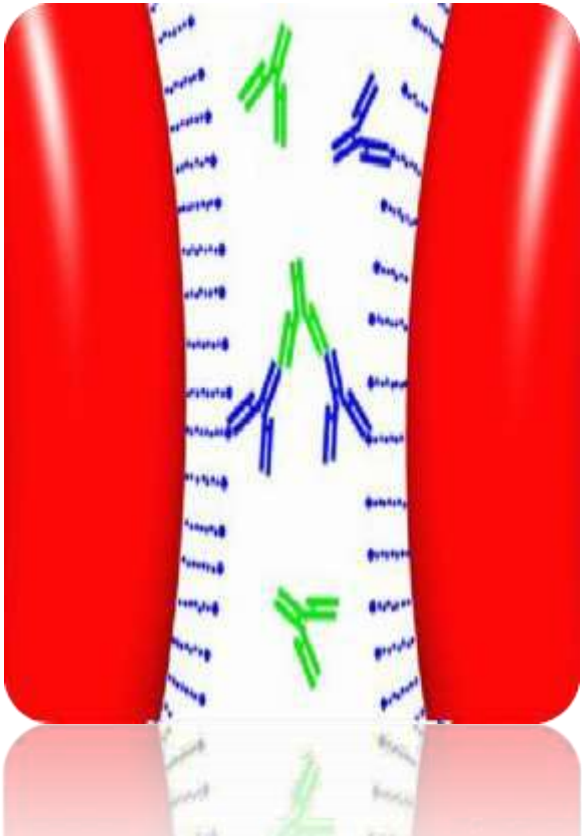
Showing incomplete and complete Agglutination Reactions



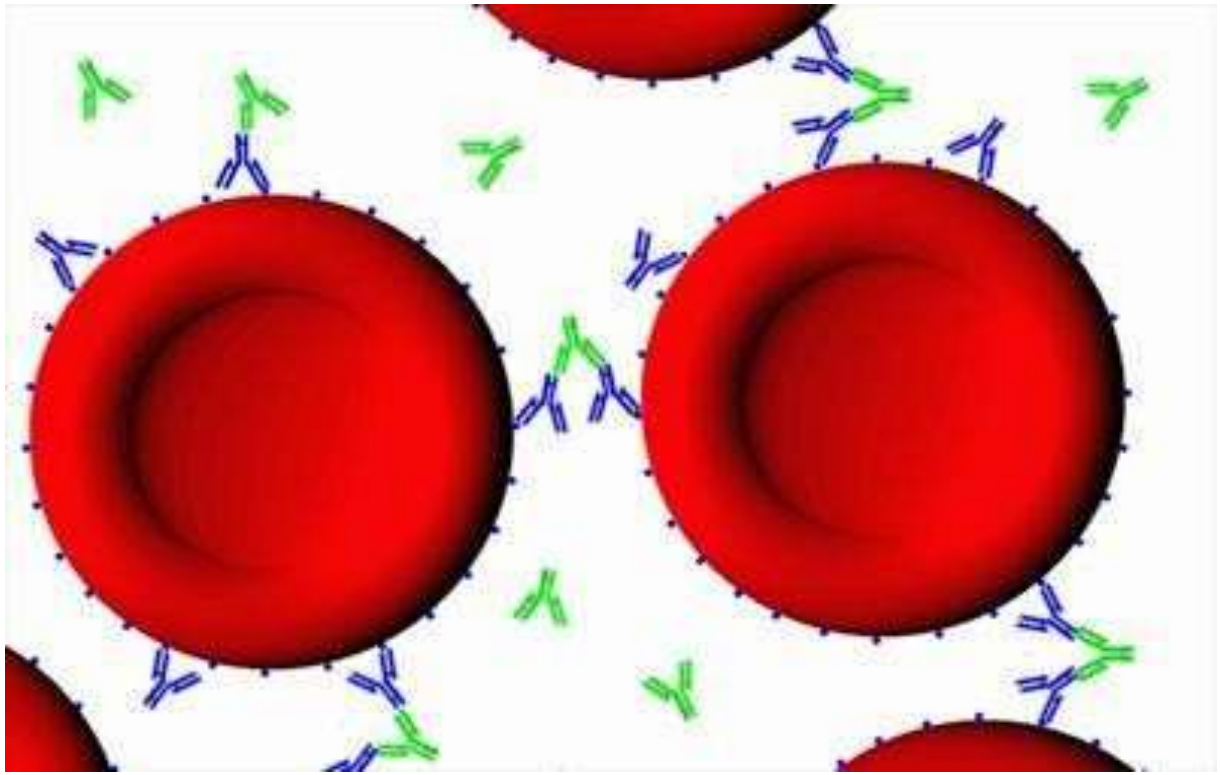


Adding of Antiglobulin serum completes the reaction

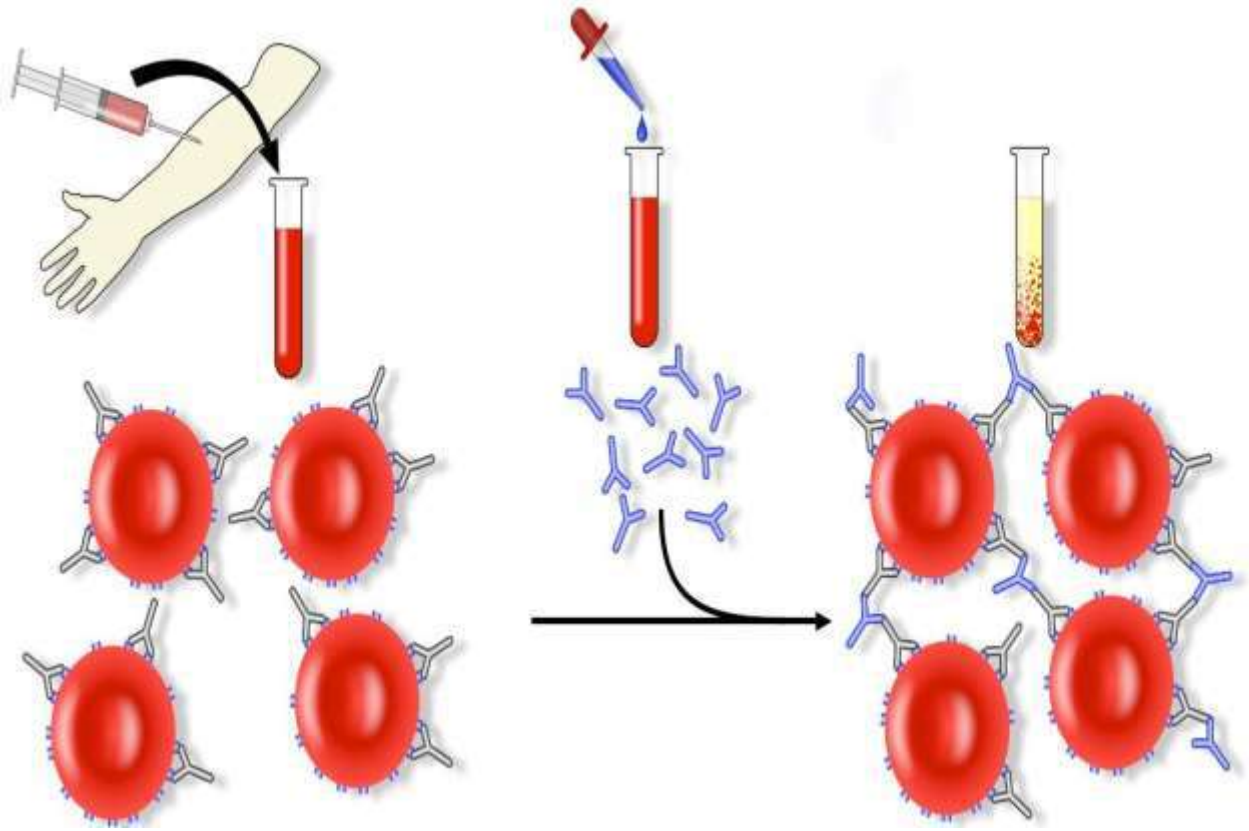
- When AHG serum is added to the washed sensitized cells, the Fab portion of the AHG molecule (anti-IgG) reacts with the Fc portions of two adjacent IgG molecules attached to red cells thereby bridge the gap between sensitized red cells and cause agglutination



Showing a Complete Reaction with Coombs Serum



Showing a Complete Reaction with Coombs Serum



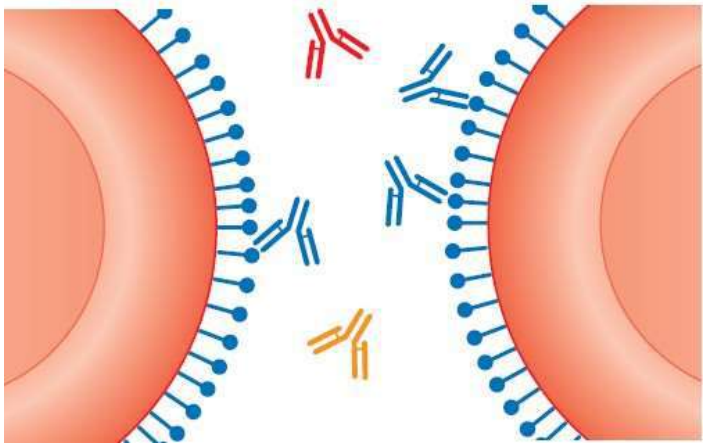
Indirect Coombs test (Indirect Antiglobulin test):

- This test is performed to detect presence of Rh-antibodies or other antibodies in patients serum in case of the following:

1. To check whether an Rh-negative women (married to Rh-positive husband) has developed Anti Rh-antibodies

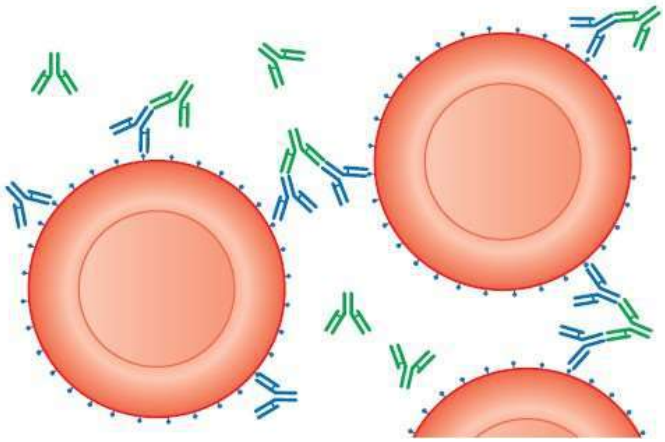
2. Anti D may be produced in the blood of any Rh-negative person by exposure to D antigen by-
   - **Transfusion of Rh positive blood**
   - **Pregnancy, if infant is Rh positive (if father is Rh-positive)**
   - **Abortion of Rh-positive fetus.**

Indirect antiglobulin test

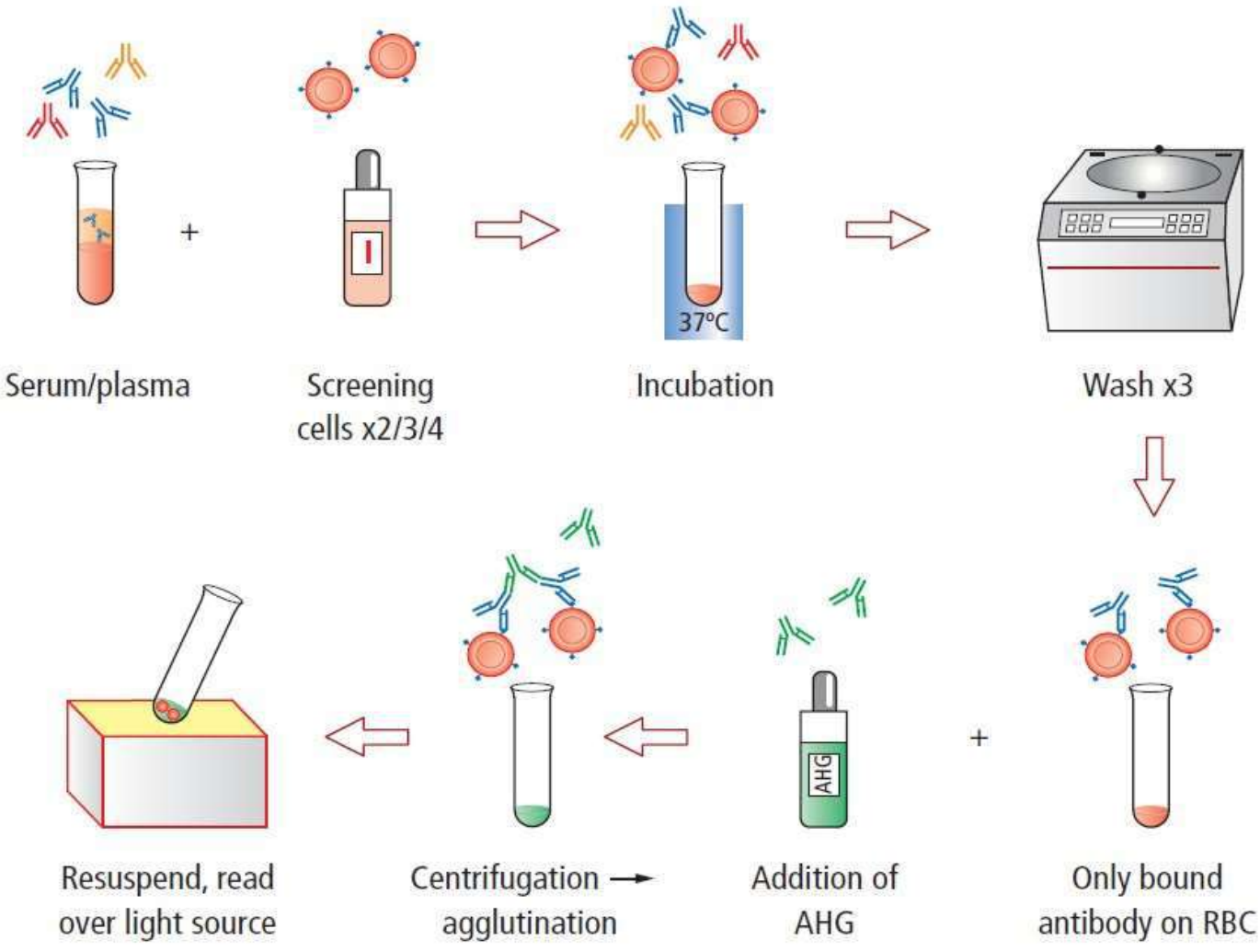


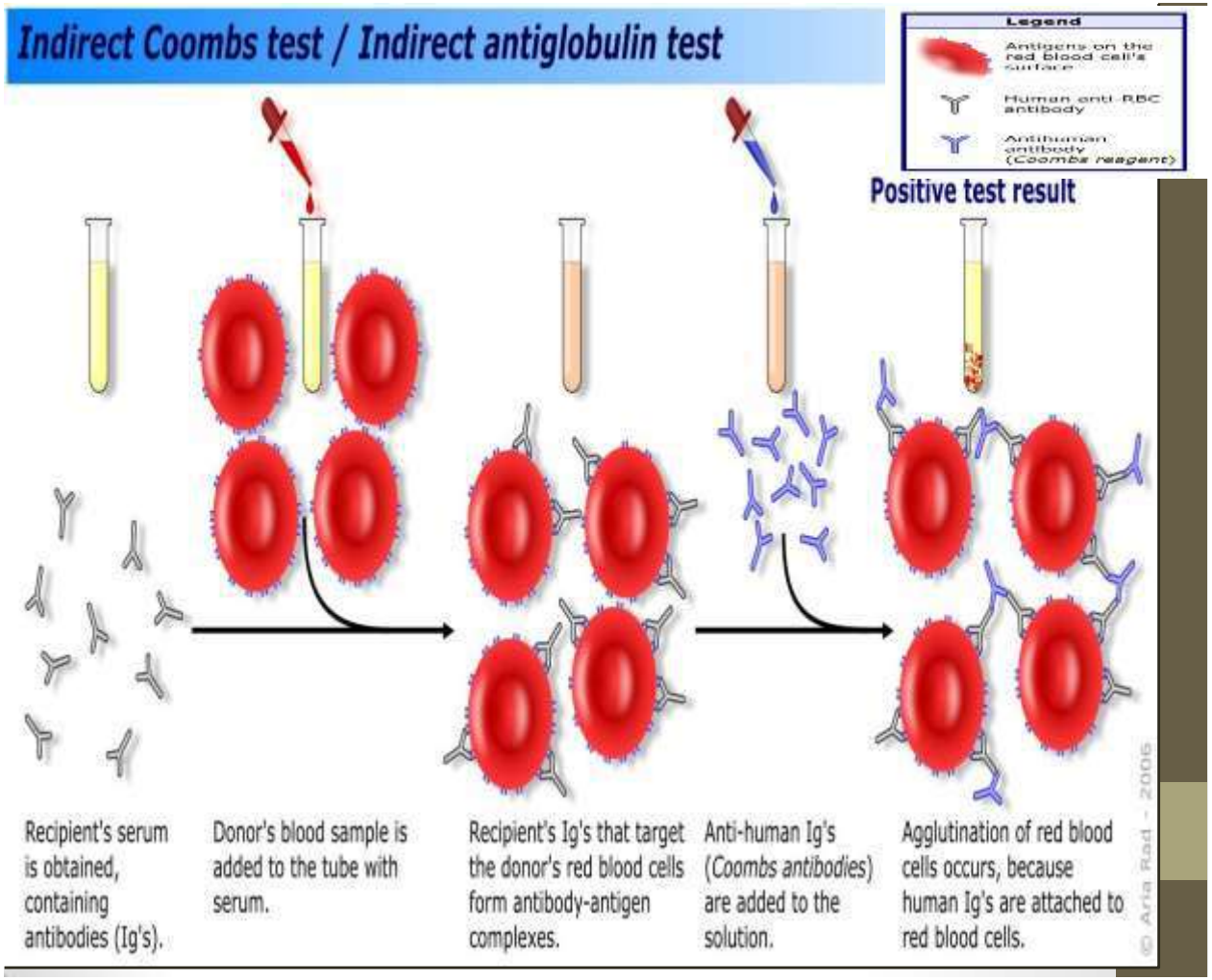
Serum with specific antibody mixed with reagent red cells

Washed x3 after incubation to remove unbound globulins



Anti-human globulin (AHG) added to promote agglutination on centrifugation


+
37°C
AHG
+
Serum/plasma
Screening cells x2/3/4
Incubation
Wash x3
Only bound antibody on RBC
Addition of AHG
Centrifugation → agglutination
Resuspend, read over light source


Indirect Coombs test / Indirect antiglobulin test
Legend
Antigens on the red blood cell's surface
Human anti-RBC antibody
Antihuman antibody (Coombs reagent)
Positive test result
Recipient's serum is obtained, containing antibodies (Ig's).
Donor's blood sample is added to the tube with serum.
Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.
Anti-human Ig's (Coombs antibodies) are added to the solution.
Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.
© Aria Rad - 2006

Direct Coombs test (direct antiglobulin test):

- This test is performed to detect anti-D antibody or other antibodies attached to the red cell surface within the blood stream.
- This occurs in the following circumstances:

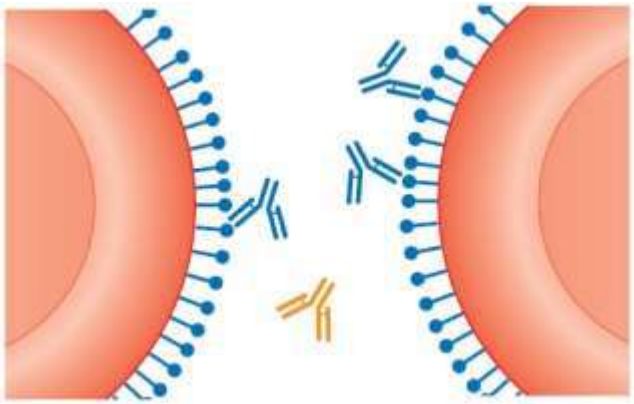
  Hemolytic disease of newborn (Rh and ABO)

  Transfusion reactions

  Drug induced red cells sensitization

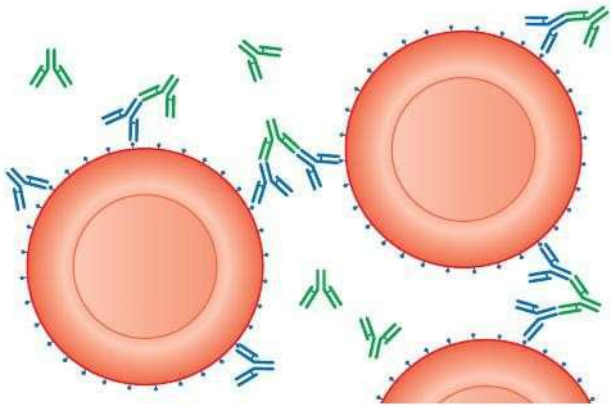
  Autoimmune hemolytic anemia

DIRECT ANTIGLOBULIN TEST (DAT)



Cells coated *in vivo*

Washed to remove unbound globulins

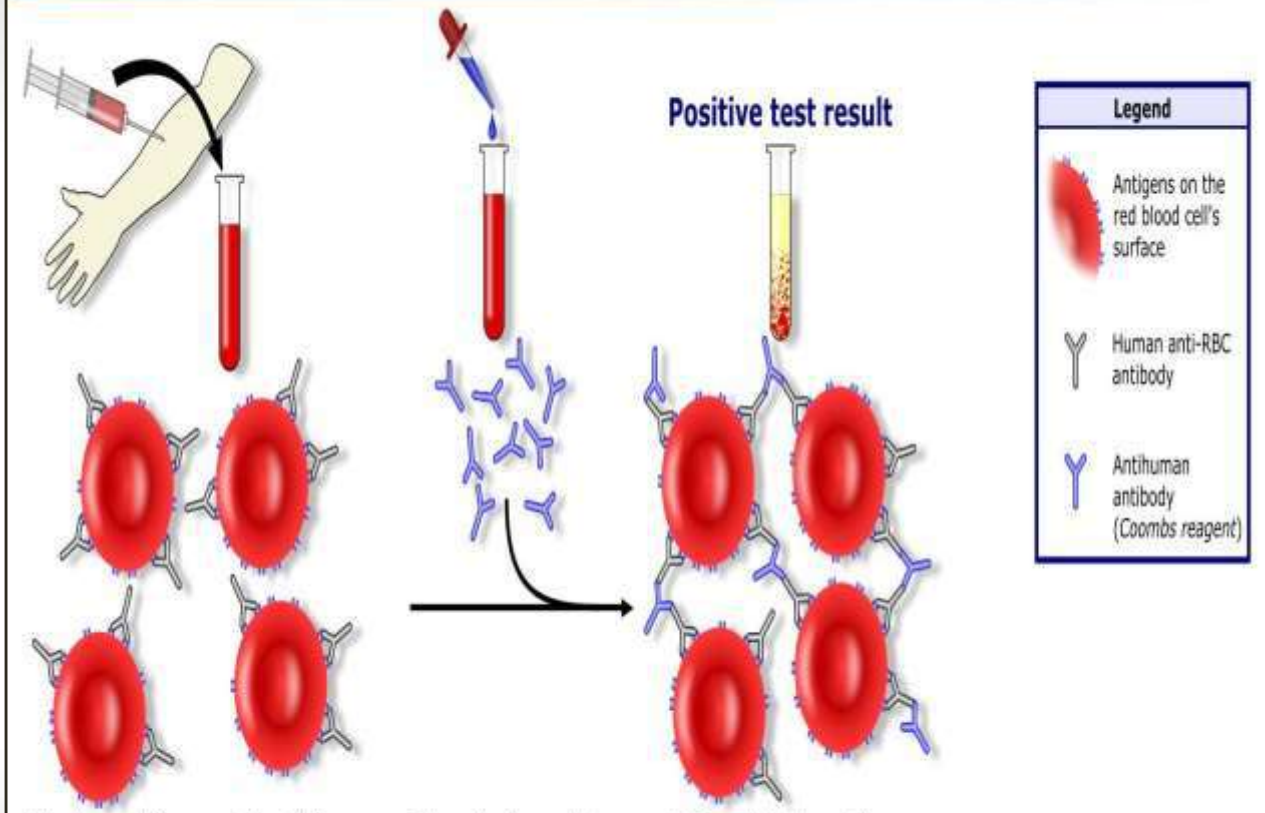


Addition of anti-human globulin (AHG) promotes agglutination after centrifugation

Direct antiglobulin test (DAT)

- The direct antiglobulin test (DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.
- In vivo coating of red cells with IgG and/or complement may occur in any immune mechanism is attacking the patient's own RBC's.
- **This mechanism could be autoimmunity, alloimmunity or a drug-induced immune-**

Direct Coombs test / Direct antiglobulin test



Blood sample from a patient with immune mediated haemolytic anaemia: antibodies are shown attached to antigens on the RBC surface.

The patient's washed RBCs are incubated with antihuman antibodies (*Coombs reagent*).

RBCs agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs.

Thank You

EXCHANGE TRANSFUSION

DEFINITION

Withdrawing a baby's blood which has high bilirubin content and replacing it with fresh blood through umbilical vein.

AIMS

1.To correct anemia by replacing the Rh positive sensitized red cells.

2.To remove the circulatory antibodies

3.To eliminate circulatory bilirubin

INDICATIONS

1Non obstructive jaundice with serum bilirubin level of 20mg/dl or more in full term and 15mg/dl in preterm infants, e.g. Rh or ABO incompatibility.

2. Kernicterus ir. respective of serum bilirubin level.

CONTD.

3.Hemolytic disease of the newborn under following situations:

- Cord Hb 10g/dl or less.
- Cord bilirubin 5mg/dl or more.

CONTD.

- Rise of serum bilirubin of more than 1mg/dl/hr
- Maternal antibody titer of 1:64 or more, positive direct Coomb's test and previous history of severely affected baby

Equipment required

- Radiant warmer
- Respiratory support : Ventilators, ET tube, AMBU bag etc.
- Suction equipment
- Multi-Channel Monitor: Heart rate, RR and SpO2
- Umbilical catheterization set
- NG tube and umbilical catheter
- Disposable syringes: 20cc, 10cc, 5cc, 2cc
- Sterile gloves
- I/V tubings
- Waste recipticle

Estimated Blood Volume

Age Group	Volume (mL/kg)
Premature	90-100
Neonate (< 1 month)	80-90
Infant (3 months - 3 years)	75-80
Children > 6 yrs.	65-70

CHOICE OF DONOR BLOOD

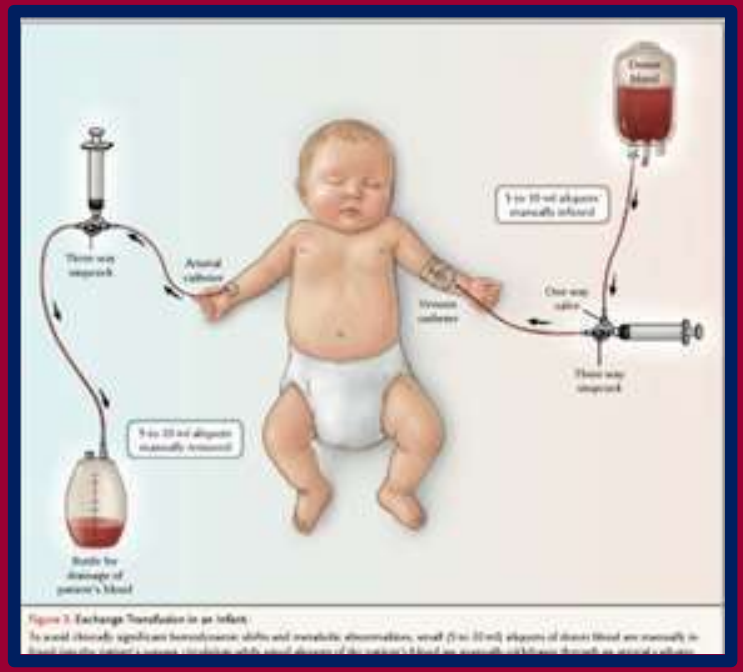
- The donor blood should be fresh(less than 3 days old)
- The amount needed for an adequate exchange is about 160ml/kg(double the blood volume of newborn)
- The blood should be cross matched against infant's blood.
- It should be made sure that blood is slowly warmed to infant's temperature.

CONTD.

- Fresh heparinized blood or blood preserved with acid citrate dextrose is used.
- In Rh incompatibility the transfusions are performed with group O, Rh negative blood whereas in case of ABO incompatibility and G-6-PD deficiency the procedure has to be performed with the same ABO and Rh groups of the baby.

CONTD.

10 – 20 ml are replaced each time



PROCEDURE

NURSING ACTION

- Explain procedure to parents.
- Get informed consent from the parent.
- Collect the blood from blood bank and check the blood type and group against the neonate's blood before administering.

CONTD.

- Procedure should be carried out in an incubator maintaining the temperature at 27 – 30°centigrade.
- NPO should be maintained 4 hours before procedure. Stomach should be aspirated before the exchange.
- Expose and immobilize the baby on cross splint.

CONTD.

- Open dressing pack and assist in cleaning of umbilical stump.
- Assist in cleaning the umbilical cord.

CONTD.

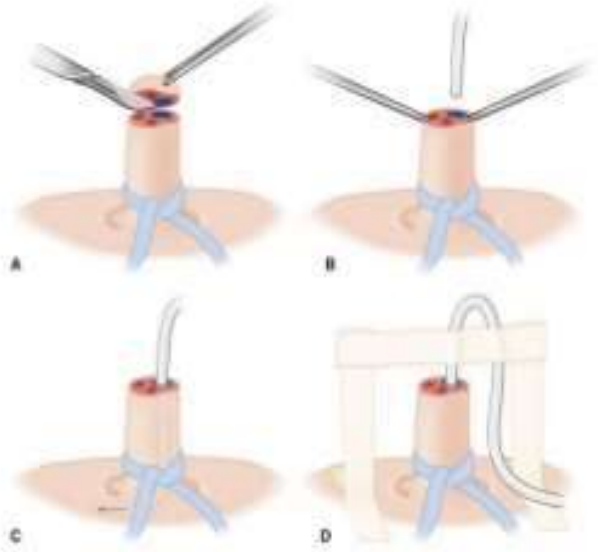
- Umbilical cord cut to less than 2.5 cm from the skin surface.
- Attach ligature loosely round the base of the cord. Insert umbilical catheter into the vein.
- The catheter should be filled with a flushing solution, or donor blood before insertion.

CONTD.

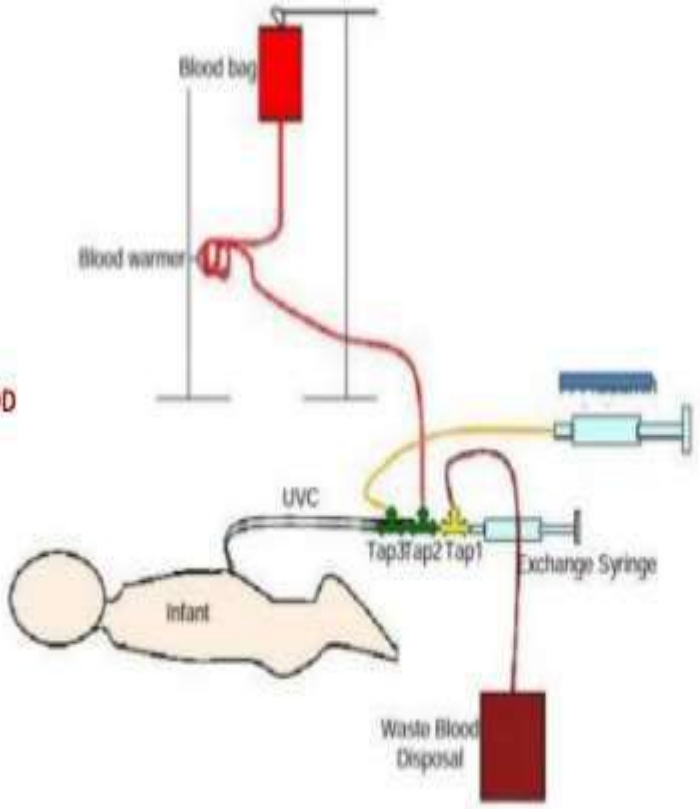
- When free flow of blood is obtained, ligature is tightened and catheter should be deep enough to reach inferior vena cava.
- Make sure that heat source is available throughout the procedure.
- Measure CVP after insertion of catheter into the umbilical vein.

Steps of procedure

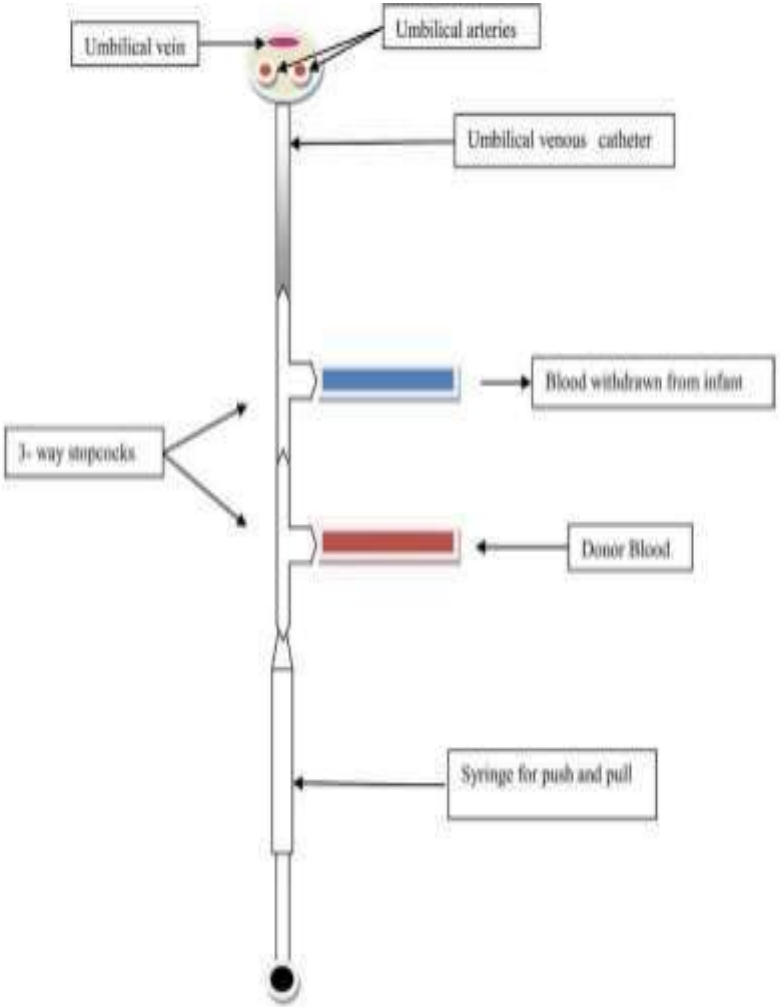
1. Umbilical Vein Catheterization



2. Exchange of blood



PUSH- PULL METHOD


Umbilical vein
Umbilical arteries
Umbilical venous catheter
Blood withdrawn from infant
3- way stopcocks
Donor Blood
Syringe for push and pull

CONTD.

- Take sample of pre exchanged blood as well as after exchange for investigation.
- Monitor heart rate, respiratory rate and condition of the baby hourly during the procedure.

CONTD.

- The physician removes 10 ml of the umbilical blood and replaces with 10ml of fresh blood immediately, until calculated volume is exchanged
- Apply cord tie at umbilicus, seal umbilicus with tincture benzoin, apply small gauze and secure with adhesive.

CONTD.

- Replace equipment and start phototherapy.
- Document time of starting, duration, completion time, amount and type of blood exchanged, condition of baby during and after procedure, drugs given during procedure and samples sent to the lab.

POST TRANSFUSION CARE

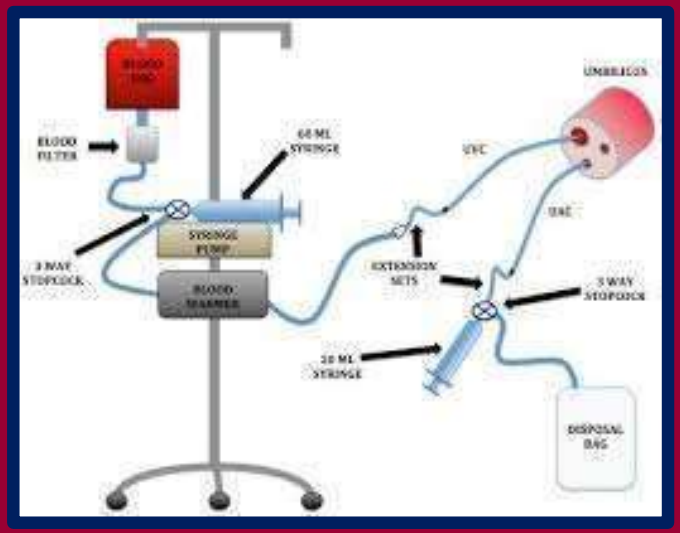
- Place the baby in a radiant warmer.
- Inspect umbilicus for evidence of bleeding.
- Repeat serum bilirubin as required.
- Check infant’s blood glucose regularly.

COMPLICATIONS

- Bacterial sepsis
- Thrombocytopenia
- Portal vein thrombosis
- Umbilical vein perforation
- Dysrhythmia
- Cardiac arrest

CONTD.

- Hypocalcemia
- Hypoglycemia
- Hypomagnesemia
- Metabolic acidosis
- Alkalosis
- HIV, Hepatitis B infection



SPECIAL CONSIDERATIONS

- If citrated or heparinized donor blood is used, one should be prepared for hypocalcemia, hypoglycemia, hyperkalemia and metabolic acidosis.
- Citrated blood leaves the infant with low calcium levels, so as a precaution calcium Gluconate at regular intervals should be given.

CONTD.

- For every 100ml of blood transfused one mili equivalent of sodium bicarbonate is given to combat metabolic acidosis.

Haemolytic Anaemias due to Extrinsic Factors

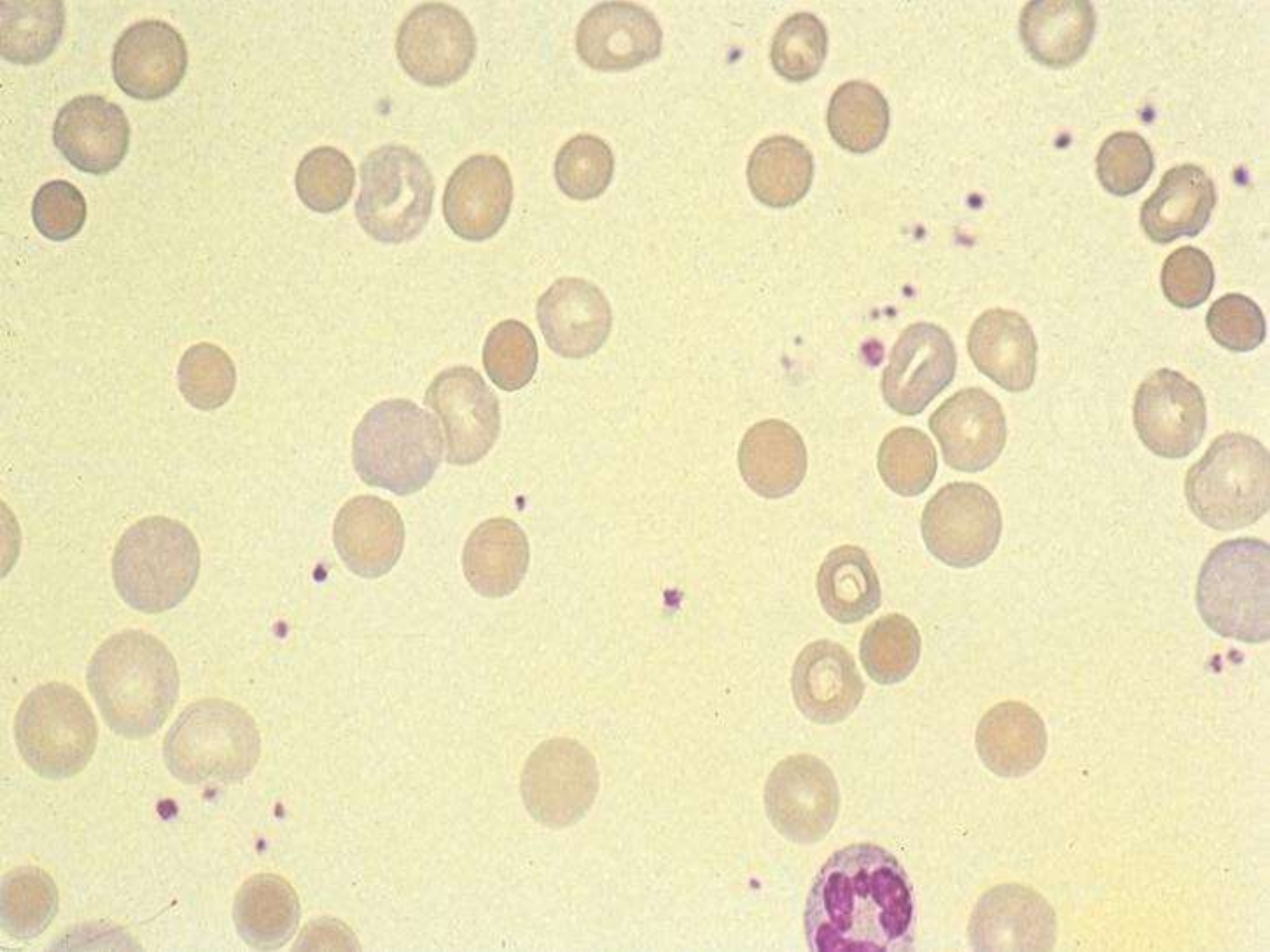
Immune Haemolytic anaemias (IHA)

Definition and Classification

- **Immune haemolysis is defined as red cell destruction brought about by antibody antigen reaction, antibodies are usually directed against red cell antigens. The defining character of all IHA is a positive direct antiglobulin (DAT or Coombs) test.**
- **Classification:**
  - **Autoimmune H. A.: Antibodies produced by the individual himself**
    - **Warm antibody type**
    - **Cold antibody type (cold agglutinin syndromes)**
  - **Alloimmune H.A : antibodies and antigens belong to different individuals:**
    - **Haemolytic disease of the newborn (HDN).**
    - **Incompatible blood transfusion.**
  - **Drug induced IHA:**

Warm AB type AIHA

- **The antibody is IgG and has a maximal activity around 37^0C. Antibody coated RBCs are destroyed extravascularly by the cells of the RE system mainly in the spleen.**
- **The disease affects females more commonly, the onset is usually insidious with jaundice , anaemia and splenomegaly.**
- **Haematologically: RBCs are normochromic, normocytic with spherocytosis, normoblastaemia and marked reticulocytosis.**



Aetiology of warm type AIHA

- **Idiopathic in 30 % of cases.**
- **Secondary to:**
  - **Lymphoproliferative disorders (CLL, HD and NHL)**
  - **Autoimmune disorders (SLE, RA and ulcerative colitis).**
  - **Infections (viral)**
  - **Carcinomas (ovarian ca.)**
  - **Drugs (methyldopa)**

Diagnosis of warm type IHA

- **Diagnosis depends on:**
  - **Clinical findings**
  - **Classical red cell morphology.**
  - **A positive direct Coombs test**

- **If transfusion is needed, these patients present a problem to the blood bank as it is almost impossible to find a compatible blood, usually the least incompatible unit is chosen from a panel of blood units.**

Cold antibodies immune haemolytic anaemia

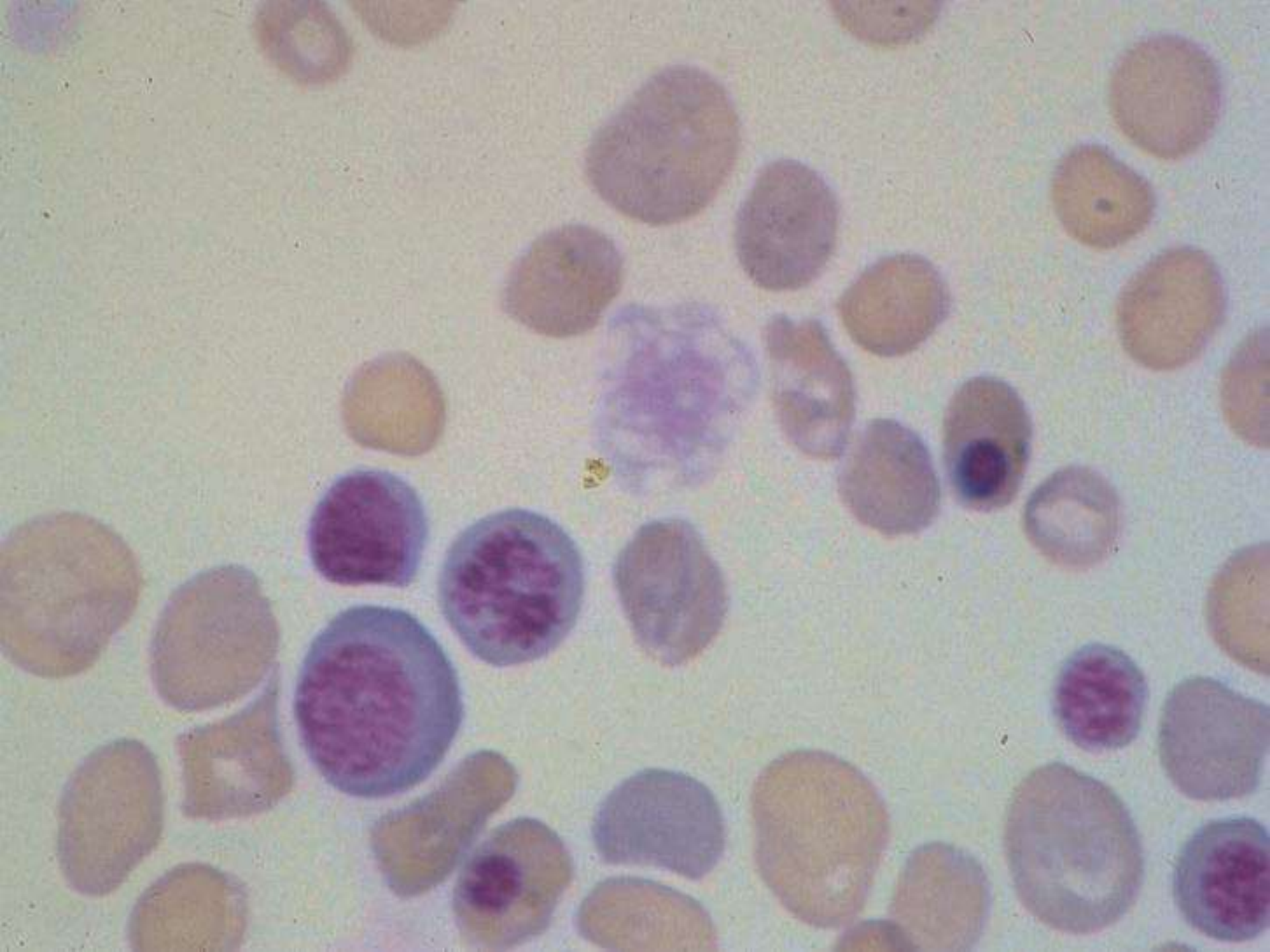
- 1) Cold haemagglutinin disease
  Can be primary or secondary to lymphoma
- Adenocarcinoma
- Mycoplasma pneumoniae
- Clinically , Acrocyanosis
- 2) Paroxysmal cold haemoglobinuria

Haemolytic Disease of the Newborn (HDN)

- **Destruction of fetal RBCs by maternal AB. Maternal IgG AB can pass the placental barrier and react with fetal red cell antigens, more commonly with antigens in the ABO and Rh systems.**
- **ABO HDN occur in blood group O^+ mothers who have in their sera immune anti-A & anti-B antibodies and carry a blood group A , B or AB fetus, the disease is most commonly mild and presents as NNJ, rarely needs exchange transfusion, it can affect the first pregnancy.**

Rh HDN (Erythroblastosis fetalis)

- **This is more serious than ABO HDN, first born baby is not affected, but at the time of delivery fetal RBCs pass to maternal circulation and the mother may become sensitized (produces anti-D antibodies), the second baby will usually have severe anaemia with severe jaundice (2^{nd} or 3^{rd} day) and may develop kernicterus with severe neurological defects unless promptly treated by exchange transfusion, subsequent deliveries result in still birth, the fetus has gross pallor, oedema , jaundice and gross abdominal distension with a bulky placenta (hydrops fetalis).**
- **Rh HDN affects only about 30% of Rh^{-ve} mothers carrying Rh^{+ve} babies, ABO fetomaternal incompatibility reduces sensitization.**
- **The blood picture shows anaemia with reticulocytosis and normoblastaemia (erythroblastosis fetalis).**



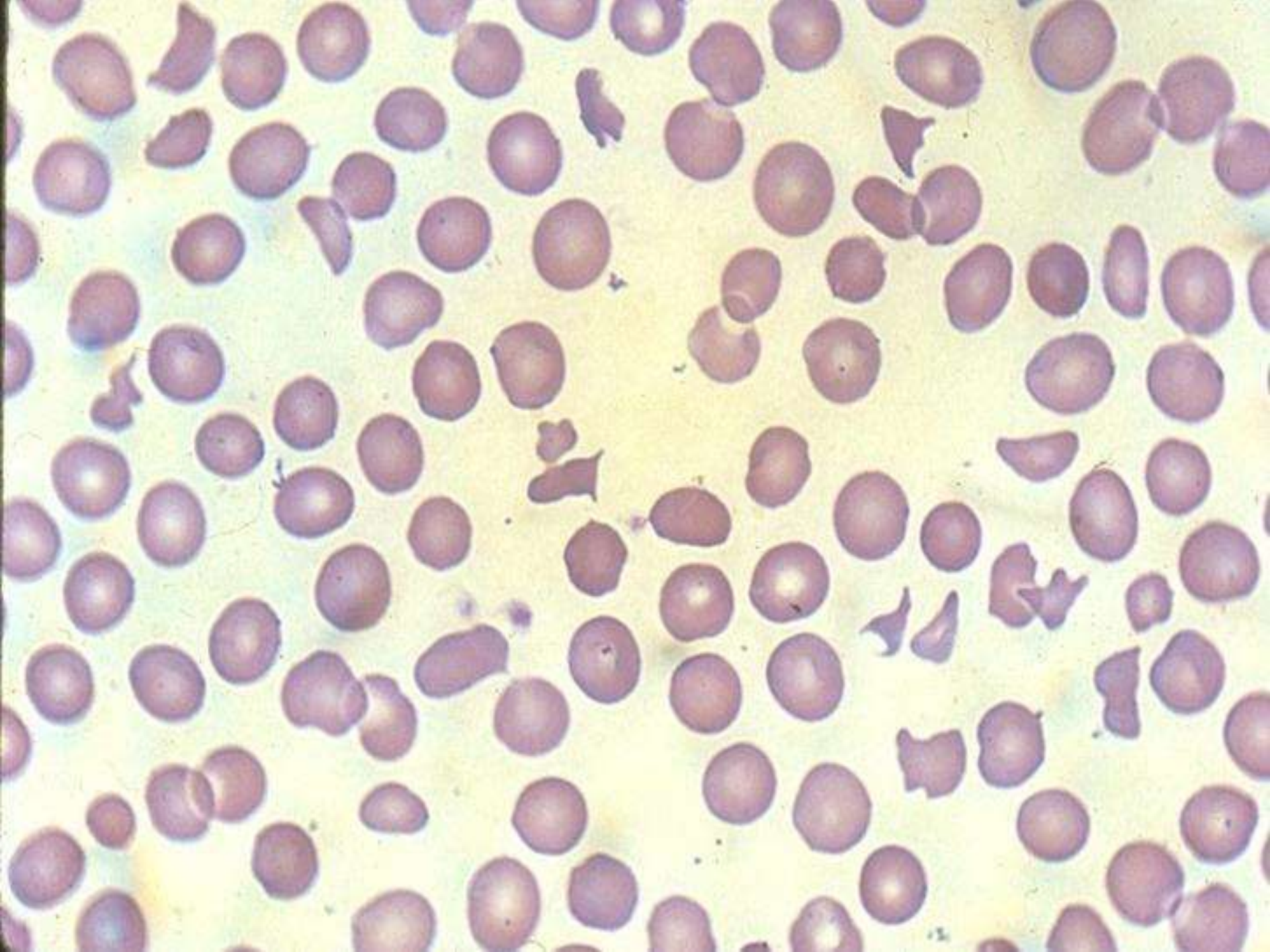
Mechanical anaemias
Fragmentation Anaemias

- **Fragmentation anaemias are group of haemolytic anaemias characterized by presence of fragmented RBCs in the peripheral blood (Schistocytes)and intravascular haemolysis.**
- **Fragmentation anaemias could result from:**
    - **Prosthetic cardiac replacements (valves and patches), associated with turbulent blood flow (cardiac haemolysis)**
    - **Red cell destruction in the small blood vessels " micro-angiopathic haemolytic anaemia (MAHA) " as a result of :**
        - **Wide spread fibrin deposition (DIC)**
        - **Abnormal platelet aggregation (platelet aggregate syndromes ; HUS & TTP).**
        - **Abnormal vascular endothelium (vasculitis)**

MAHA is characterized by thrombocytopenia in addition to schistocytosis & features of intravascular haemolysis.

March haemoglobinuria

- In long marches or marathon running
- Karate sports



Miscellaneous other acquired

- Haemolytic toxins & chemical
- Cl welchii
- Lead poisoning
- Spider & snake venoms
- Black water fever (falciparum malaria)
- Paroxysmal nocturnal haemoglobinuria

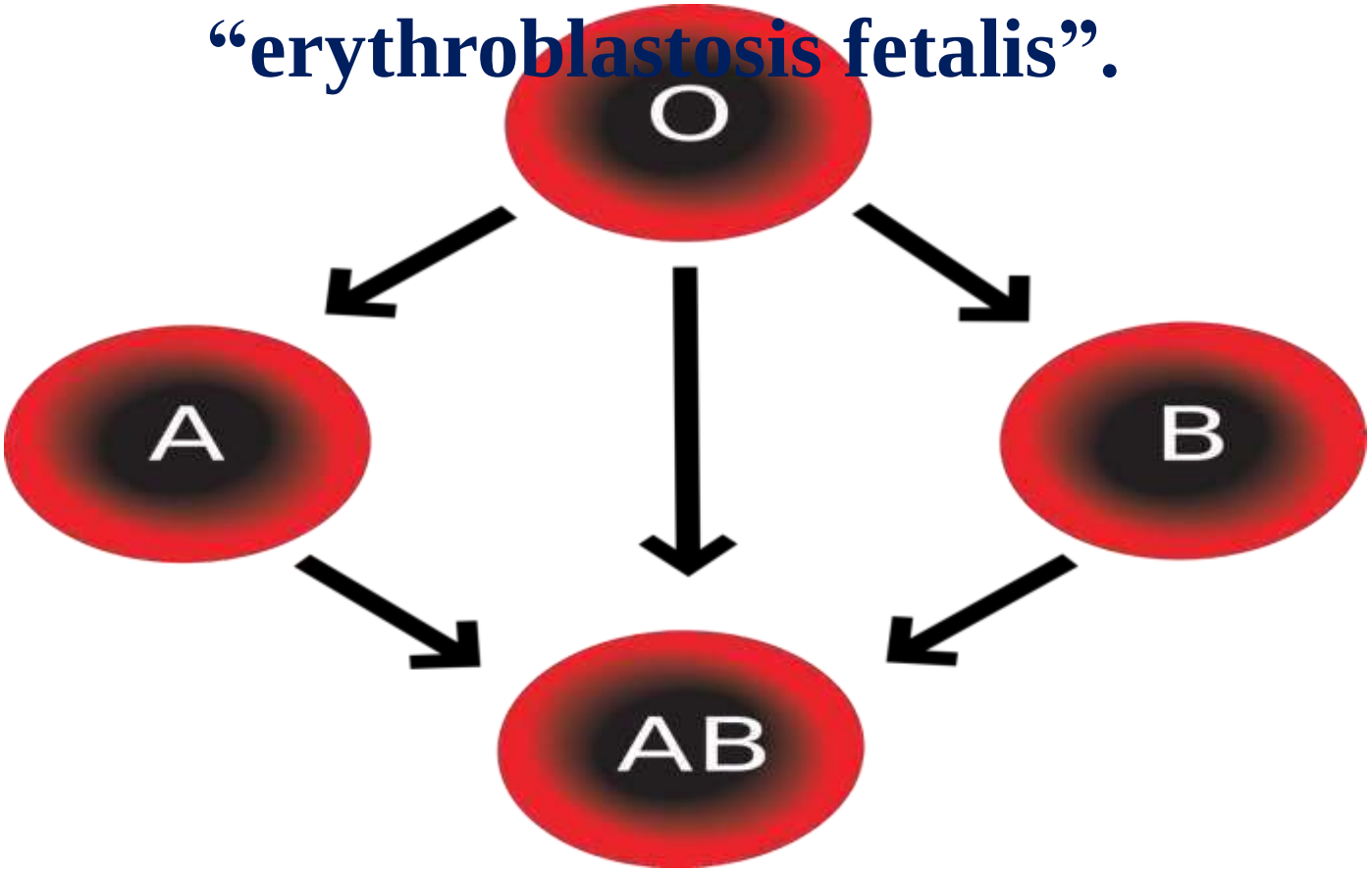
Hemolytic disease of newborn

Hemolytic disease of newborn

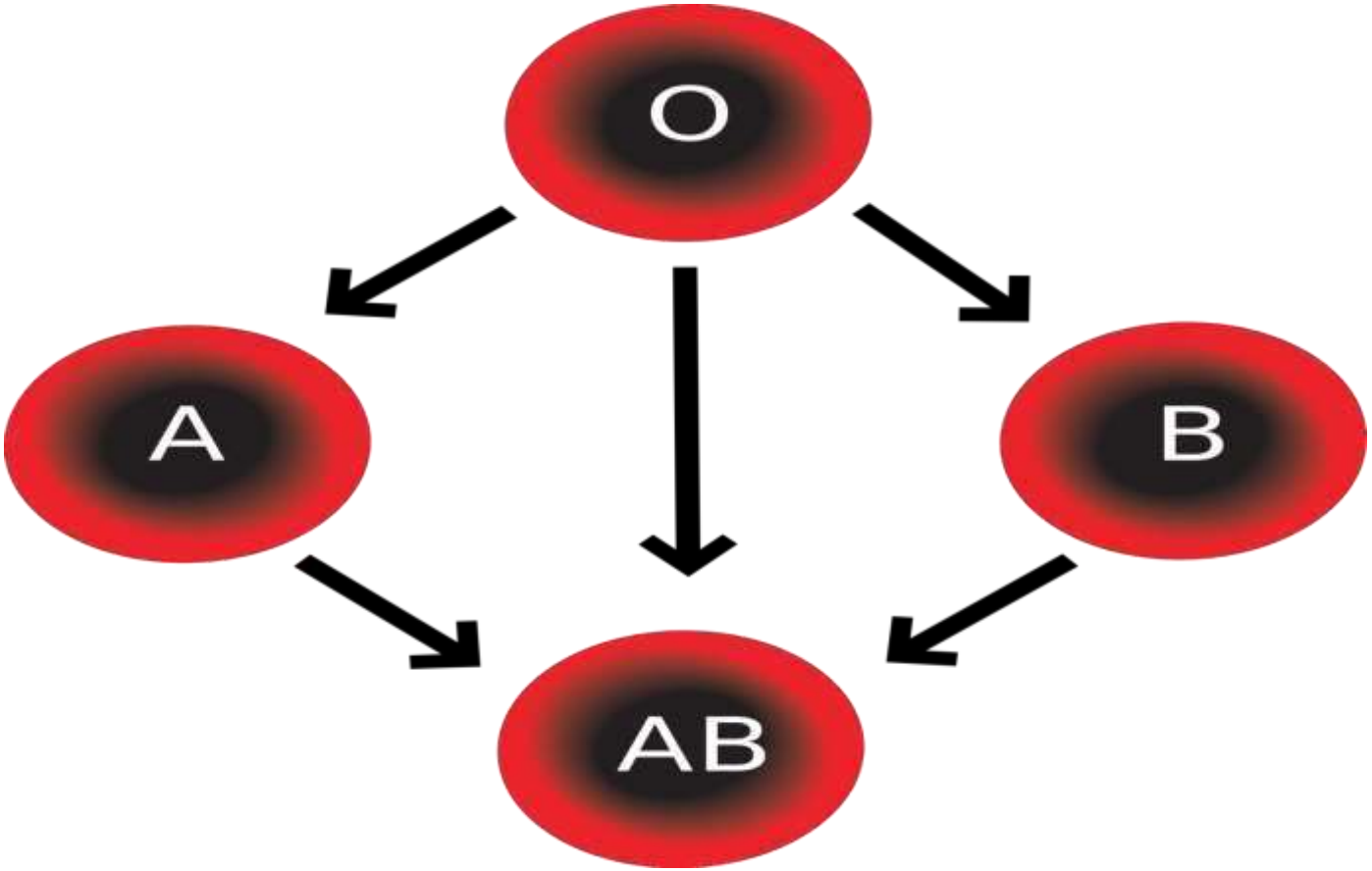
Hemolytic disease of the new born and fetus (HDN) is a destruction of the red blood cells (RBCs) of the fetus and neonate by antibodies produced by the mother

It is a condition in which the life span of the fetal/neonatal red cells is shortened due to maternal allo-antibodies against red cell antigens acquired from the father

increases production of red cells ,many of which enter the circulation prematurely as nucleated cells hence the term "erythroblastosis fetalis".



- **Also called Hydrops fetalis as Severly affected fetuses may develop generalized edema, called “Hydrops fetalis”**



Types of HDN

- ABO hemolytic disease of New born
- Other blood group HDN

E.g. Rh blood group, kell blood group, kid etc

Pathogenesis

↓

Fetomaternal Hemorrhage

↓

Maternal Antibodies formed against fetus derived antigens

↓

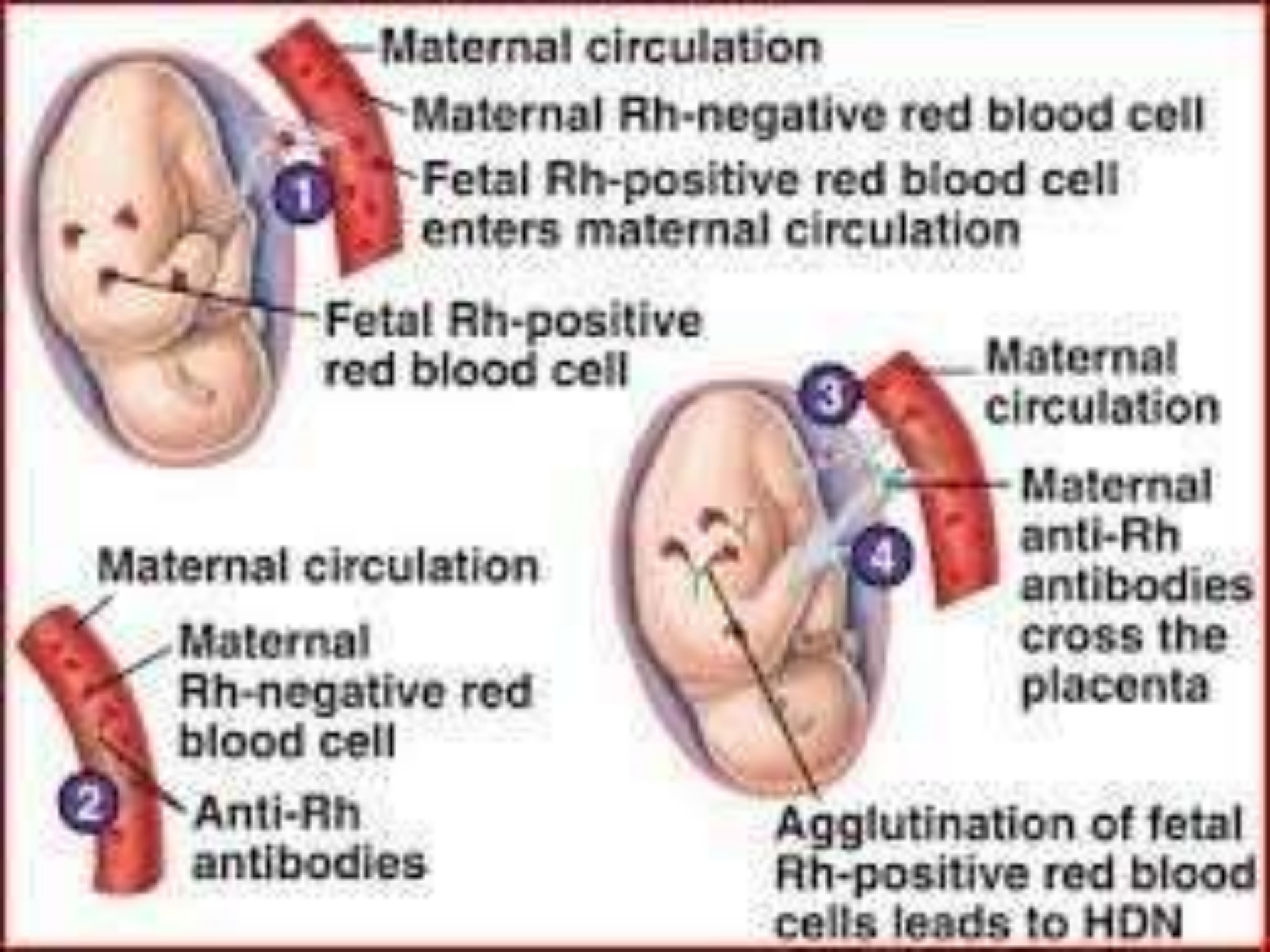
During subsequent pregnancy, placental passage of maternal IgG antibodies

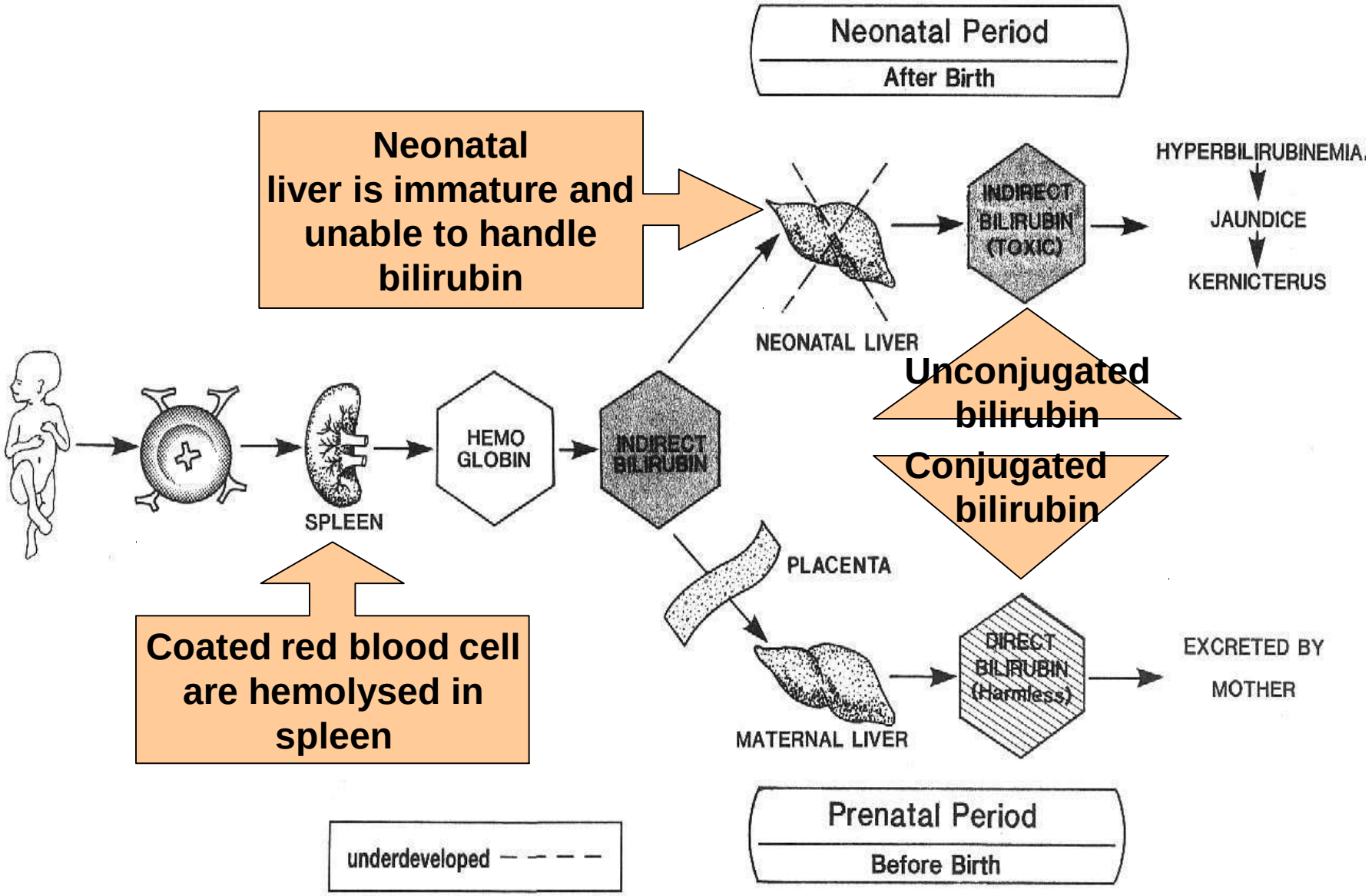
↓

Maternal antibody attaches to fetal red blood cells

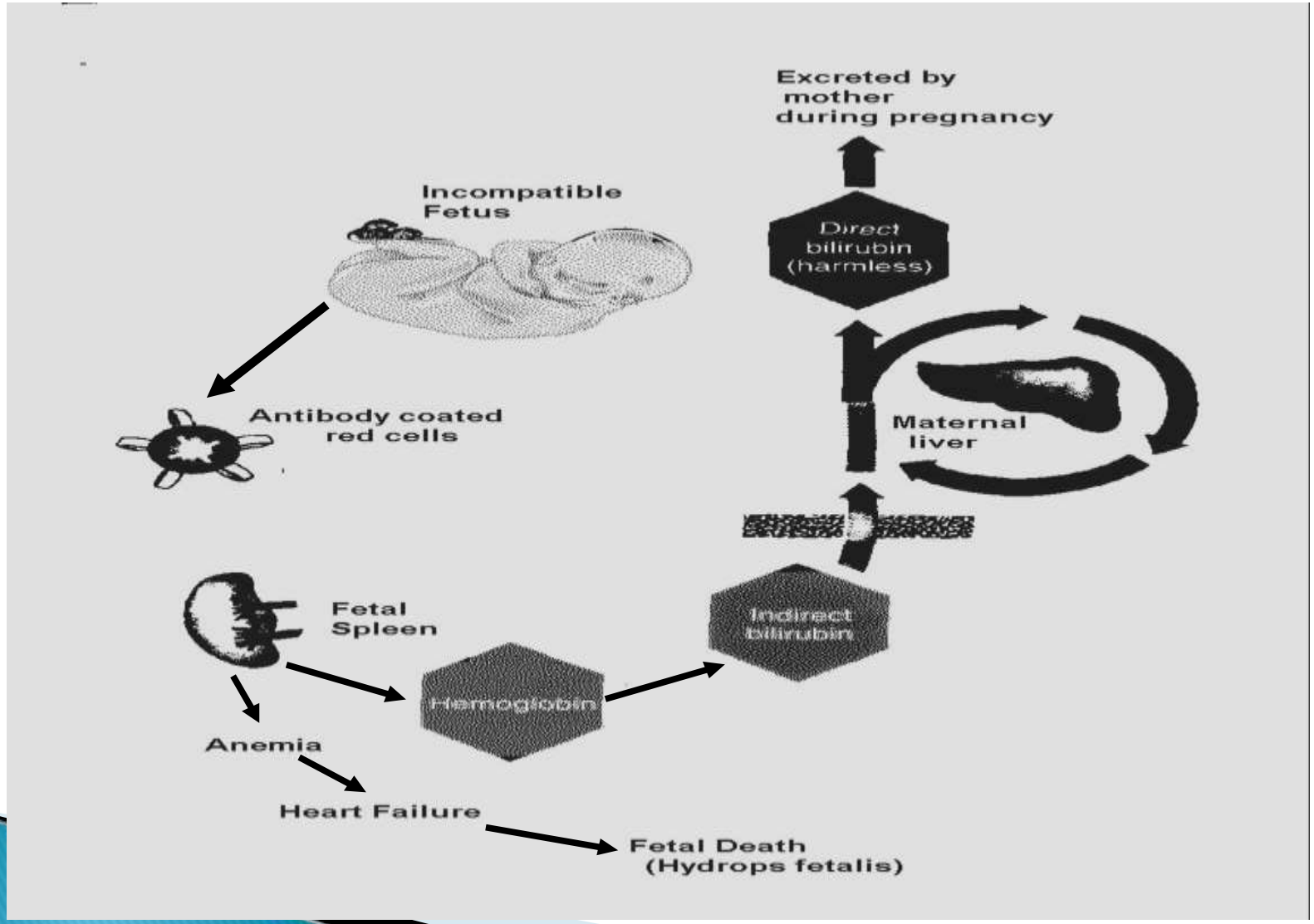
↓

Fetal red blood cell hemolysis

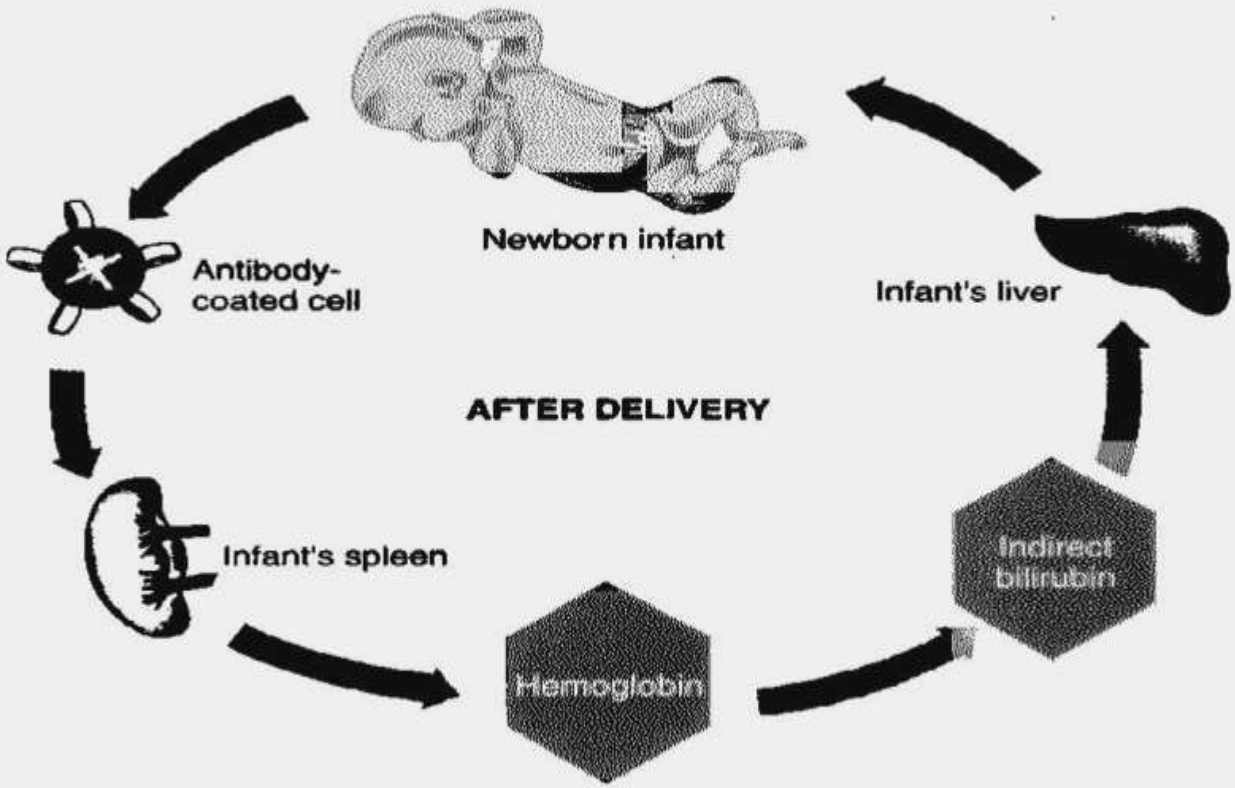

Maternal circulation
Maternal Rh-negative red blood cell
Fetal Rh-positive red blood cell enters maternal circulation
1
Fetal Rh-positive red blood cell
Maternal circulation
Maternal Rh-negative red blood cell
2
Anti-Rh antibodies
3
Maternal circulation
Maternal anti-Rh antibodies cross the placenta
4
Agglutination of fetal Rh-positive red blood cells leads to HDN


Neonatal Period
After Birth
Neonatal liver is immature and unable to handle bilirubin
HYPERBILIRUBINEMIA
JAUNDICE
KERNICTERUS
INDIRECT BILIRUBIN (TOXIC)
NEONATAL LIVER
Unconjugated bilirubin
Conjugated bilirubin
HEMO GLOBIN
INDIRECT BILIRUBIN
SPLEEN
PLACENTA
Coated red blood cell are hemolysed in spleen
DIRECT BILIRUBIN (Harmless)
EXCRETED BY MOTHER
MATERNAL LIVER
underdeveloped
Prenatal Period
Before Birth

Pathogenesis; before birth

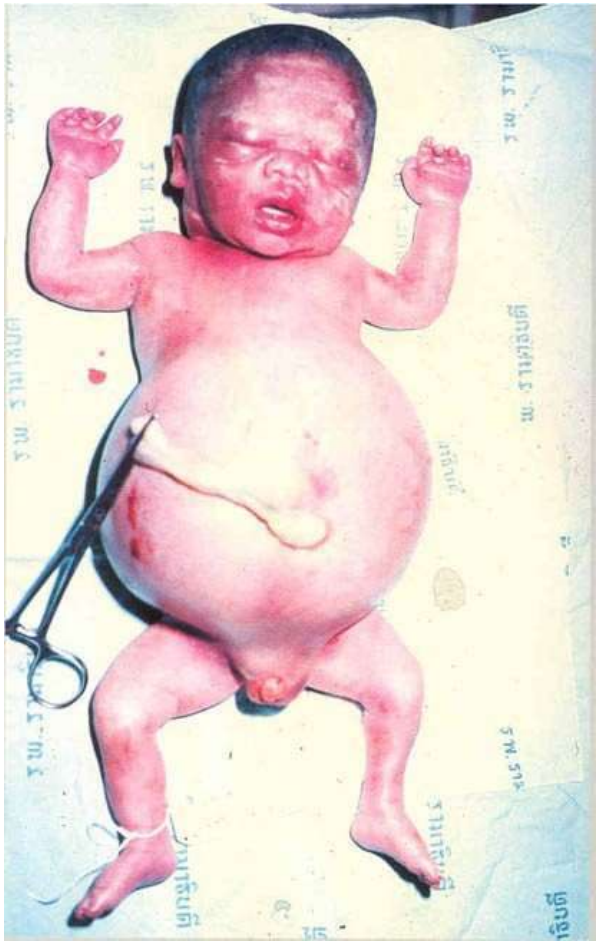


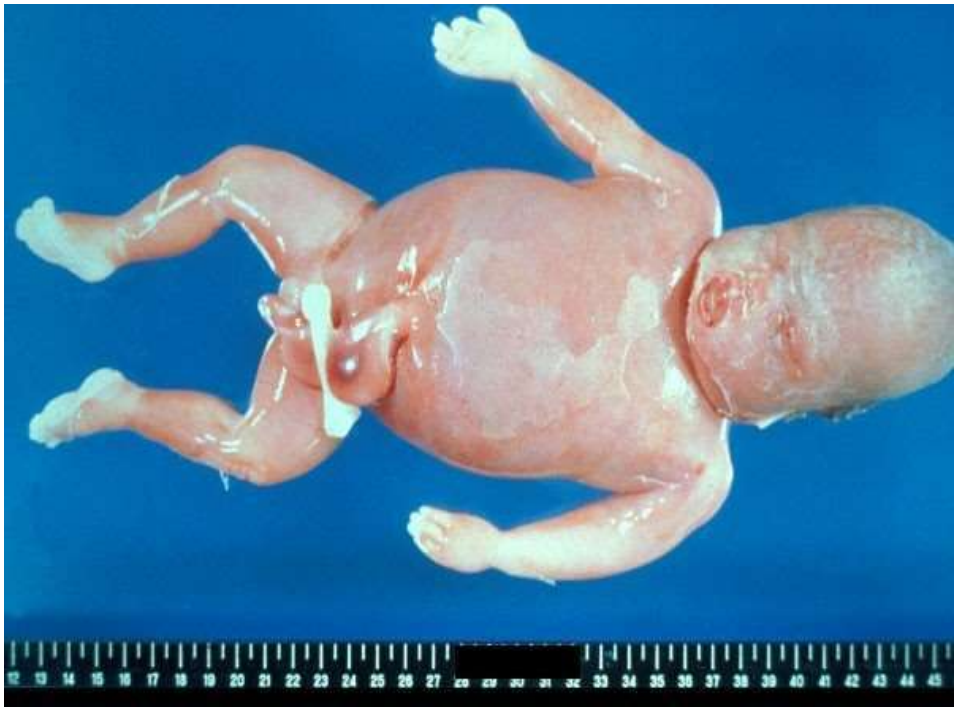
Pathogenesis; after delivery



Factors affecting immunization and severity

- Antigenic exposure
- Host factors
- Antibody specificity





Clinical Presentation

- Varies from mild **jaundice** and **anemia** to **hydrops fetalis** (with ascites, pleural and pericardial effusions)
- Chief risk to the **fetus is anemia**.
- Extramedullary hematopoiesis due to anemia results in **hepatosplenomegaly**.
- **Postnatal problems** include:
  - **Asphyxia**
  - **Pulmonary hypertension**
  - **Pallor** (due to anemia)
  - **Edema** (hydrops, due to low serum albumin)
  - **Respiratory distress**
  - **Coagulopathies** (↓ platelets & clotting factors)
  - **Jaundice**
  - **Kernicterus** (from hyperbilirubinemia)

Kernicturus

- **Kernicterus** (bilirubin encephalopathy) results from high levels of indirect bilirubin (**>20 mg/dL in a term infant with HDN**).

- Affected structures have a bright yellow color.
- **Unbound unconjugated bilirubin crosses the blood-brain barrier** and, because it is lipid soluble, it penetrates neuronal and glial membranes.
- Bilirubin is thought to be toxic to nerve cells
- The mechanism of neurotoxicity and the reason for the topography of the lesions are not known.

Laboratory Findings

- CBC:

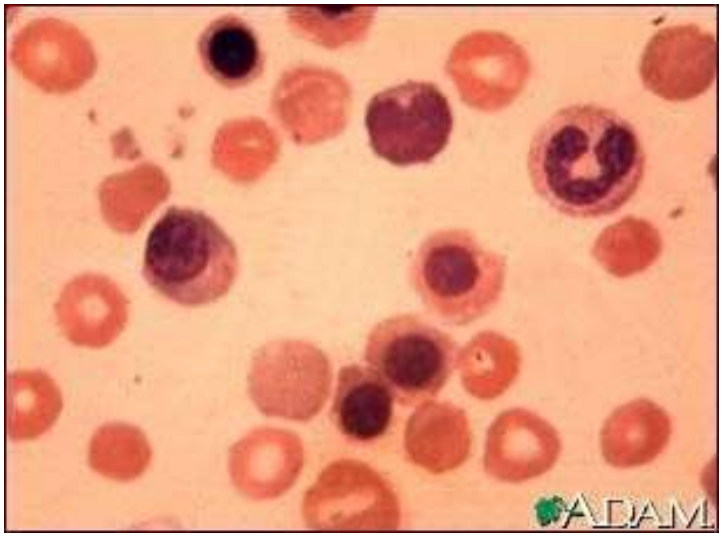
TLC: normal Hb: Decrease

MCV, MCH, HCHC : Normal or Increase

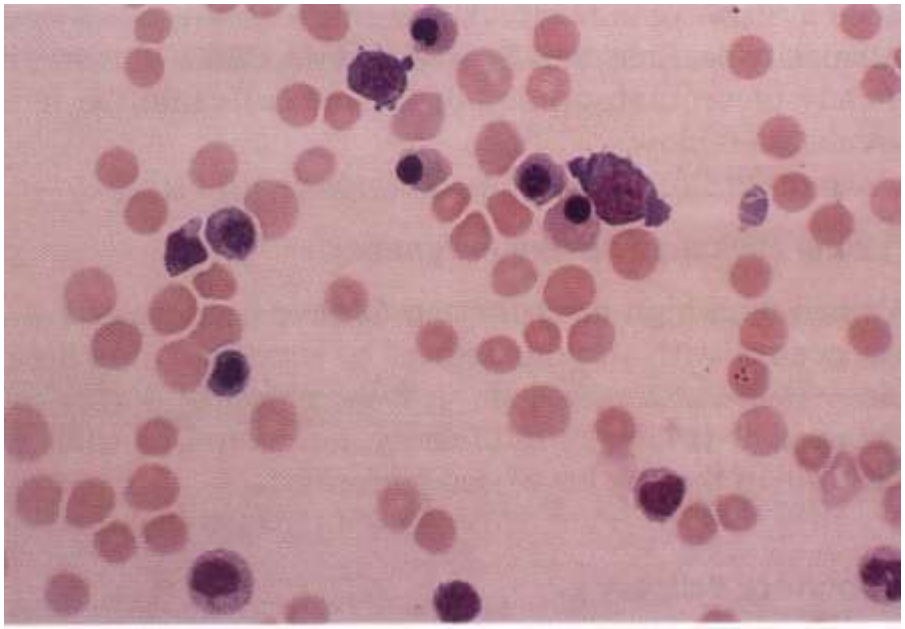
Platelets: Normal to Decrease

Reticulocytosis (6 to 40%)

Blood Smear



- Polychromasia
- Anisocytosis
- Increase NRBCs
- no spherocytes



- Blood Banking test: Blood grouping
- Mother: Rh Negative
- Father: Rh Positive
- Baby: Rh Positive
- Direct Coombs test: Positive

Biochemical test

- **Hyperbilirubinemia**
- **Hypoalbuminemia**
- **LDH: Increase**
- **Haptoglobin Decrease**

Prevention of Rh- HDN

- Prevention of active immunization
  - Administration of corresponding RBC antibody (e.g anti-D)
  - Use of high-titered Rh-Ig (Rhogam)

- Calculation of the dose
  - Kleihauer test for fetal Hb

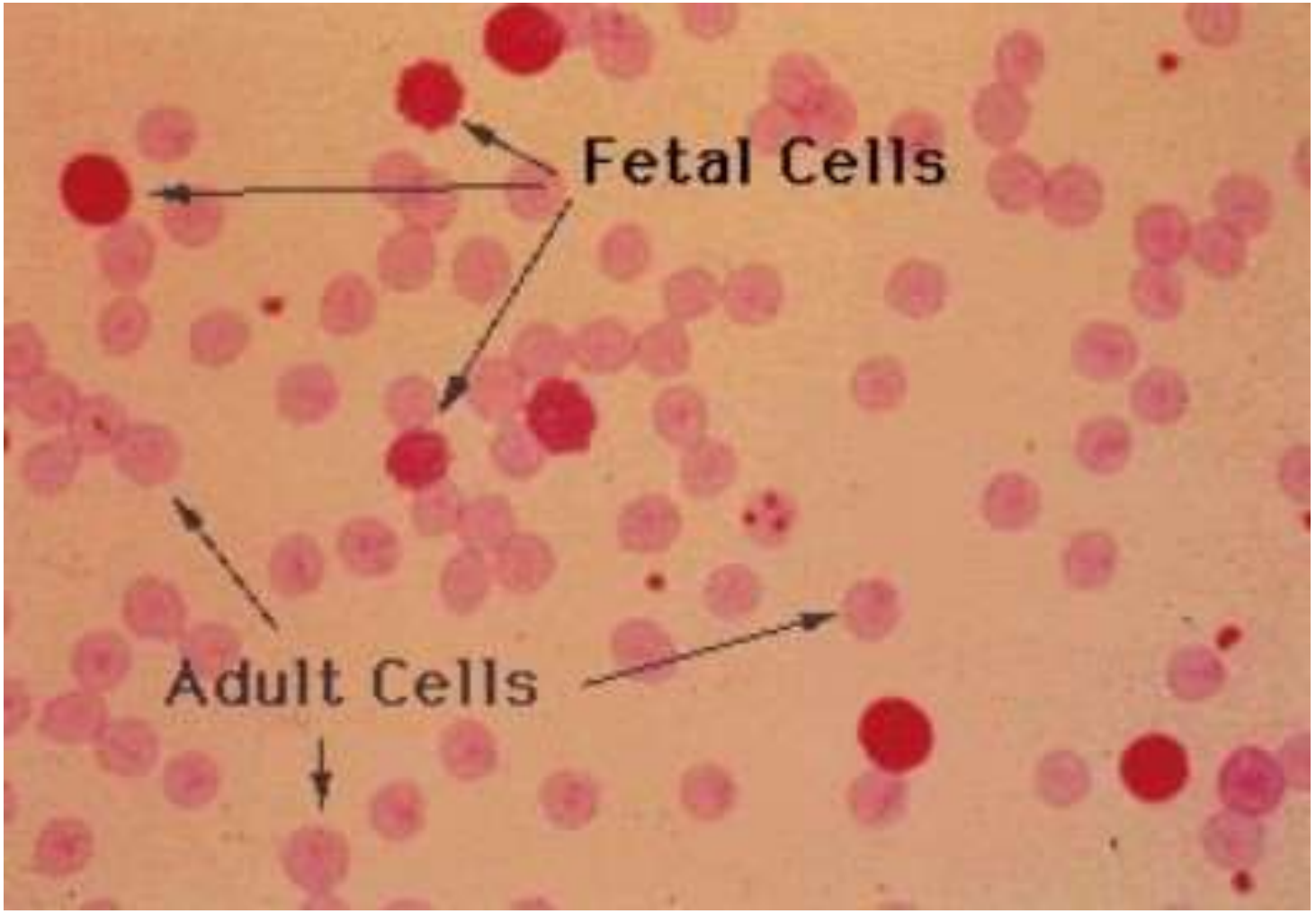
MEASUREMENT OF FETOMATERNAL HAEMORRHAGE

KLEIHAUER-BETKE TEST – Based on acid elution technique. Fetal and maternal RBC have different response to acid elution.

Maternal cells (adult Hb) get eluded leaving behind only cell membrane and hence appear as swollen round large "*GHOST CELLS*" against normal fetal cells whose Hb remain unaltered hence look as red refractile round cells .

If in 40 low power fields of maternal peripheral blood 80 fetal RBC's are found- it is estimated that 4ml of fetomaternal hemorrhage has occurred.

For 1ml of fetal blood 10ug of Rh anti D is needed. Thus 300ug anti D will be sufficient for 30 ml of fetal blood which has entered the maternal circulation.


Fetal Cells
Adult Cells

ABO HDN

In a group O mother with naturally occurring anti-A and anti-B of the IgG subclass which can cross the placenta.

HDN due to ABO incompatibility occurs when a group O mother with IgG anti-A or IgG anti-B is carrying a fetus of blood group A or blood group B respectively.

The most common presentation of ABO HDN is jaundice (un-conjugated hyperbilirubinaemia).

ABO HDN contd.

- Signs and symptoms
  - Two mechanism protects the fetus against anti-A and anti-B
    - Relative weak A and B antigens of fetal red cells
    - Widespread distribution of A & B antigen
    - in fetal tissue diverting antibodies away from fetal RBCs
  - Anemia is most of the time mild
  - ABO- HDN may be seen in the first pregnancy

Summary.

- Hemolytic disease of newborn occurs when IgG antibodies produced by the mother against the corresponding antigen which is absent in her, crosses the placenta and destroy the red blood cells of the fetus.
- Proper early management of Rh- HDN saves lives of a child and future pregnancies
- ABO- HDN is usually mild
- Other blood group antigens can also cause HDN

Haemorrhagic Disorders

Dr. Bashar
Department of Pathology
Mosul Medical College

Haemorrhagic Disorders

These include

- Disorders of platelets.
- Disorders of blood vessels.
- Disorders of coagulation & fibrinolysis.

Platelet Disorders

- Quantitative : Thrombocytopenia.
- Qualitative : Platelet defects.

Thrombocytopenia

- Thrombocytopenia exists when platelet count is less than 150×10^9 /L .
- Normal platelet count = $150 – 400 \times 10^9$ /L
- Bleeding is unusual when count is $>50 \times 10^9$ /L
- Spontaneous bleeding occurs when count is $< 20 \times 10^9$ /L

Causes of Thrombocytopenia

- 1.***decresed platelet production***

Characterized by reduction of megakaryocytes in bone marrow & by small mean size of circulating platelets (Mean Platelet Volume –MPV) and association with anaemia and leucopenia :

a. Aplastic anaemia.

b. Megaloblastic anaemia (decrease Vit. B12 or /and decrease folic acid).

c. Bone marrow infiltration by neoplasms.

d. Cytotoxic drugs (Dose Dependant).

e. Ionizing radiation (Dose Dependant).

f. Drugs; cause thrombocytopenia in some recipients : Metheprim, Phenylbutazone, Gold compounds .

g. Alcohol.

2. *Increased destruction of platelets*

Characterized by normal or increased numbers of megakaryocytes in bone marrow , circulating platelets appear larger than normal (raised MPV) and that platelets are usually only affected (no anaemia or leucopenia).

Causes of Increased Destruction of Platelets

- **hypersensitivity to drugs**

  Occurs suddenly following single dose drugs act as a hapten forming antigenic complex by binding to plasma protein and then antibody (usually IgG) is formed against this complex , this antigen-antibody complex then binds to platelets leading to destruction by phagocytosis usually in the spleen .

- Drugs : Chlorothiazides , Digoxin , Methyl-dopa ,PAS (para-aminosalicylic acid), Quinine, Quinidine, Sulphonamides .

Autoimmune Thrombocytopenia

Autoantibodies usually of IgG class either as

- isolated disorder :idiopathic (immune) thrombocytopenic purpura (ITP)
- in association with other autoimmune disorders : SLE ,myasthenia gravis ,Evan's syndrome(autoimmune hemolytic anemia + autoimmune thrombocytopenia), lymphoma , chronic lymphocytic leukaemia

ITP (Idiopathic {Immune} Thromocytopenic Purpura)

- Occurs chiefly in children and young adults

Character	Children	Adults
Behavior (onset)	Acute (sudden)	Chronic (insidious)
Peak age incidence	2-8 years	20-40 years
Sex	F=M	3F:1M
Duration	<6 months (usually weeks)	> 6 months (often years)
Associated disorders	Preceding viral infection	None

- Responsible antibody usually belongs to subclass 3 of IgG.
- Clinically
  - Varies from mild cutaneous bleeding to gross uterine or GIT hemorrhage .
  - In severe cases it lead to intracerebral hemorrhage .
- Treatment
  - Steroids
  - Immunosuppressive drugs
  - Splenectomy

Blood: Hb – Normal

WBC – Normal

Platelet count reduced

In severe cases 20 – 50 x 10^9/L.

In moderate cases (50 – 80) x 10^9/L.

Bleeding:

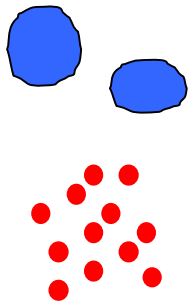
- Epistaxis
- GIT, GUT bleedings.

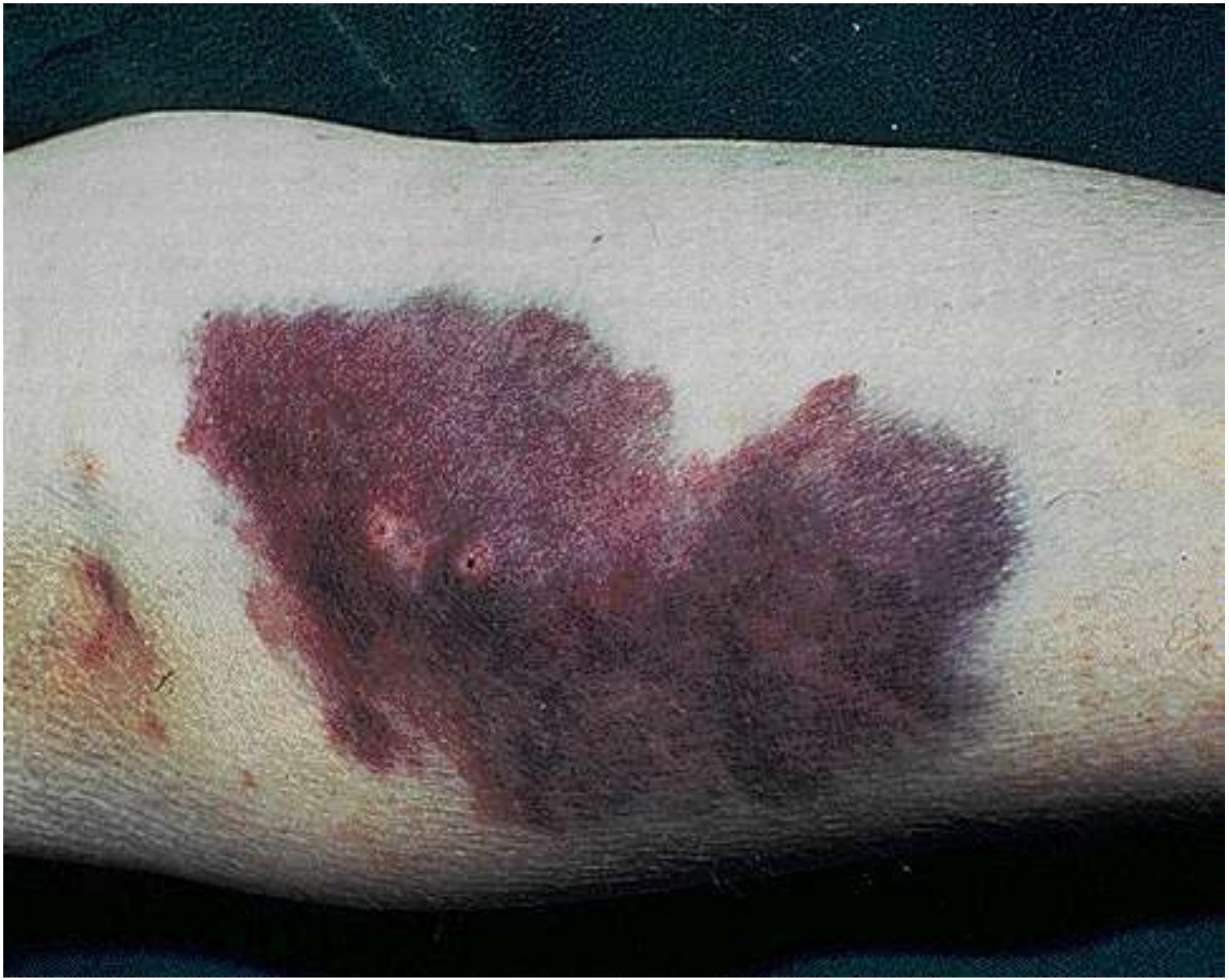
CNS " fatal" very rare.

Skin:

Ecchymosis (Bruises)

Petechie





- 3. ***Hypersplenism***

  Clinical syndrome :

- Enlargement o f the spleen.
- Reduction in one or more of cell lines of blood (anemia, leucopenia, thrombocytopenia).
- Normal bone marrow.
- Cure after splenectomy.

- 4.***DIC(disseminated intravascular coagulation)***

  This causes thrombocytopenia by excessive utilization & destruction of platelets .

- 5. ***Massive blood transfusion***

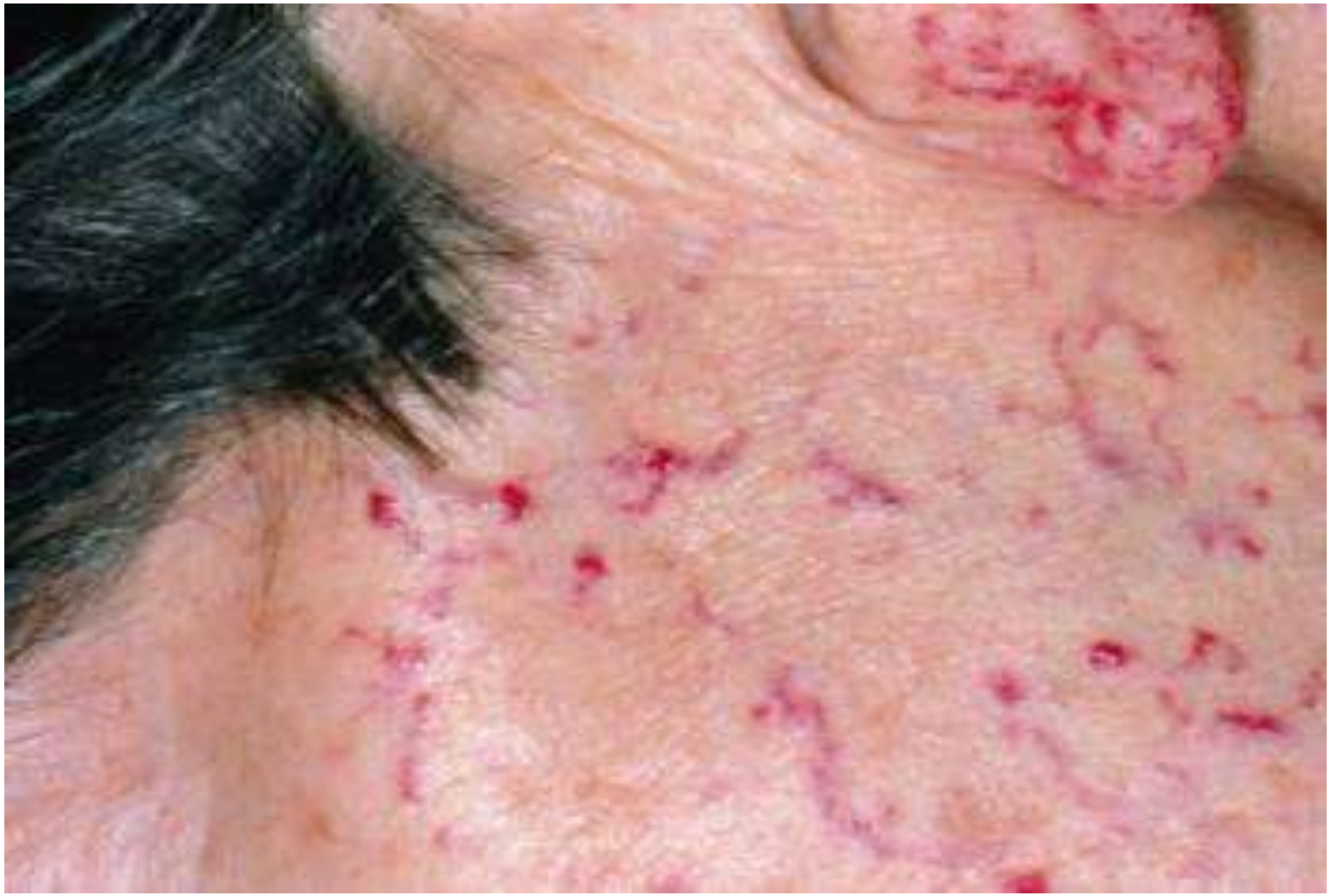
Qualitative Platelet Defects

- Platelet count is normal ,but there is defect in platelet aggregation .
- e.g. Glanzmann's disease (thrombosthenia, autosomal recessive)

Disorders of Blood Vessels (Vascular Purpra)

- ***Congenital :***
  - Hereditary Hemorrhagic Telagiectasia
    - Autosomal dominant
    - Clinically: usually epistaxis , multiple telangiectatic spots in the skin & mucus membranes leading to hemorrhage & iron deficiency anemia ,haemoptysis.


Baseline
After 3 doses of IV bevacizumab



- ***Acquired :***
- Purpura simplex in women .
- Senile purpura :on the dorsum of hands & arms due to poor capillary support from collagen as also in :
- Steroid therapy or Cushing syndrome
- Scurvy ,vit. C needed for polymerization of mucopolysaccharides necessary for collagen synthesis .

- Henoch Schonlein Purpura : necrotizing vasculitis give rise to small hemorrhages especially in the skin & gut ,there may be associated glomerulonephritis ,usually follow streptococcal infection.
- Damage to capillaries as in :
  - severe acute bacterial infection: septicaemia.
  - subacute bacterial endocarditis .

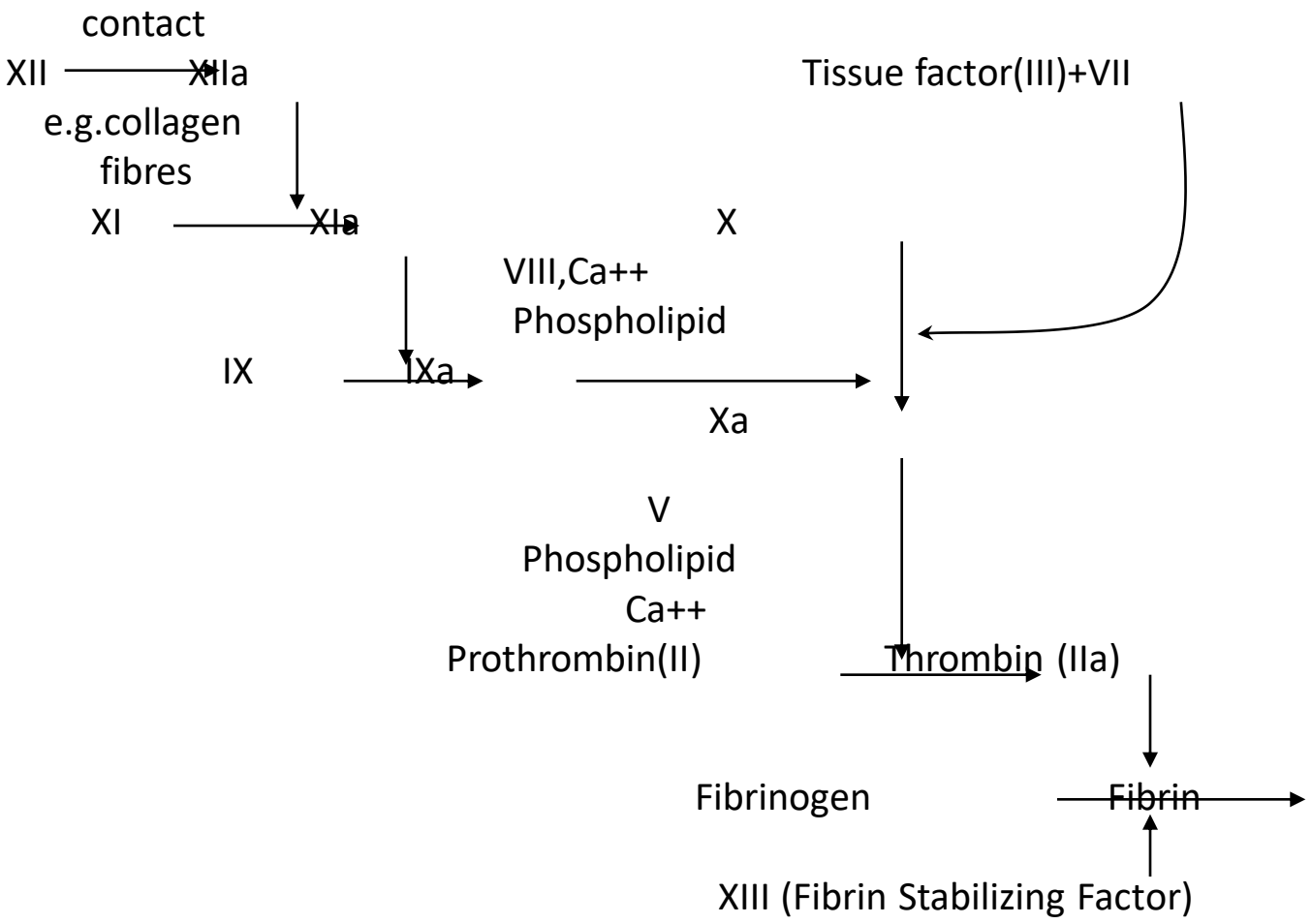
Henoch-Schonlein purpura

Symptoms, Diagnosis and Treatments

Neelima Hospital

promise of good health

Disorders of Coagulation



Inherited Disorders of Coagulation

- Of these coagulation factors deficiencies factor VIII deficiency is important .it can lead to Haemophilia A and von Willebrand's disease .

Structure of factor VIII

- Plasma factor VIII is now considered to be a complex of two components ;the larger of the two ,factor VIII /von Willebrand factor (VIII R: WF) is coded by autosomal genes and is deficient in von Willebrand ‘s disease , it promotes primary haemostasis by interacting with platelets and also appears to function as a carrier of smaller component factor VIII coagulant (VIII C) which is coded by an X chromosome which participates directly into cascade clotting reaction & is deficient in classical haemophilia ,when assayed immunologicaly these two components are expressed as antigen (Ag)

  i.e. VIII R: Ag and VIII C : Ag .

Haemophilia A

- Hereditary abnormality of coagulation.
- Sex linked : affect ♂ ,while ♀ are carriers .

- All sons of diseased ♂ are normal .
- All daughters of diseased ♂ are carriers .

X° Y XX

YX YX X° X X° X

Normal ♂ Carrier ♀

- 50% of daughters of carrier female are carriers .
- 50% of sons of carrier female are diseased .

XY X♌ X

XX♌ XX YX♌ YX

Clinically

- Male child will suffer from bleeding following circumcision , haemarthrosis usually after crawling .
- Severity of haemophilia is graded according to the level of VIII C into:

i. Severe (VIII C < 1% of normal).
ii. Moderate (2-3% of normal).
iii. Mild (5-20% of normal).

Diagnosis

- APTT ↑
- Clotting time either normal or ↑
- Bleeding time normal
- VIII C activity ↓
- VIII C : Ag ↓
- VIII R: Ag normal

Von Willebrand's Disease

- Inherited hemorrhagic disease in which bleeding time is prolonged due to deficiency of von Willebrand's factor (vllll R) as this factor is important for platelet adhesion to vascular subendothelium.

Comparison Between Haemophilia & von Willebrand's Disease

Character	Haemophilia A	Von willebrand's disease
Inheritance	Sex linked (♂ affected)	Autosomal (♂ & ♀)
Bleeding time	Normal	Prolonged
VIII C	↓	↓
VIII C: Ag	↓	↓
VIII R	Normal	↓

Factor IX deficiency (Haemophilia B or Christmas Disease)

- Inherited disorder shows the same pattern of inheritance as haemophilia A (sex linked).
- Same clinical picture but incidence of disease = 1/5th of the haemophilia A .
- Treated by factor IX concentrate .

Acquired Disorders Of Coagulation

- **Vitamin K deficiency**

Vitamin K is necessary for γ carboxylation of precursors of factor II (prothrombin) & some other coagulation factors. It is fat soluble ,present in leaf vegetables & also synthesized by the normal intestinal flora.

Dietary deficiency of sufficient severity to produce bleeding is well recognized in:

- Neonates (Haemorrhagic Diseases of the newborn) in whom normal bacterial flora is not yet established.
- In children & adults(malnourishment).
- ↓ absorption in billiary obstruction, coeliac disease.

Liver disease

- Liver is the site of synthesis of most coagulation factors.
- Severe impairment of liver lead to combined factor deficiency particularly II , VII ,IX ,X, &
- I (fibrinogen).

Renal Impairment

- Lead to thrombocytopenia ,platelet dysfunction ,(II ,VII ,IX ,X ,XIII) ,DIC.

Warfarin therapy

- Oral anticoagulant act as competitive inhibitor of vit. K ,suppressing the synthesis of four vit. K dependant clotting in the liver prothrombin (factor ll ,VII ,IX & X .

Control of Warfarin Therapy by

- Doing prothrombin time
- Control = seconds.
- Test = seconds.
- Test/control ratio (R) =
- INR (international normalized ratio) =
- Accepted INR = 2 - 3.5
- INR = (R)^s
- S= sensitivity index ,fixed figure provided by manufacturer of the kit (e.g S = 2)

Heparin therapy control

- Coagulation (Clotting) time
- Thrombin time
- Activated Partial Thromboplastin Time (APTT)

Disseminated Intravascular Coagulation (DIC)

- wide spread deposition of fibrin in the small vessels of many organs causing tissue necrosis & multiple organ dysfunction and subsequent bleeding state due to consumption of platelets & clotting factors and secondary enhancement of fibrinolytic activity . Microangiopathic haemlytic anaemia is a common accompaniment.

Causes of DIC

- Extensive burn
- Septicaemia
- Shock
- Liver disease
- Renal disease
- Complications of labour : retroplacental haemorrhage & aminotic fluid embolism.

DIC: Disseminated Intravascular Coagulation:

1) Bleeding: " Consumption coagulopathy"

↓ Platelets (severe)

↓ Coagulation factors (I, II, VIII, IX, X)

↑ Fibrinolysis

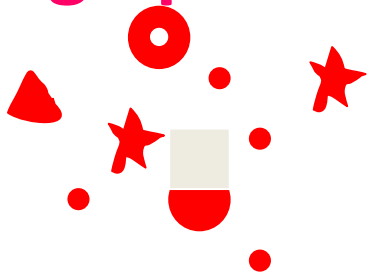
2) Haemolytic Anemia " Microangiopathic"

↓ Hb * RBC Fragmentation

↓ PCV * ↑ Retic

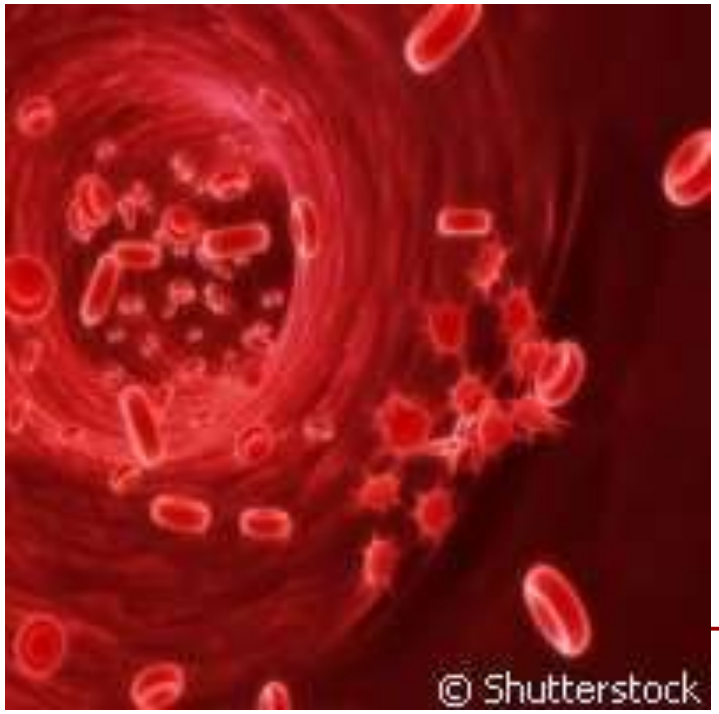
* ↑ Indirect S. Bilirubin.

* Hb uria



3) Thrombotic manifestations:

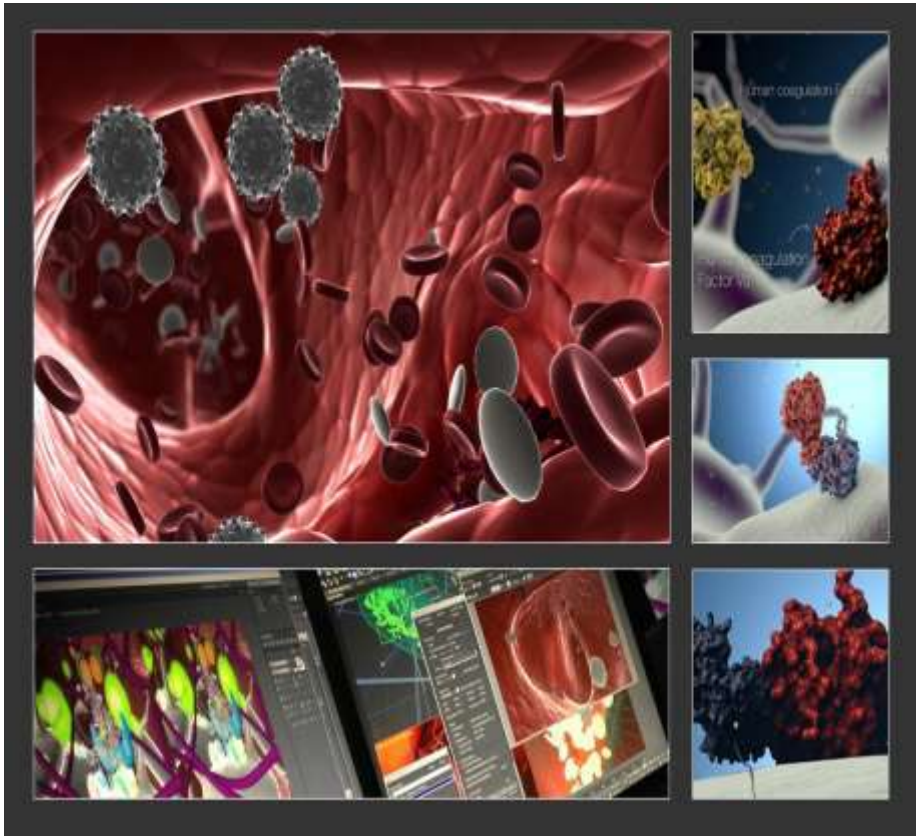
1) Acute Renal failure
2) Skin Necrosis.
3) CNS ischemia
4) Respiratory Distress.



HAEMOSTASIS- I

OBJECTIVES

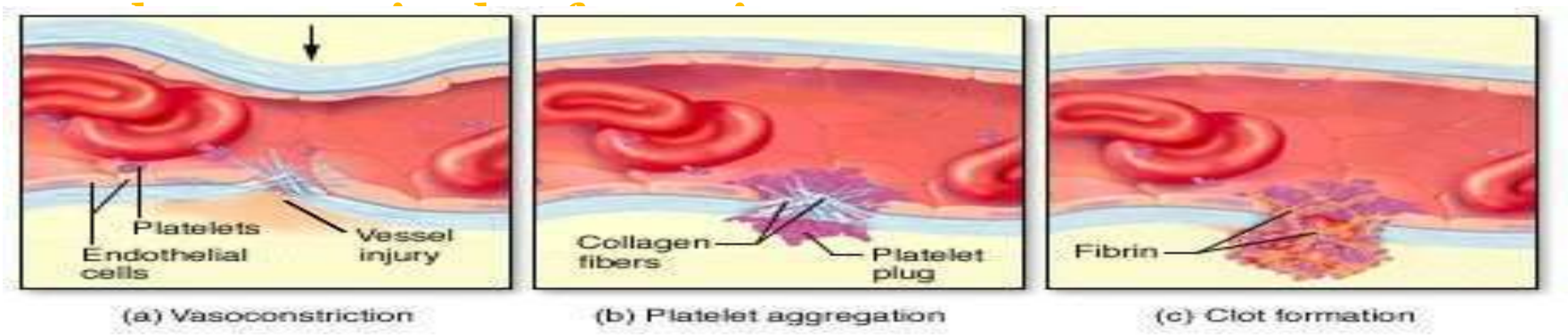
- **Haemostasis**
- **Blood coagulation**
- **Anti-haemostatic mechanism**
- **Bleeding disorders.**
- **Laboratory tests.**



HAEMOSTASIS

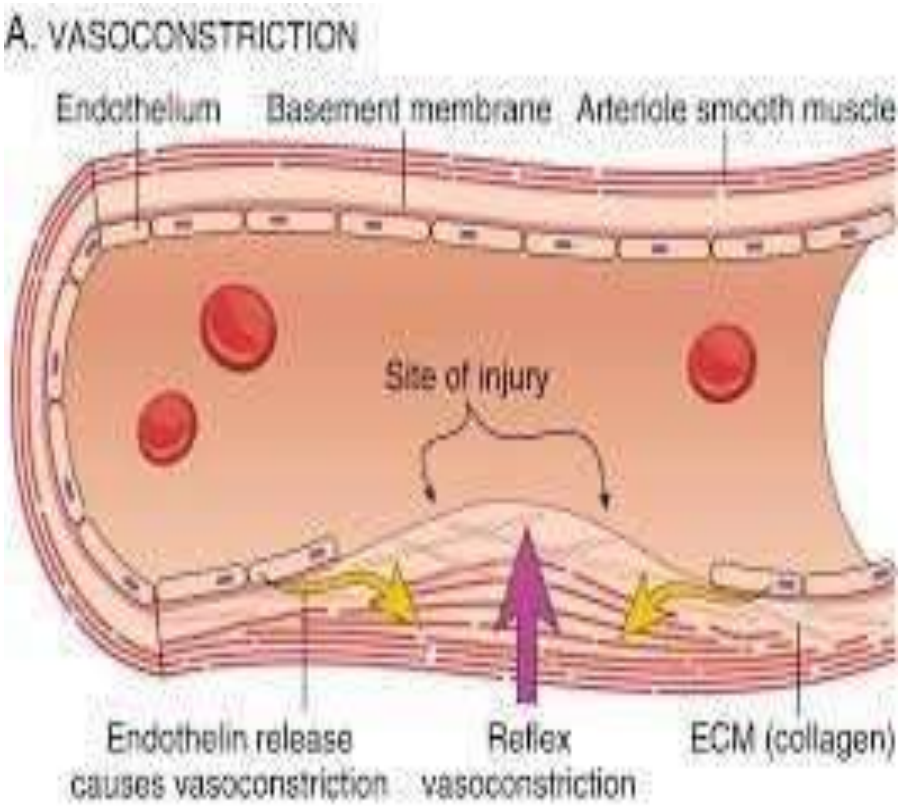
- **Definition**

-
  - **Vasoconstriction**
  - **Formation of temporary haemostatic plug**
  - **formation Formation of Definitive**

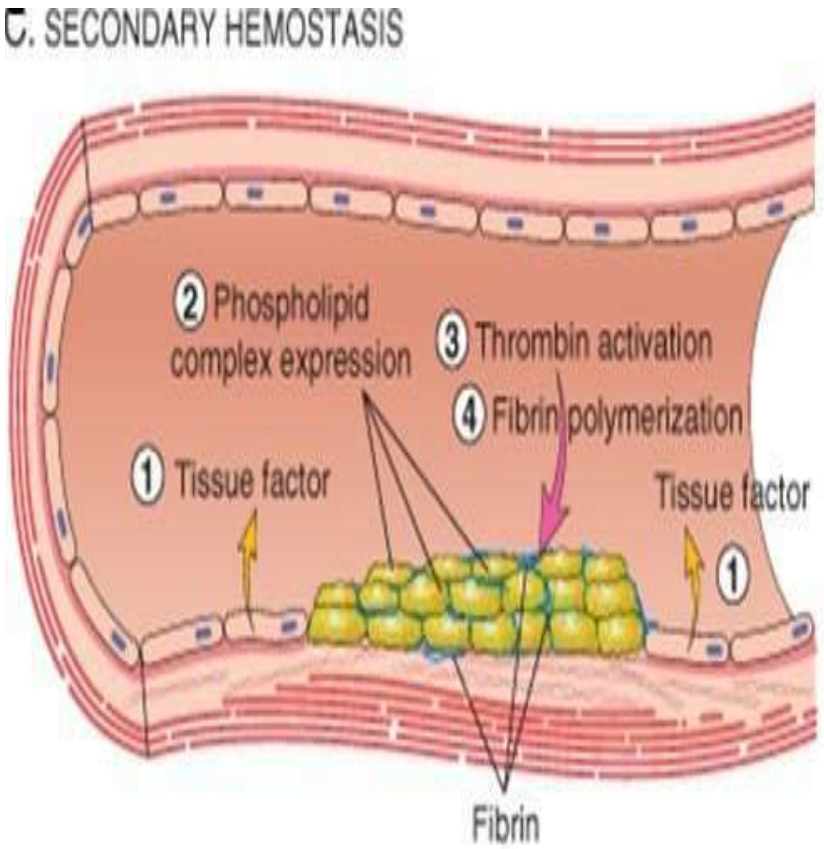


VASOCONSTRICTION

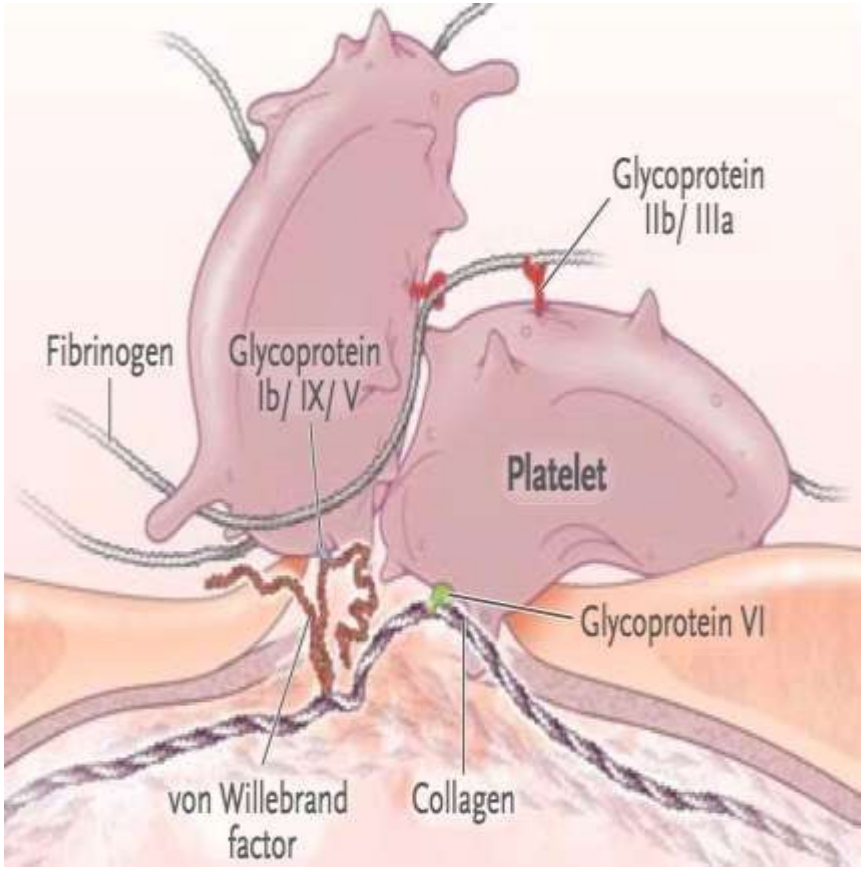
- **Immediate**
  -
  -
- **Later by humoral**
  -



FORMATION OF TEMPORARY HAEMOSTATIC PLUG FORMATION

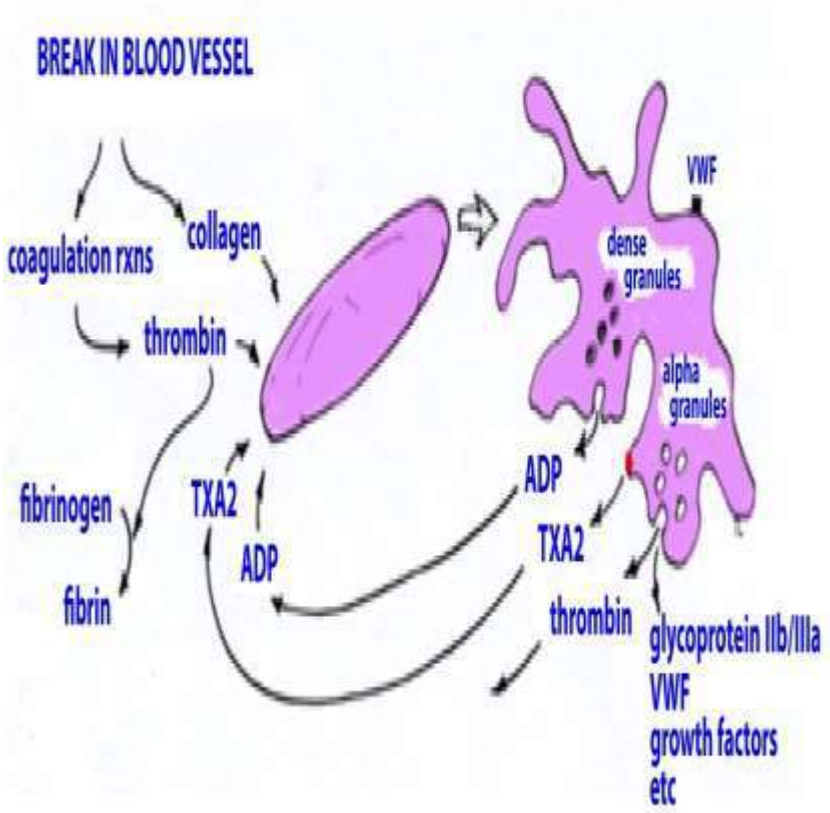


PLATELET ADHESION



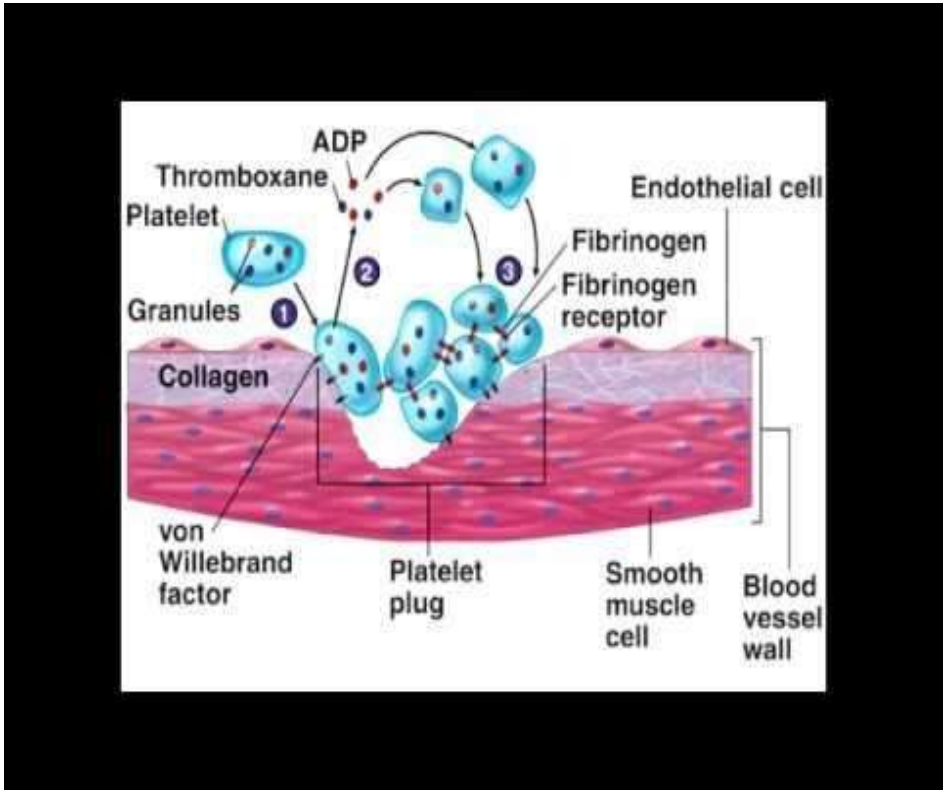
PLATELET ACTIVATION

- ⬇
- **ADP & Thromboxane A2**
- ⬇
-

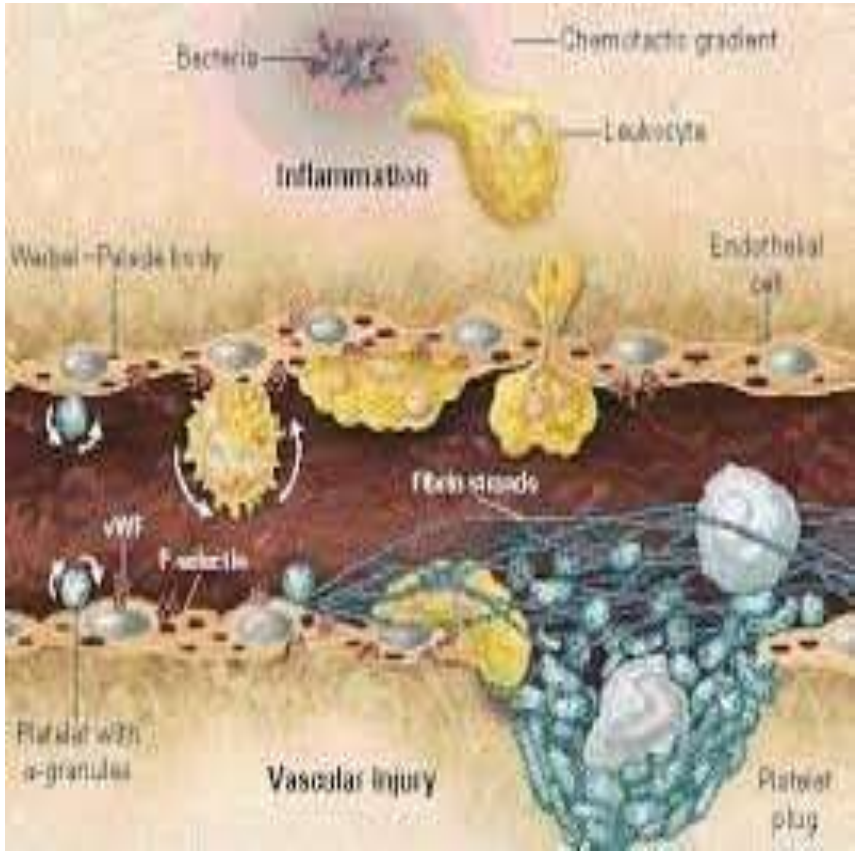


PLATELET AGGREGATION

- **Platelet Activating Factor (PAF)**

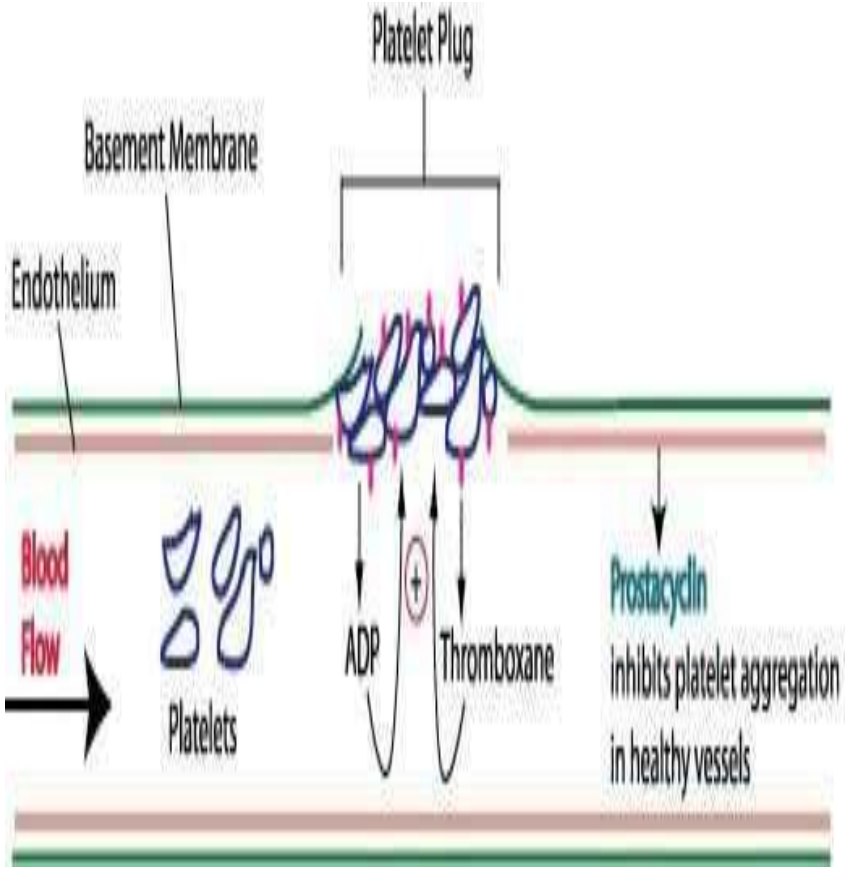


FORMATION OF TEMPORARY HAEMOSTATIC PLUG

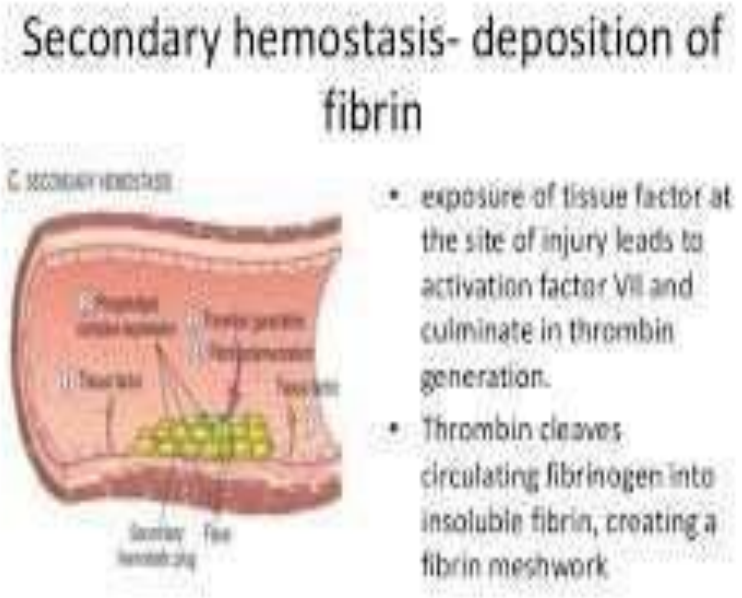


INHIBITION OF FURTHER PLUG FORMATION.

- **Prostacycline**
- **Inhibit**

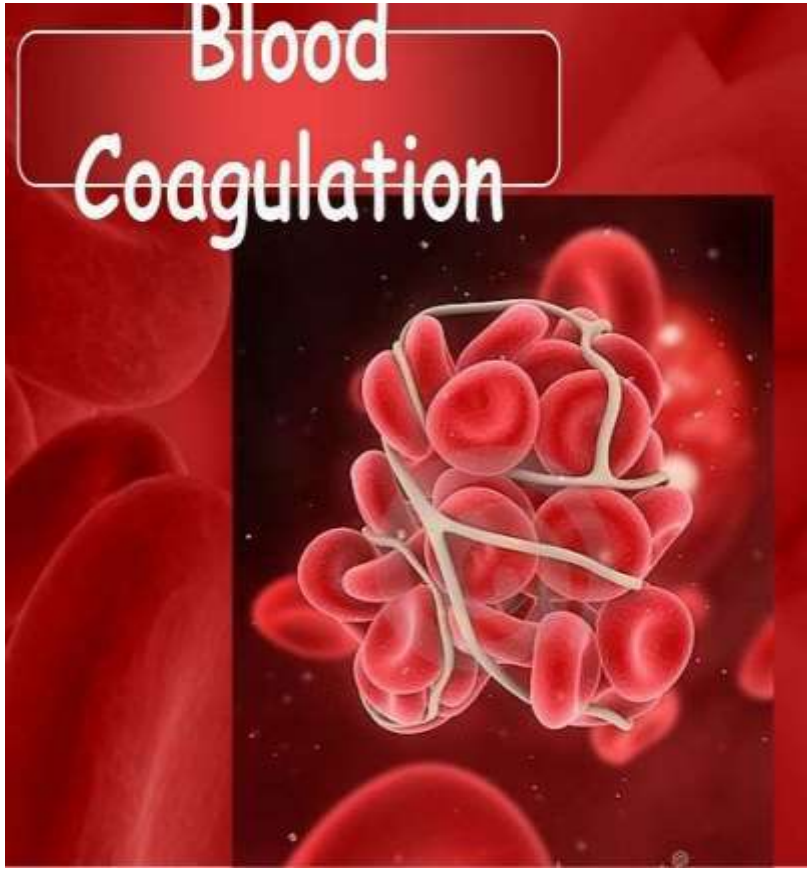


FORMATION OF DEFINITIVE HAEMOSTATIC PLUG FORMATION



COAGULATION OF BLOOD

-

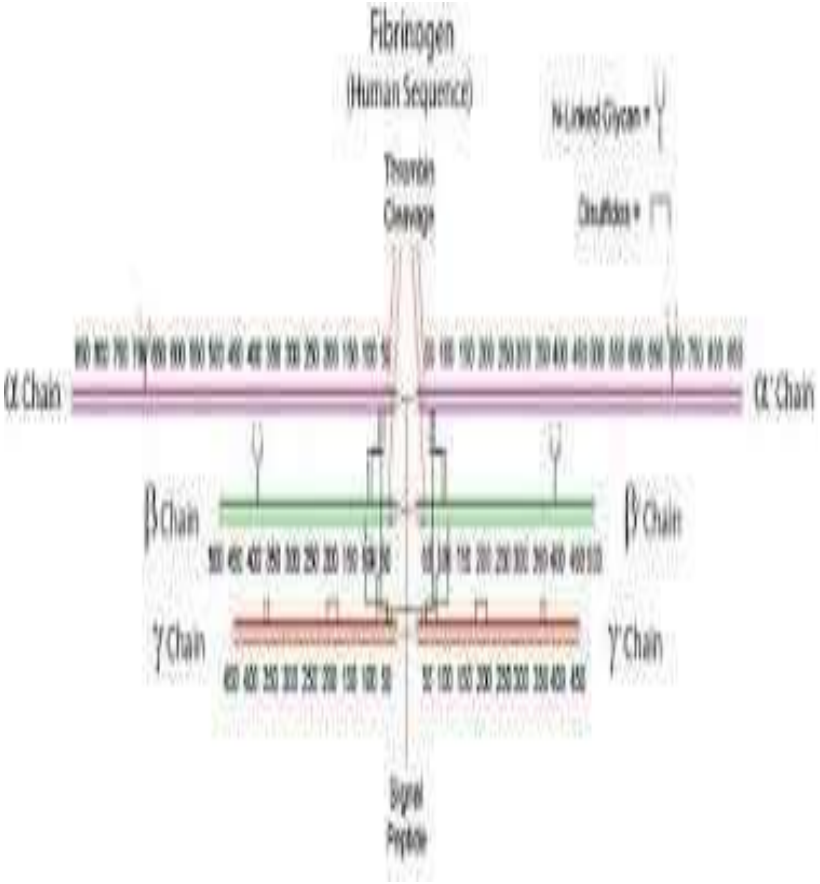


- **Fluidity**
- **Coagulation**

COAGULATION OF BLOOD

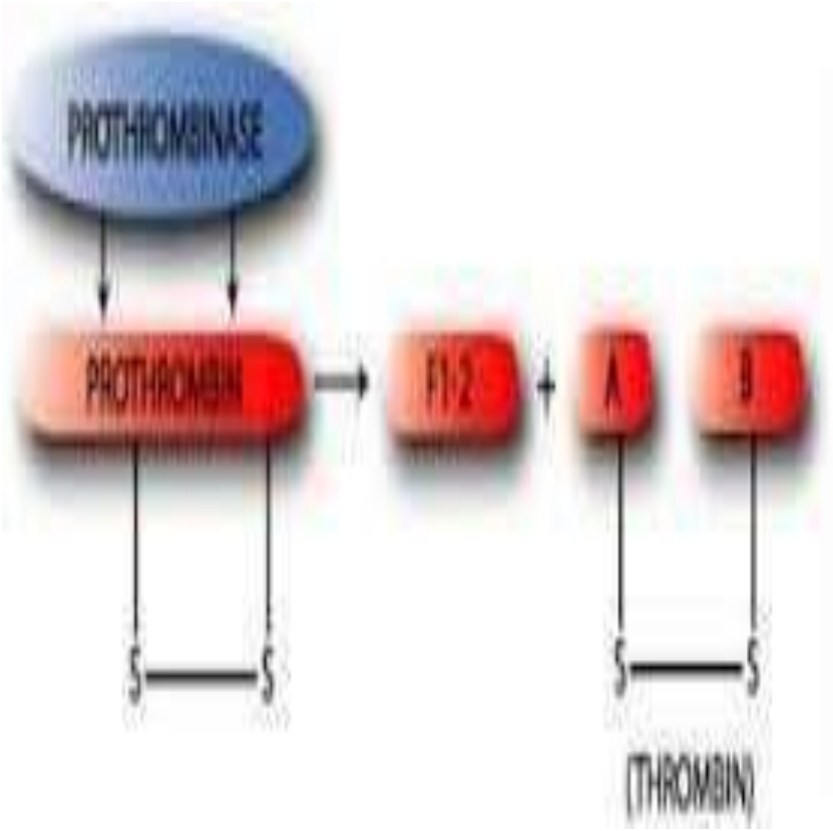
CLOTTING FACTORS

- **I – FIBRINOGEN**
  -
-
-
  -



CLOTTING FACTORS

- **II- PROTHROMBIN**
  -
  -
  -



CLOTTING FACTORS

- **III – THROMBOPLASTIN**
  -
  -

CLOTTING FACTORS

- **IV – CALCIUM**
  -

CLOTTING FACTORS

- **V – LABILE FACTOR / PROACCLERIN**
  -
  -

CLOTTING FACTORS

- **VII – STABLE FACTOR / PROCONVERTIN**

CLOTTING FACTORS

- **VIII – ANTI-HAEMOPHYLLIC FACTOR A / ANTI- HAEMOPHLIC GLOBULIN.**

CLOTTING FACTORS

- **IX–ANTI-HAEMOPHILIC FACTOR B/ PLASMA THROMBOPLASTIC COMPONENT**

CLOTTING FACTORS

- **X –STUART-PROWER FACTOR**

CLOTTING FACTORS

- **XI – PLASMA THROMBOPLASTIN ANTICEDENT / ANTI-HAEMOPHILIC FACTOR C**

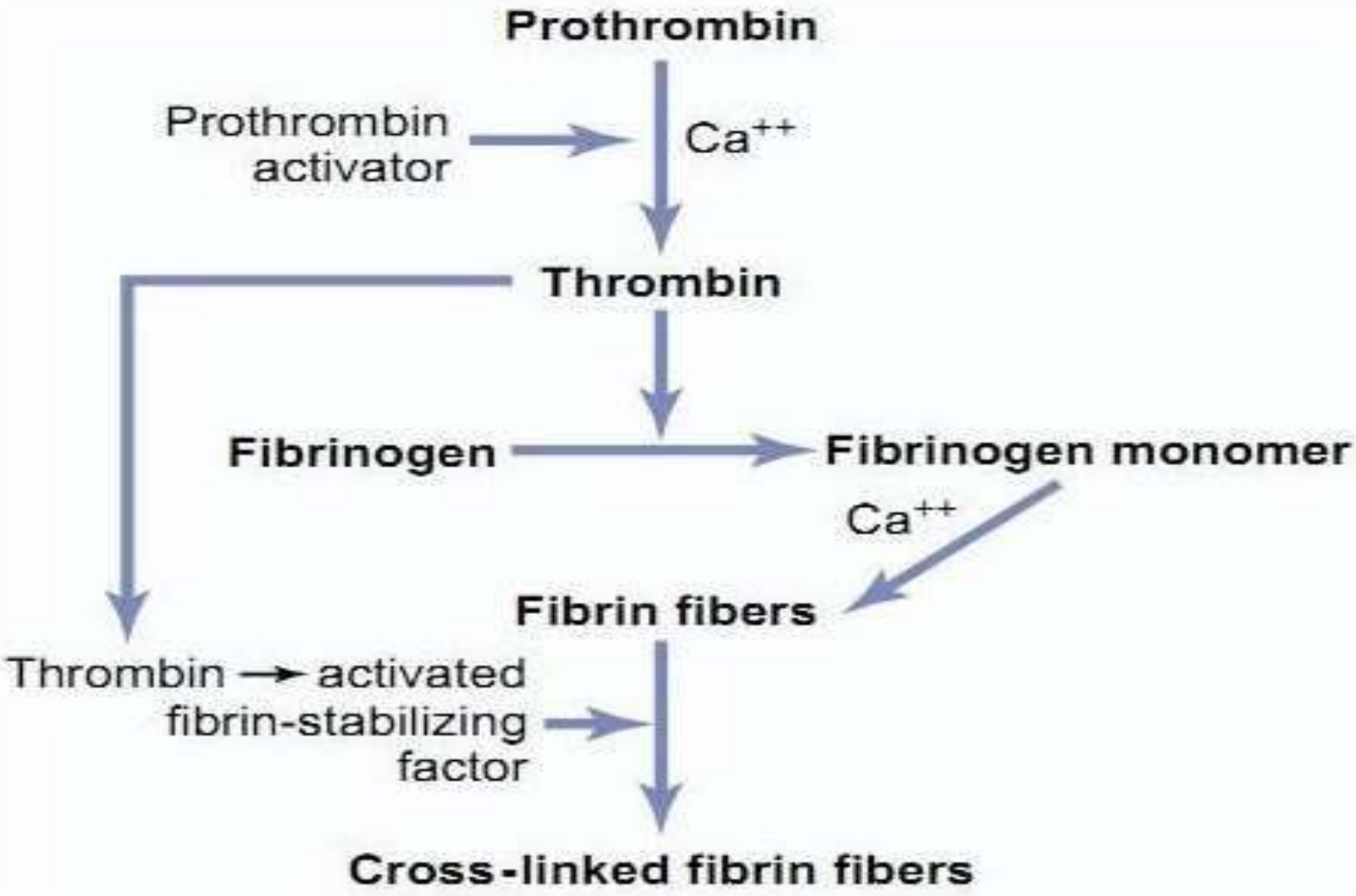
CLOTTING FACTORS

- **XII – HAGEMAN FACTOR/ GLASS FACTOR /**

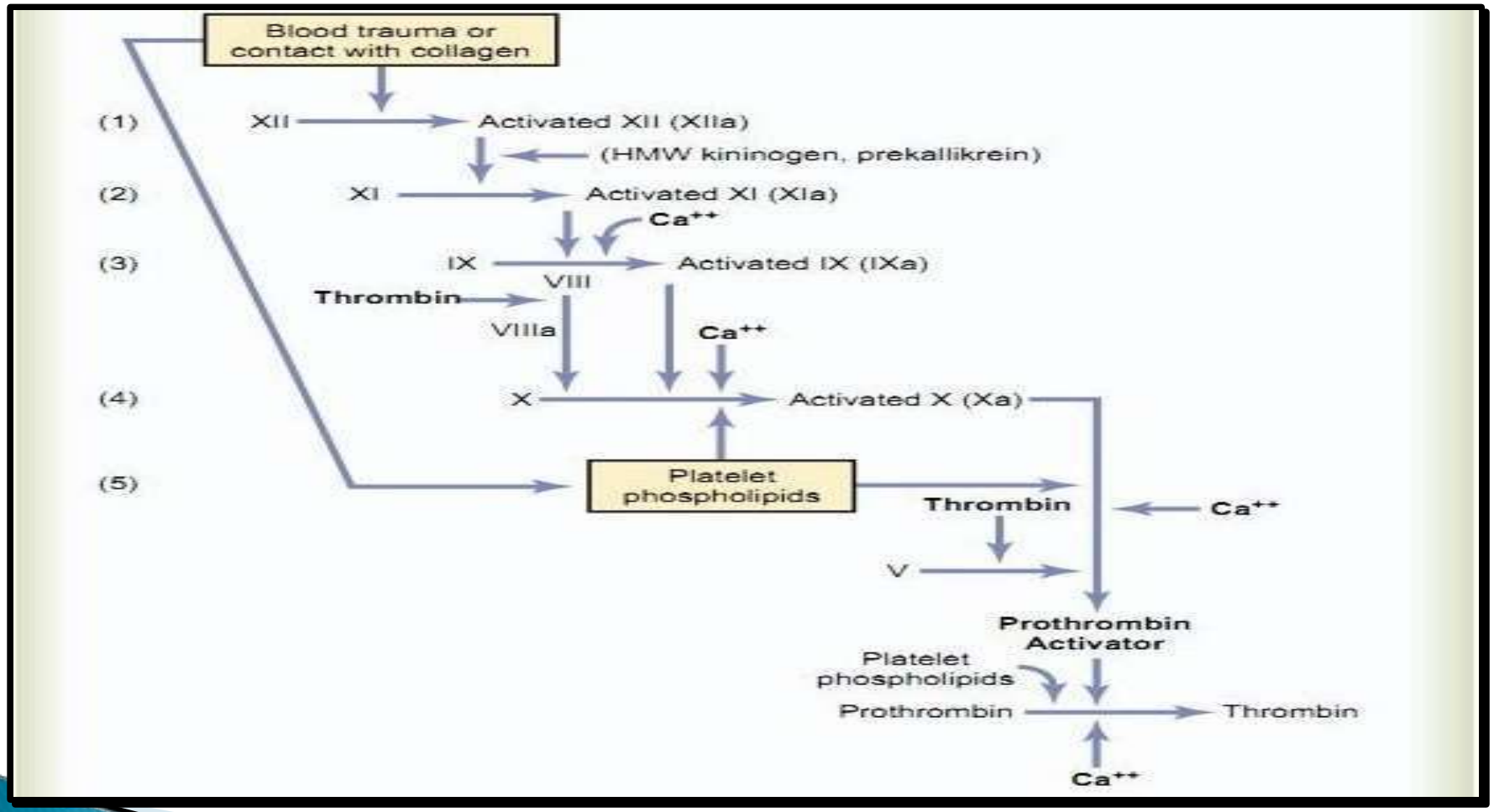
CONTACT FACTOR

CLOTTING FACTORS

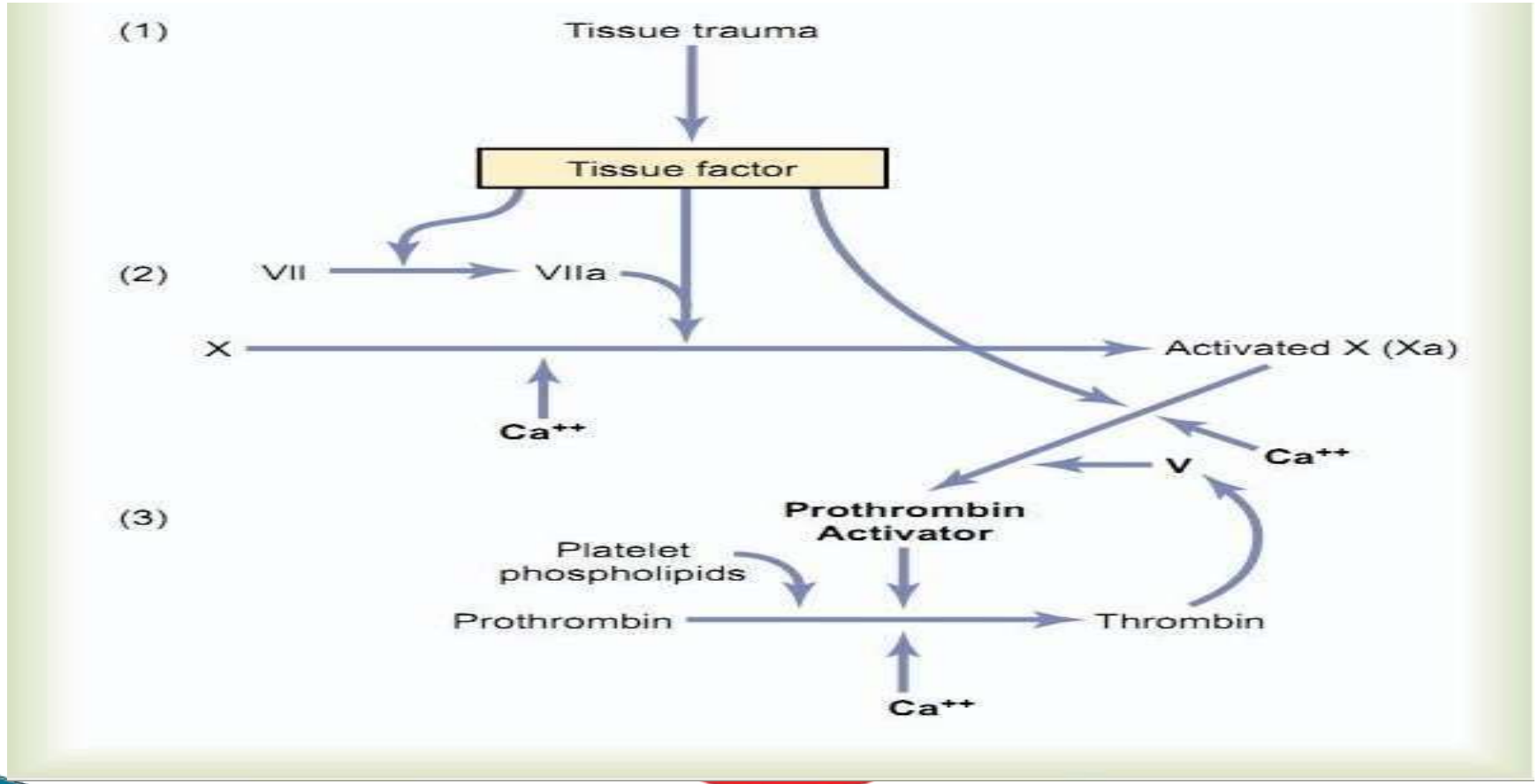
- **XIII – FIBRIN STABILIZING FACTOR / FIBRINASE**


Prothrombin
Prothrombin activator
Ca++
Thrombin
Fibrinogen
Fibrinogen monomer
Ca++
Fibrin fibers
Thrombin → activated fibrin-stabilizing factor
Cross-linked fibrin fibers

MECHANISM OF COAGULATION- INTRINSIC PATHWAY.



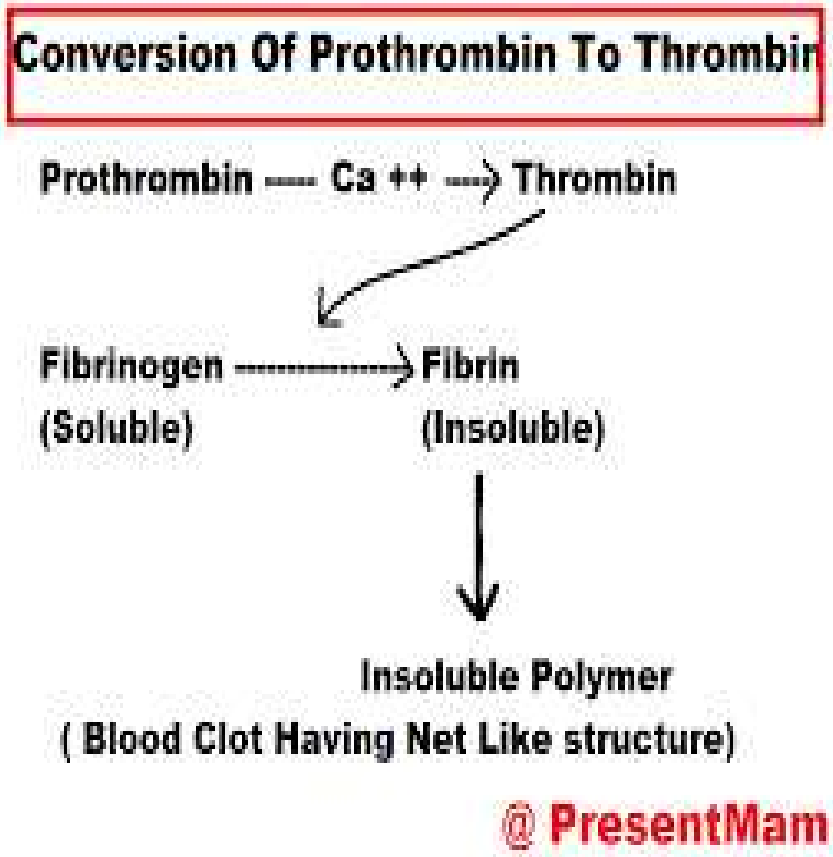
EXTRINSIC PATHWAY



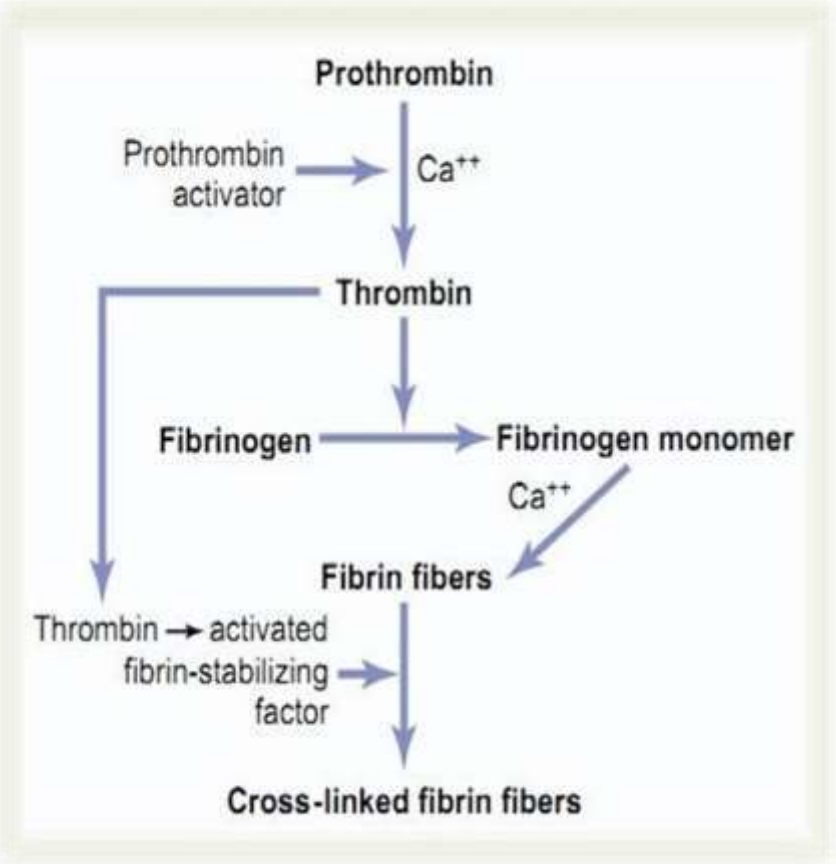
DIFFERENCE.

EXTRINSIC PATHWAY.	INTRINSIC PATHWAY.

CONVERSION OF PROTHROMBIN TO THROMBIN



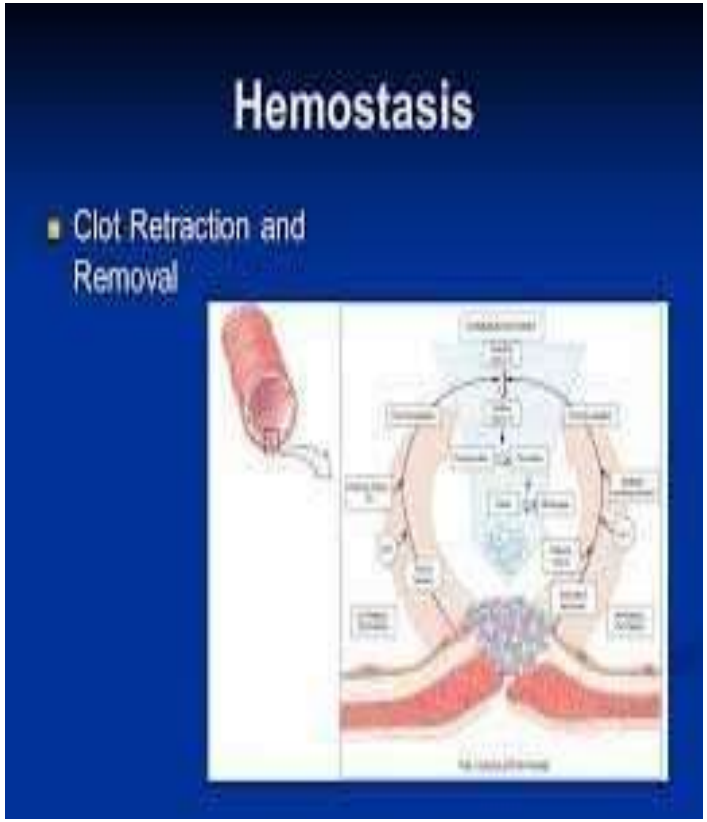
THROMBIN.


Prothrombin
Prothrombin activator
Ca++
Thrombin
Fibrinogen
Fibrinogen monomer
Ca++
Fibrin fibers
Thrombin → activated fibrin-stabilizing factor
Cross-linked fibrin fibers

I – FIBRINOGEN

FIBRINOGEN TO FIBRIN.

BLOOD CLOT RETRACTION.



ROLE OF CALCIUM.

-
- **Ca removal causes Anticoagulation.**
-

ROLE OF VITAMIN K.

- **Chemical structure.**
  -
  -
- **Sources**
  -
- **Role of vitamin K**
  -
  -

ROLE OF VITAMIN K.

ROLE OF LIVER.

- **Synthesis of procoagulants**

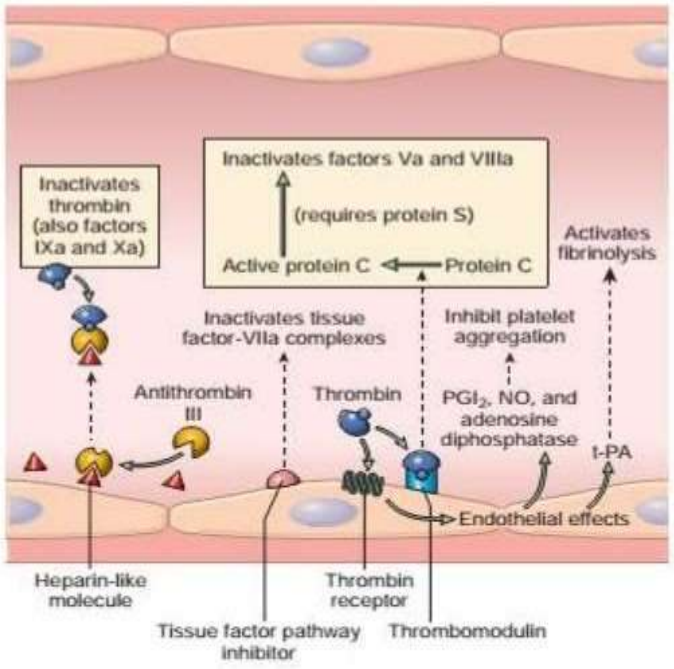
- **Removal**

- **Synthesis of anticoagulants**

ROLE OF BLOOD VESSELS.

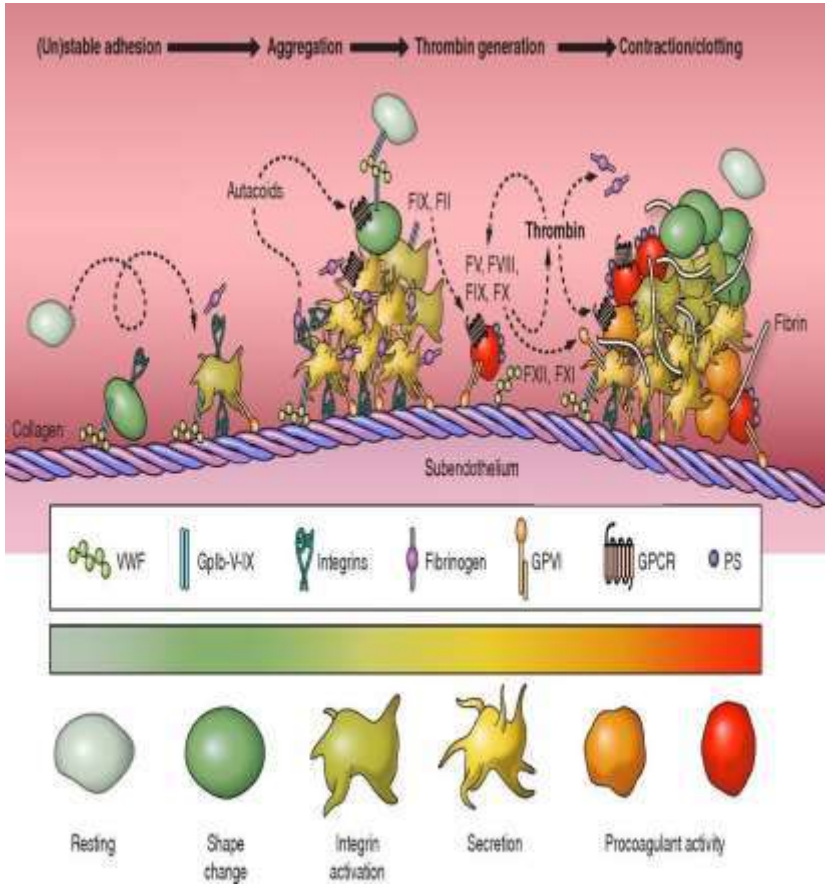
- **Endothelium**
  -
    -
    -
    -
    -

Anticoagulant activities of normal endothelium

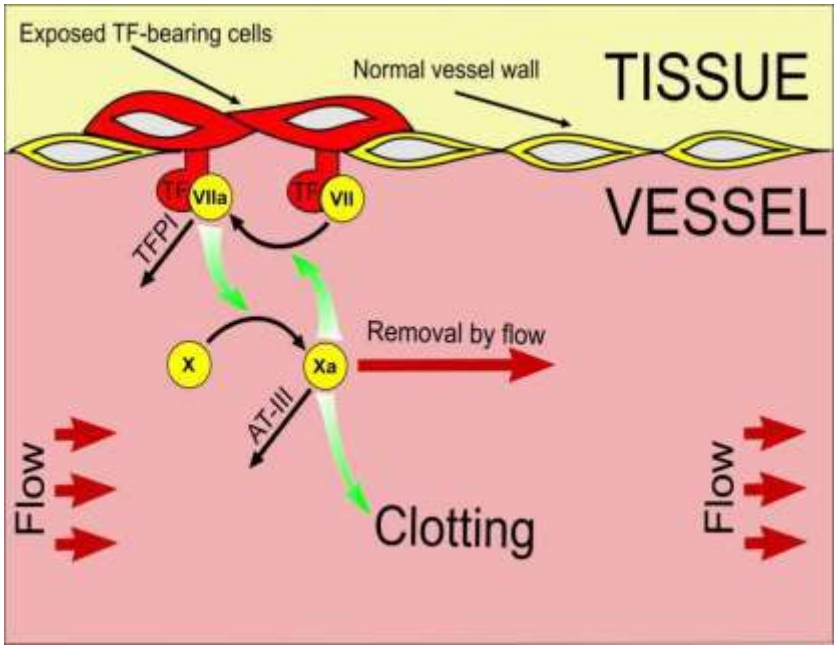


ROLE OF BLOOD VESSELS.

- **Coagulant**
  -
  -
  -
- **Sub endothelial tissue – collagen fibres**
  -
  -



BLOOD IN FLUID STATE



VELOCITY OF CIRCULATION.

-

-

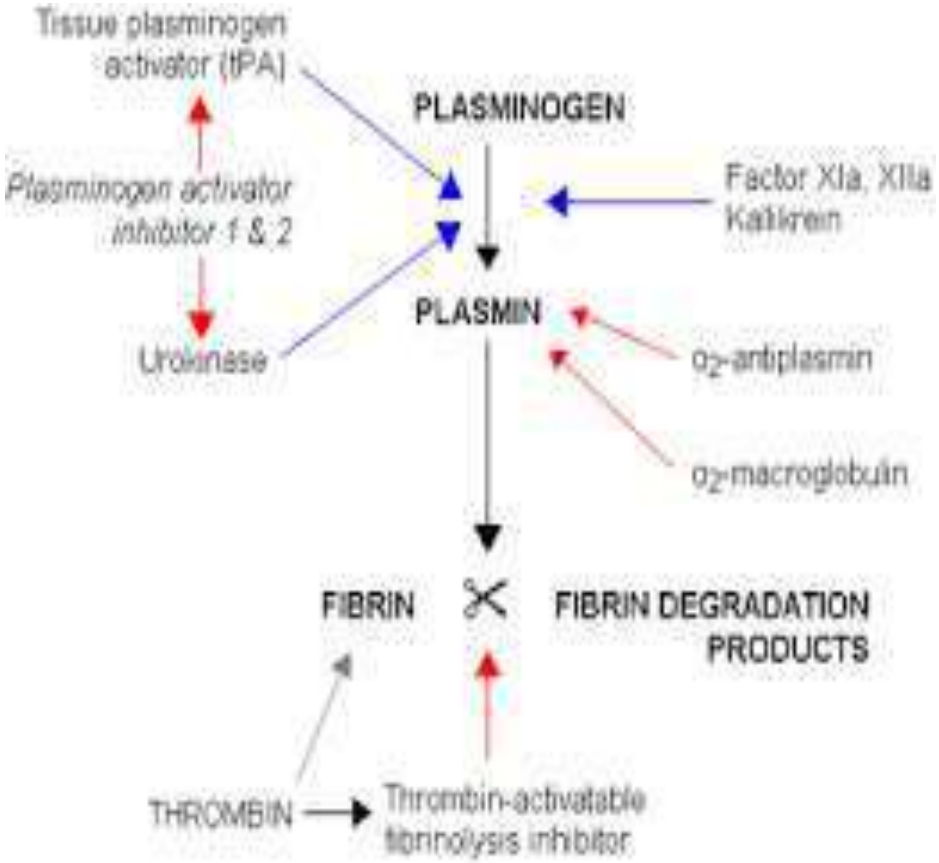
-

SURFACE EFFECT OF ENDOTHELIUM. GLYCOCALYX

- Endothelial lining smoothness – prevents adhesion & intrinsic mechanism.
- **Glycocalyx** --inner layer of endothelium negatively charged repel clotting factors &Y platelets & prevent clotting.
- **Intact Endothelium** – barrier between collagenous tissue & blood

CIRCULATORY ANTICOAGULANTS.

FIBRINOLYTIC MECHANISM.



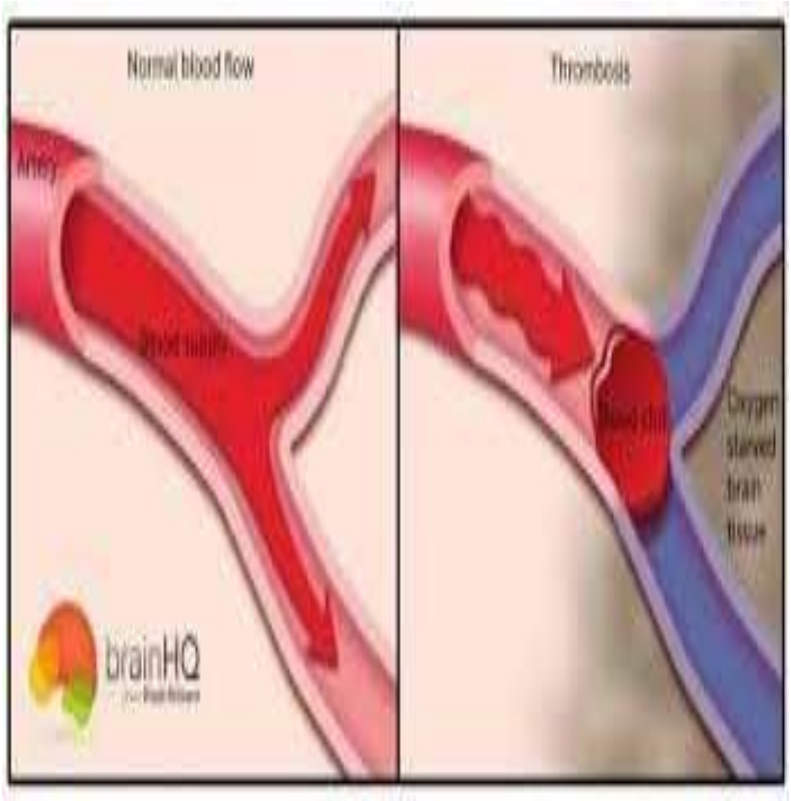
REMOVAL OF ACTIVATED CLOTTING FACTORS.

-
-
-

THROMBOSIS

- **Def**

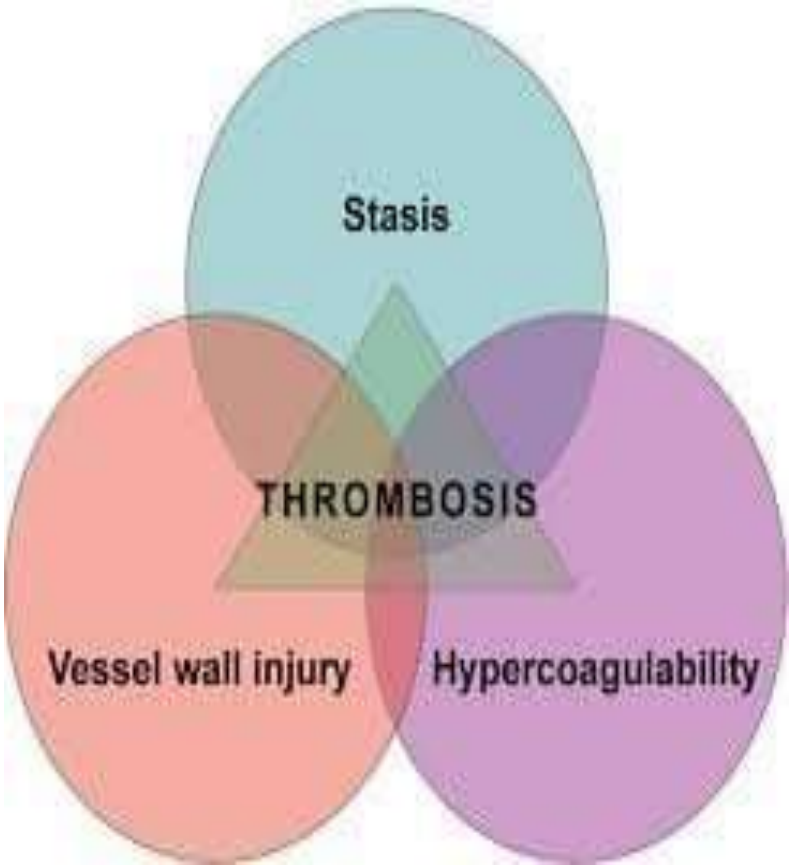
- **Thrombus**



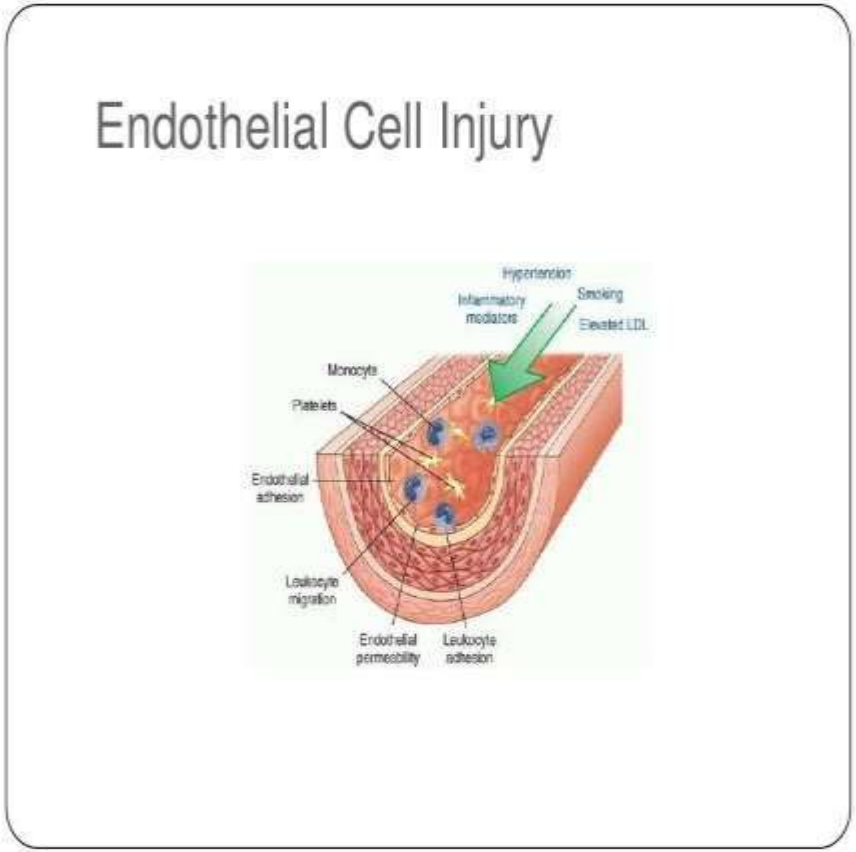
THROMBOSIS

- **Virchow's triad**

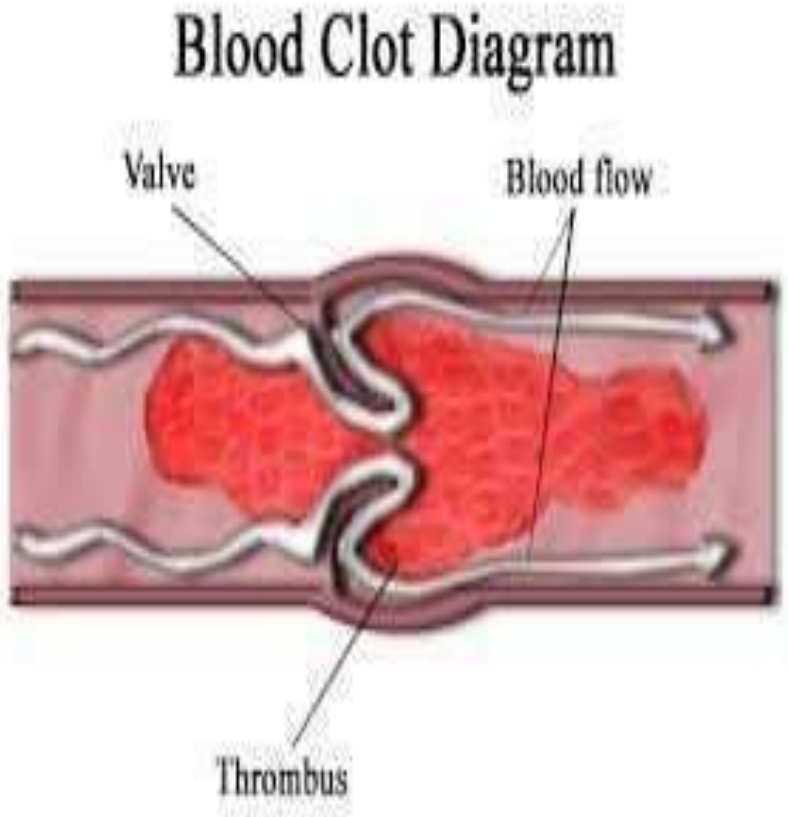
- **Predisposing factors**
  -
  -
  -



ENDOTHELIAL INJURY

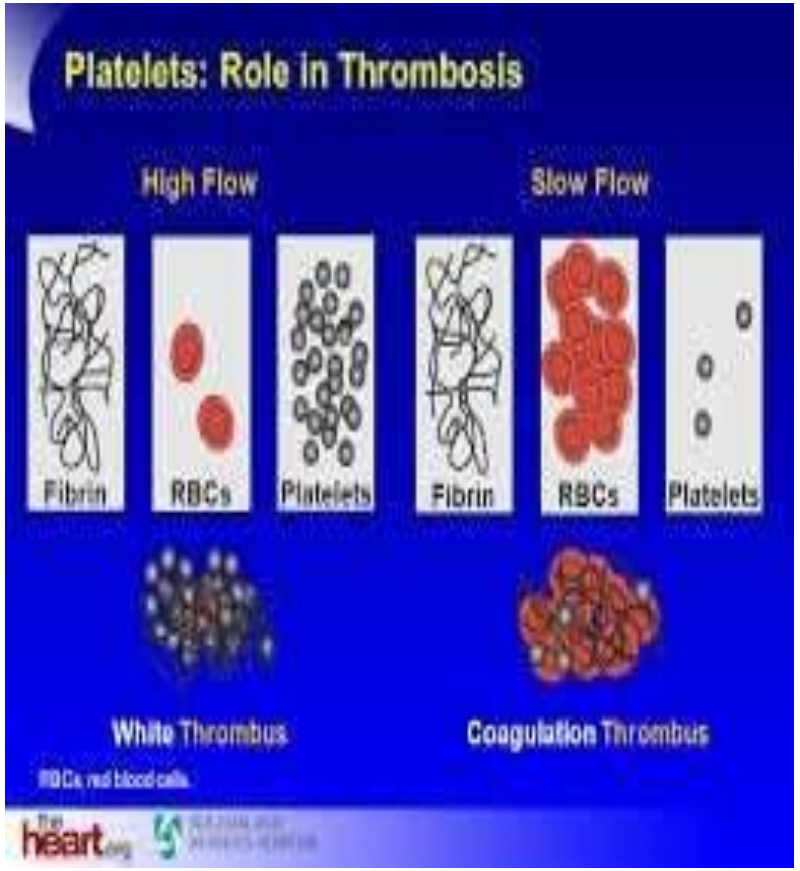


ALTERATION OF BLOOD FLOW



HYPERCOAGULABILITY OF BLOOD

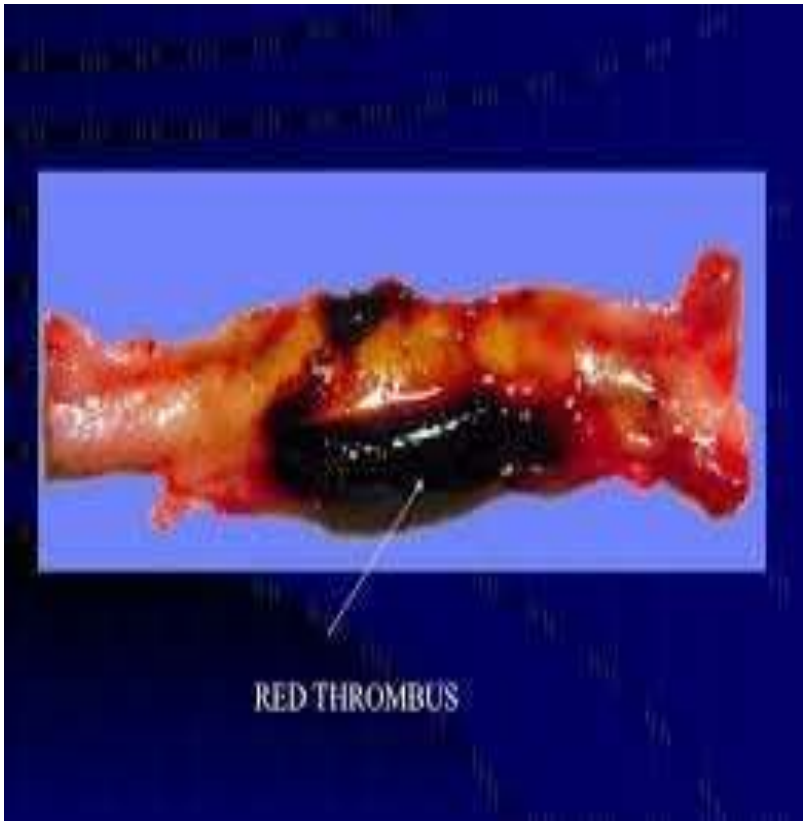
- **Increase Platelet count**
- **Increase Coagulation**
- **Decrease coagulation inhibitors**



THROMBOGENESIS

THROMBI

- **Red**
- **White**
- **Mixed or laminated**



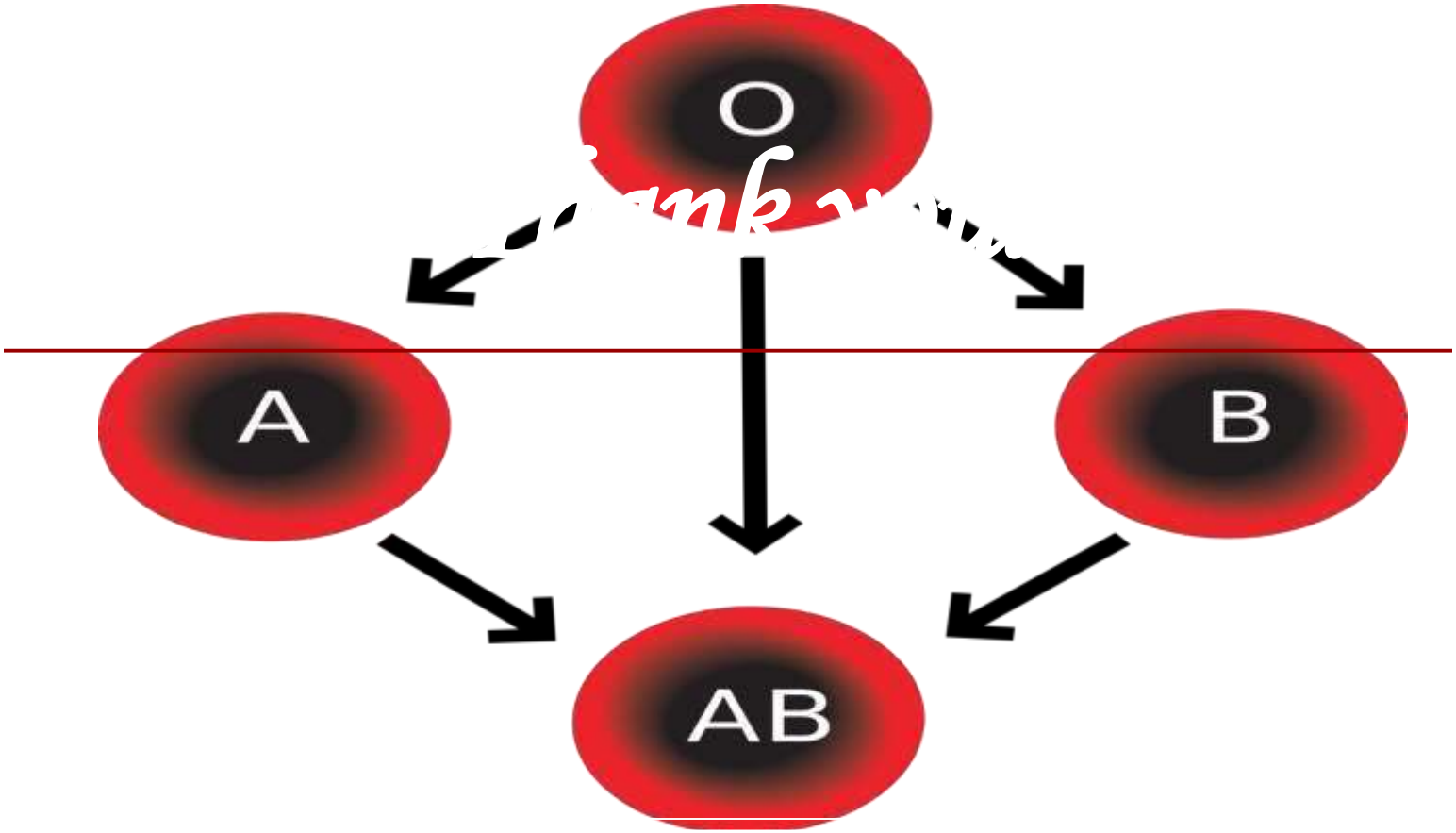
EFFECTS OF THROMBI

- **Ischemia and Infarction**
  -
  -
- **Thromboembolism**
  -
  -

PREVENTION OF THROMBI

- **Drugs**

- **Anticoagulants**
- **Intermittent compression**


O
A
B
AB

Investigations of bleeding disorders

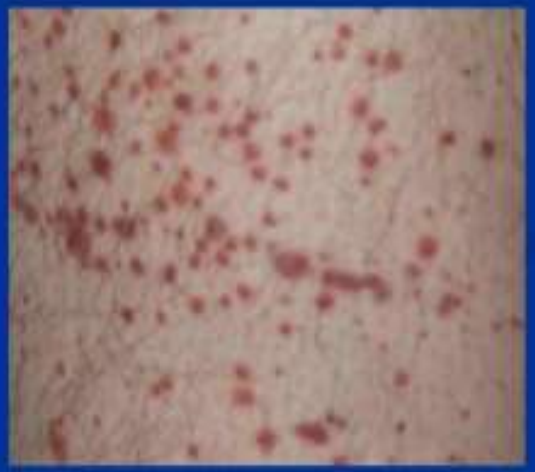
Tests of haemorrhagic disorders

Bleeding in skin

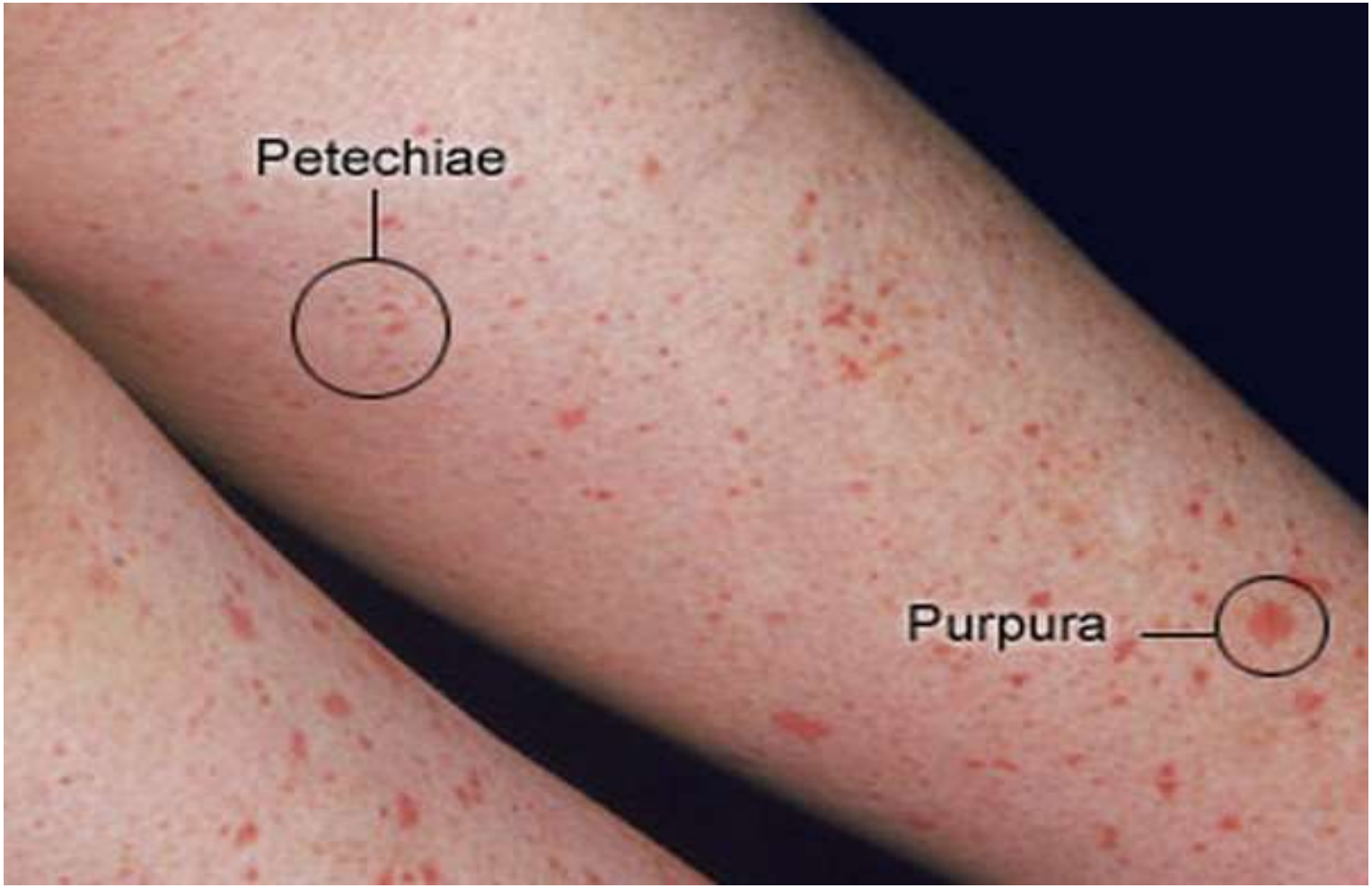
- Purpura is a condition of red or purple discolored spots on the skin that do not blanch on ... They measure 3–10 mm, whereas petechiae measure less than 3 mm, and ecchymoses greater than 1 cm.

Petechiae
(1 – 2 mm)

Purpura
(≥ 3 mm)



Ecchymosis
(> 1 – 2 cms)


Petechiae
Purpura

History

- Spontaneous or due to trauma
- Age & sex
- Family history
- Site of bleeding
- Type of bleeding : Petechiae, purpura, ecchymosis (bruisis)
- Drug history : Aspirin , warfain, heparin, steroid

CBP & Platelets count

- Diagnosis of :
- Leukaemia especially acute leukaemia
- Macrocytic (Megaloblastic Anaemia)
- Pancytopenia (Aplastic anaemia & hypersplenism)
- Thrombocytopenia (low platelets count)

Whole blood clotting time

- Lee & White method
- Normal range : 4 – 10 minutes
- Prolonged in :
- Severe haemophilia
- DIC

Bleeding time

- Ivy method in adults
- Duke method in children
- Normal range : 2 – 9 minutes
- Prolonged in :
- Thrombocytopenia
- Platelets dysfuntion (e.g. Thrombosthenia)
- Von Willebrand disease
- Aspirin
- Vascular purpura
- DIC

Prothrombin time

- Test of extrinsic pathway of coagulation
- Normal range : 10 – 15 seconds
- Prolonged in :
- Warfarin therapy
- Haemorrhagic disease of newborns
- Obstructive jaundice
- Chronic liver disease
- Vit K deficiency
- DIC

Activated Partial Thromboplastin Time (APTT)

- It is test of intrinsic pathway of coagulation
- Normal range : 25 – 35 seconds
- Prolonged in :
- Haemophilia (A , B , C)
- Von Willebrand disease
- Heparin therapy
- DIC

Other tests

- Fibrinogen level
- Thrombin Time (TT)
- D dimer
- FDP (Fibin Degregration Products)
- Platelets funcion test (platelets aggregometry)

Tests of hyper coagulable states (Thrombophilia)

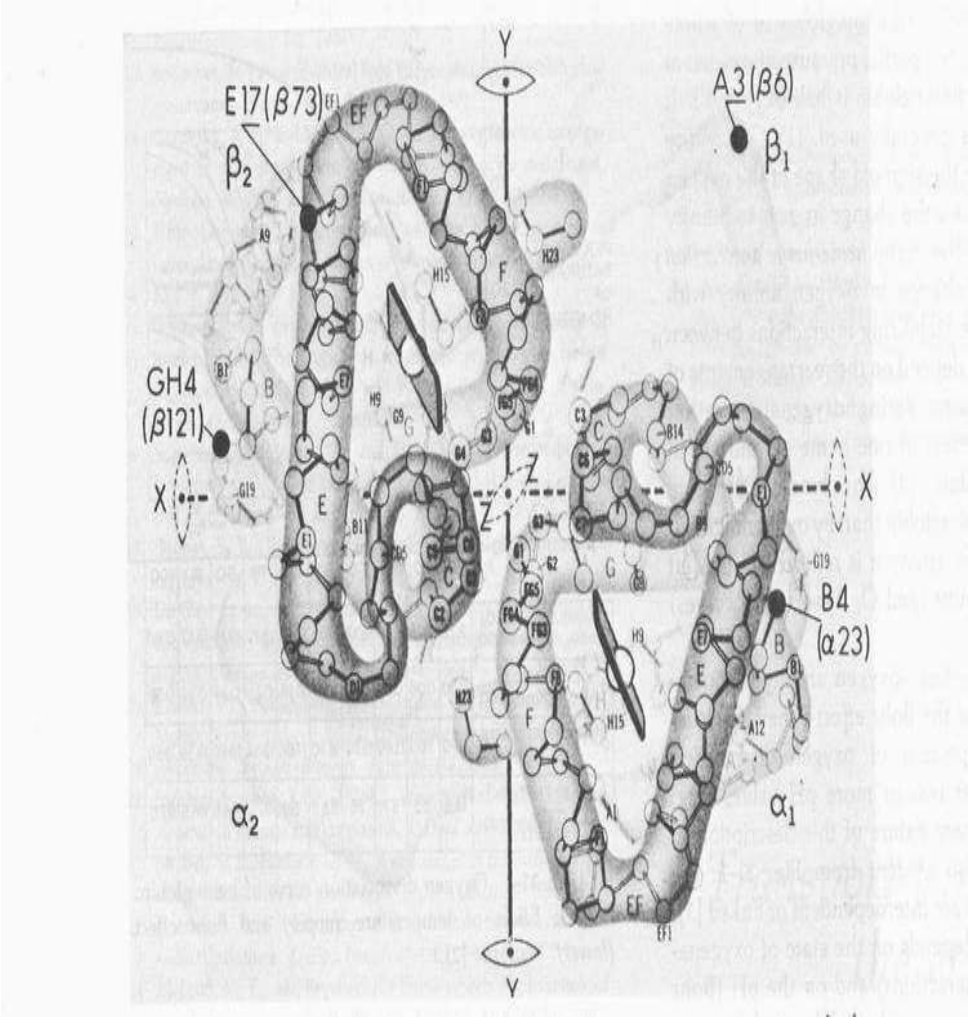
- Anti thrombin level
- Protein C level
- Protein S level
- Anti phospholipid level
- Anti cardiolipin level
- SLE tests

Haemoglobinopathies

A group of genetic disorders of Hb synthesis characterized by either a reduction of the rate of synthesis of globin chains (Thalassaemias) or the production of abnormal globin chains (Hb-variants).

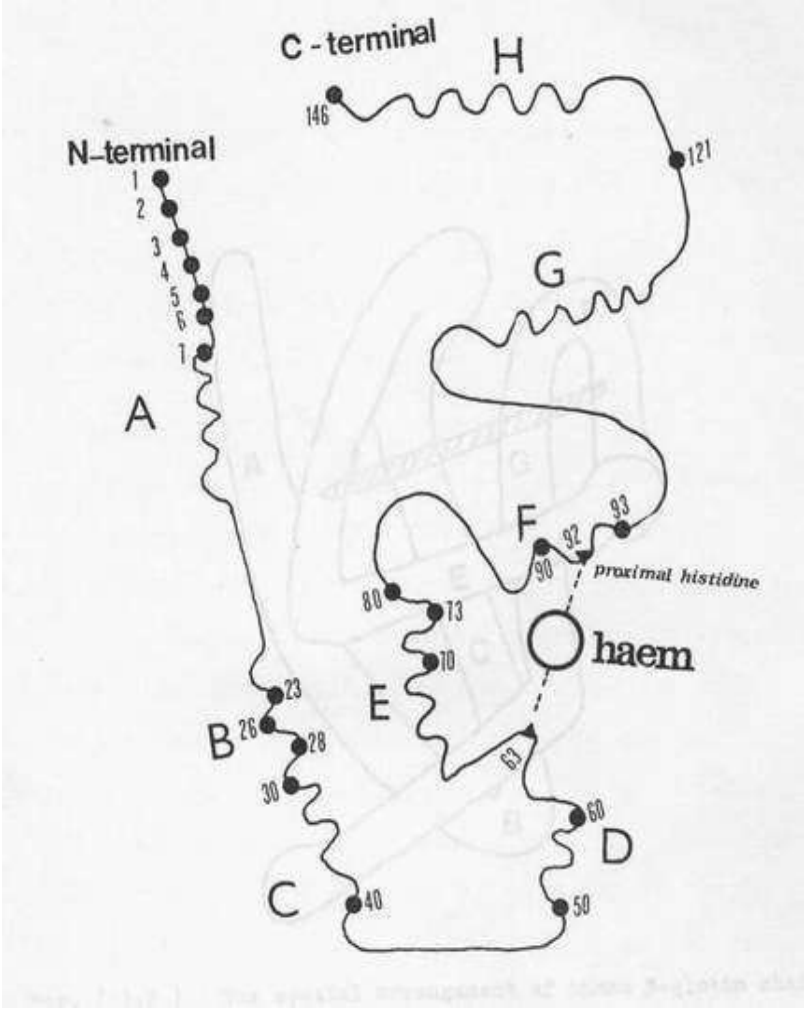
Structure of Haemoglobin

- Adult Hb (Hb-A) is a tetramer of 4 globin chains arranged in a helical form; there are 2 α and 2 β chains, (α 2 β 2)
- Other normal Hbs are :
  - Fetal Hb (Hb-F), α 2γ2
  - Hb-A2; α 2 δ2
- Normal adults have: about 98% Hb-A, 2% Hb-A2 and less than 1% Hb-F



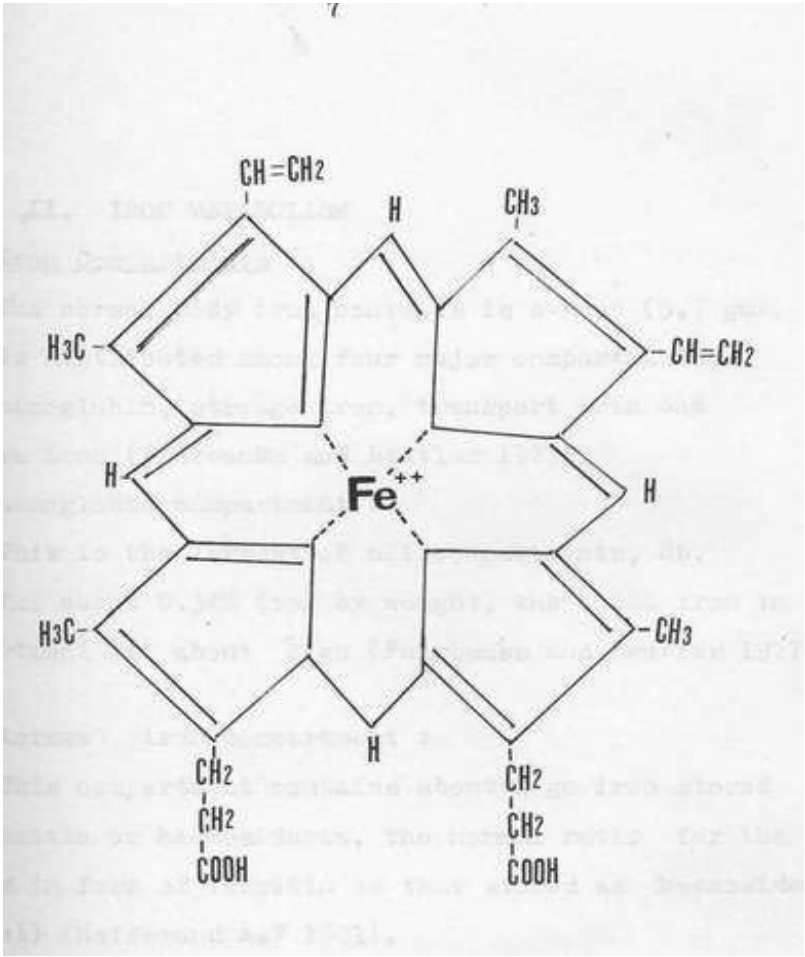
The globin chain

- The globin chain is composed of a sequence of (about 150) amino acids arranged in a tertiary manner. Amino acids facing haem are non-polar (repel water) while external ones are polar (bond with water). This arrangement will keep haem in a reduced form and the Hb soluble in the plasma.



The haem molecule

- Each globin chain carries a single haem molecule lying in a deep recess inside the helix.
- Haem is a tetrapyrol ring with a central atom of iron.
- Haem Iron has 6 bonds; 4 are connected to the pyrol rings, one to the globin chain, the 6th is the one involved in transport of O2 and CO2, attachment to these gasses is through reversible covalent bonding.



The Thalassaemia Syndromes

- **A heterogeneous group of genetic disorders of Hb synthesis characterized by reduction in the rate of synthesis (or total absence) of one or more of the globin chains; according to the chain involved they are pathologically subdivided into:**
  - **β -thalassaemias (the commonest type in Iraq)**
  - **α -thalassaemias**
  - **δ- thalassaemia**
  - **Compound inheritance of more than one thalassaemic gene (δβ, αβ) or Hb-variant (sickle cell-thalassaemia)**

Original cases described were all of Mediterranean origin hence the name thalassaemia (thalos=sea, aemia=blood)

Clinical classification

Regardless of the underlying pathology, thalassaemias are classified into 3 types according to the clinical severity:

- **Thalassaemia major:** transfusion dependent, anaemia is severe (Hb about 5 g/dl), starts within the 1st year of life, rarely live beyound the 2nd decade of life.
- **Thalassaemia intermedia:** moderate anaemia (Hb>7 g/dl), infrequent or no need for transfusions, late presentation and long survival.
- **Thalassaemia minor:** Mild or no anaemia, minimal red cell morphological changes, increased Hb-A2, also called thalassaemia trait or carrier.

B- Thalassaemia major

Also called Cooly's anaemia and homozygous B-thalassaemia, very common in Mediterranean basin, middle and south-east Asia

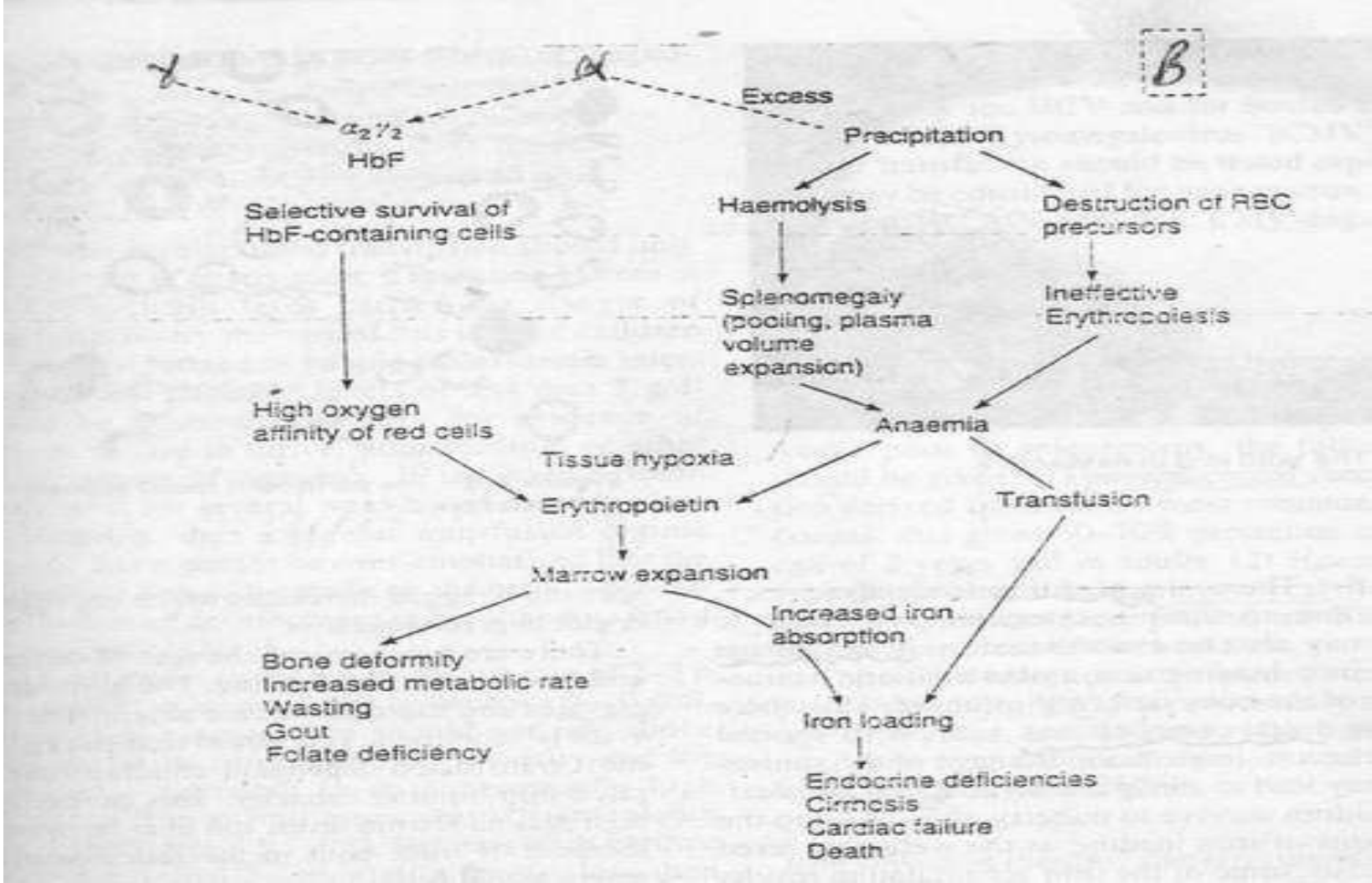
Molecular defects:

Most cases result from point mutation affecting the B-Globin gene, this may result in:

- **Failure of mRNA transcription**
- **Production of unstable mRNA.**
- **Production of unreadable mRNA**

The final result is either a reduction in the amount of B-chains synthesis (B^+ thallassaemia) or total absence of B- chains (B^0 thalassaemia), whatever chains produced are normal.

Pathophysiology



Clincal Presentation

- Anaemia with splenomegaly starting in the 1st 6 months of life
- Inadequately transfused patients suffer intercurrent infections, progressive abdominal distention and severe growth retardation with skeletal deformities.
- Adequately transfused patients have little anaemia & splenomegaly & normal growth.
- During the 2nd decade organ failure develop due to iron over load & most die for this reason.

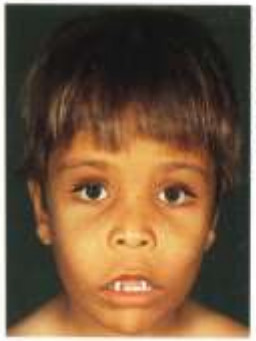


Fig. 5.6
β-Thalassaemia major: characteristic facies of a 7-year-old Middle Eastern boy includes prominent maxilla and widening of the bridge of the nose. There is also marked bossing of the frontal and parietal bones and zygomata giving a mongoloid appearance.

Fig. 5.12
β-Thalassaemia major: this 4-year-old inadequately transfused Cypriot boy has enlargement of the spleen to an unusual degree. It may be partly reversed by adequate transfusion. Splenectomy is usually required but should usually be delayed until the child is over 6 years old to reduce the incidence of postoperative fatal infection.

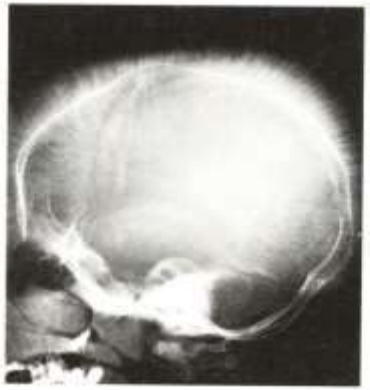
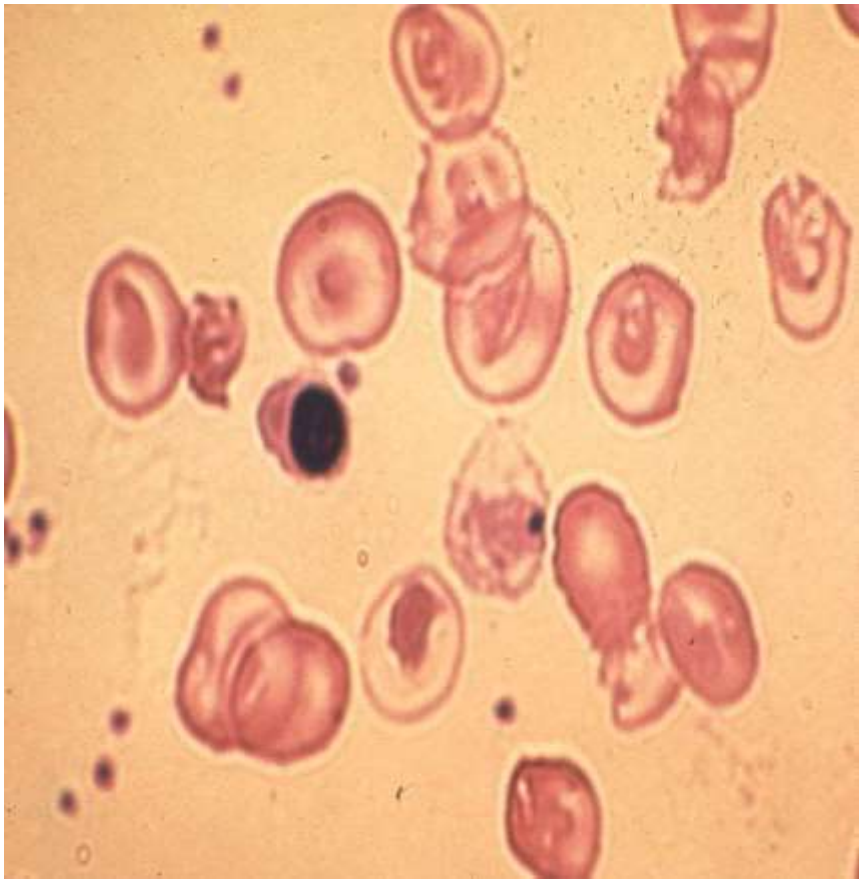


Fig. 5.7
β-Thalassaemia major: lateral radiograph of the skull (same case as Fig. 5.6) shows the typical 'hair-on-end' appearance, with thinning of the cortical bone and widening of the marrow cavity.

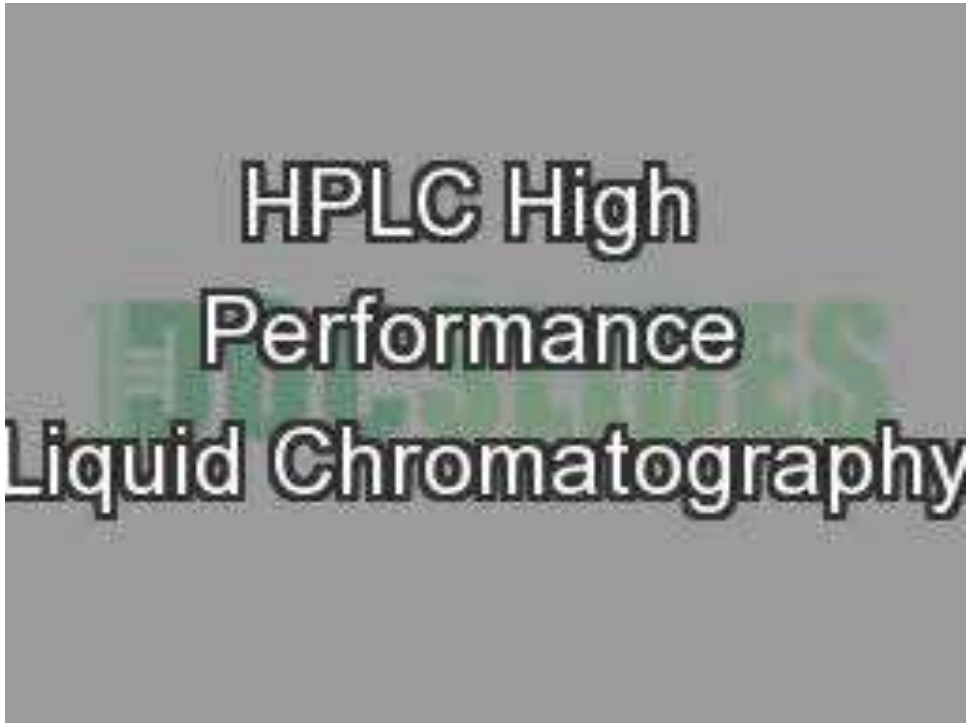
Haematological features

- Hypochromic microcytic anaemia with marked red cell distortion, target forms and normoblastaemia.
- Raised Hb-F level(10->90%)
- BM shows erythroid hyperplasia with increased marrow iron.
- Increased body iron contents
  - Increased S.ferritin
  - Increased transferrin saturation
  - Increased marrow iron



Diagnosis

- Clinical features:
  - Refractory anaemia with marked splenomegaly starting early in life
  - Positive family history.
  - Social history of Consanguineous marriage
- Haematological features:
  - Typical red cell morphological changes.
  - Raised Hb-F level on electrophoresis
  - Increased serum iron parameters.
  - Reduced B-chain synthesis rate
- Molecular techniques to demonstrate defective B- globin gene.


HPLC High
Performance
Liquid Chromatography

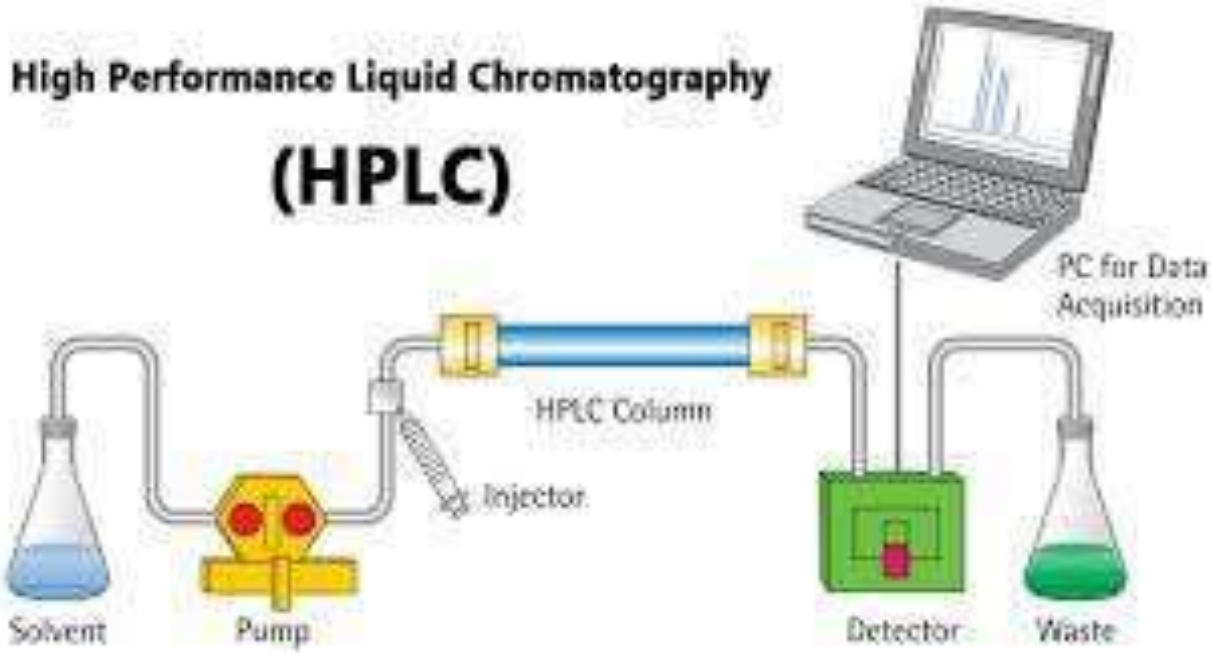
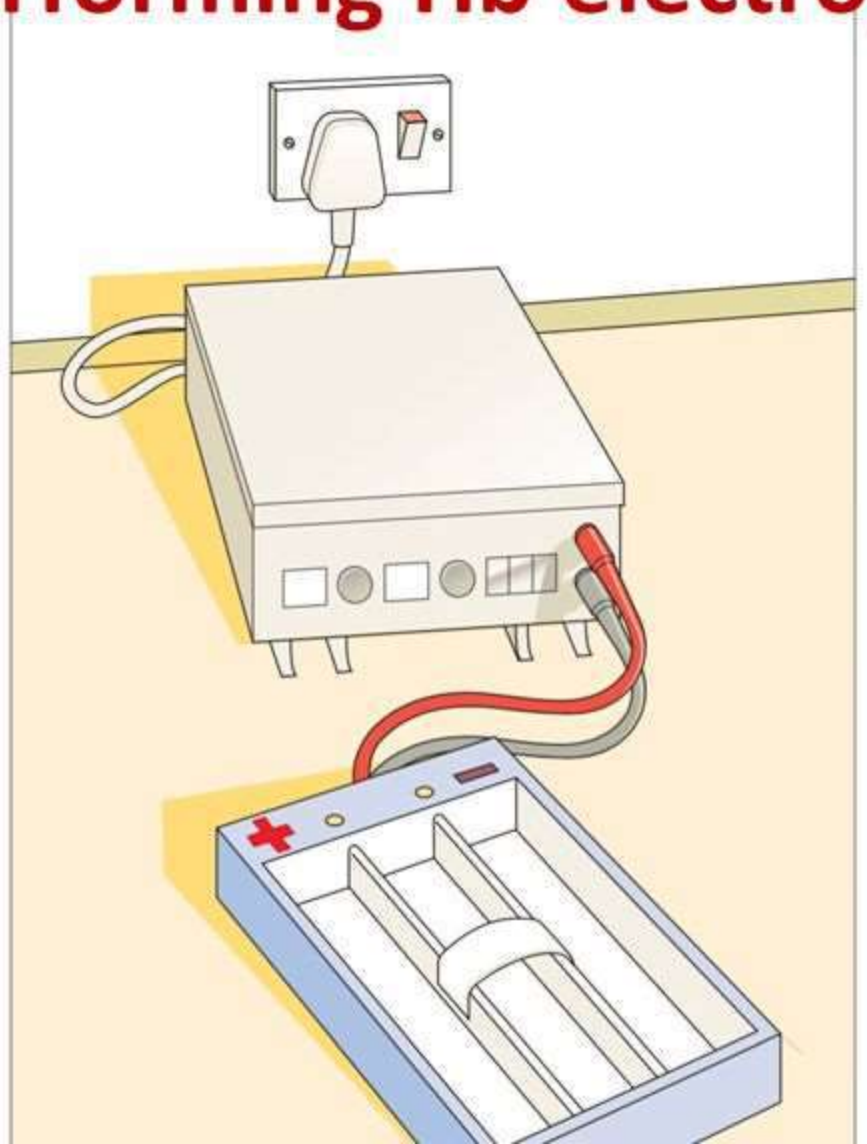

High Performance Liquid Chromatography
(HPLC)
PC for Data Acquisition
HPLC Column
Injector
Solvent
Pump
Detector
Waste

Diagram of apparatus for performing Hb electrophoresis



mangement

- Supportive measures include:
  - Blood transfusion;
    - Traditional method.
    - Hypertransfusion.
    - Supertransfusion.
  - Splenectomy
  - Iron chelation by desferal.
  - Treatment of infections, replacement therapy, folate
- BM transplantation.
- Gene therapy.

Hb – Variants ; Sickle cell disease(SCD)

SCD is the homozygous state of Hb-S, the disease is common in central Africa, middle east and in Black Americans of African origin.

Molecular defect & pathogenesis

SCD results from a point mutation in the B-globin gene that causes substitution of valine instead of glutamic acid at the 6^{th} amino position of the globin chain, this markedly reduces Hb solubility which under reduced O2 tension forms crystals (tactoids) that gives a sickle form to the cells, these cells are rigid and will occlude microcirculations (vas-occlusive phenomena) leading to multiple infarcts in the spleen, bones and elsewhere. Sickle cells are abnormal that have a shortened life span and this lead to chronic haemolytic anaemia .

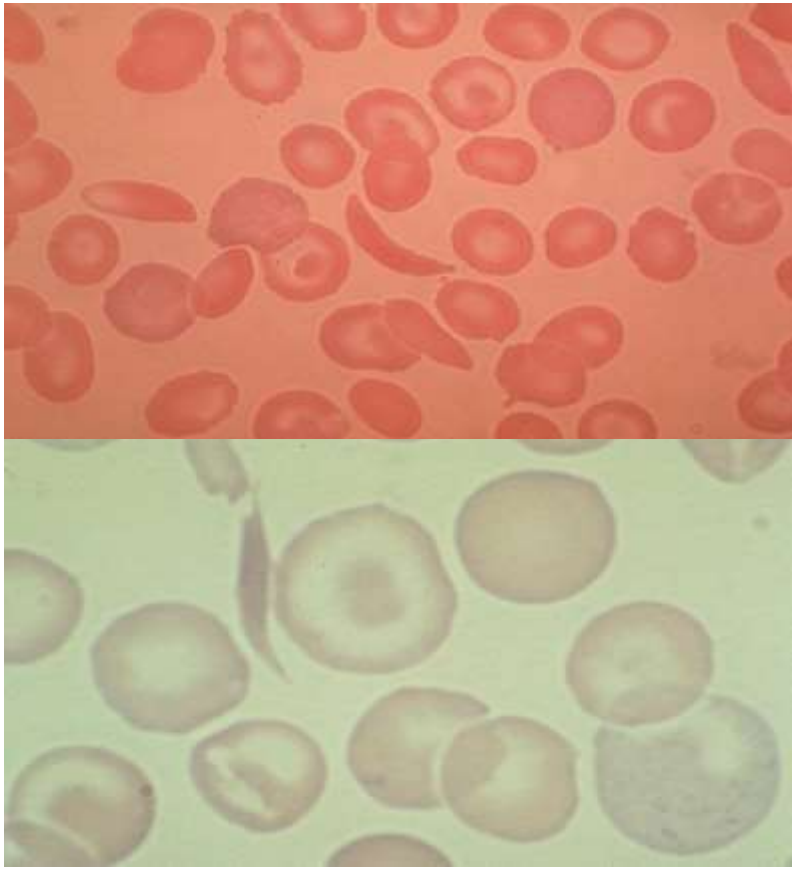
Clinical Features

The clinical featurs are those of chronic haemolytic anemia associated painful incidents (Sickle Crises) due to organ infarcts including:

- Painful abdominal crises due to splenic infarcts or mesenteric thrombosis. They loose their spleens by the age of 5 years (autosplenectomy)
- Painful dactylitis due to infarcts of small bones of the hand and feet (hand and foot syndrome)
- Painful chest crisis
- CVA
- Priapism
- These painful crises are associated with anaemia and jaundice

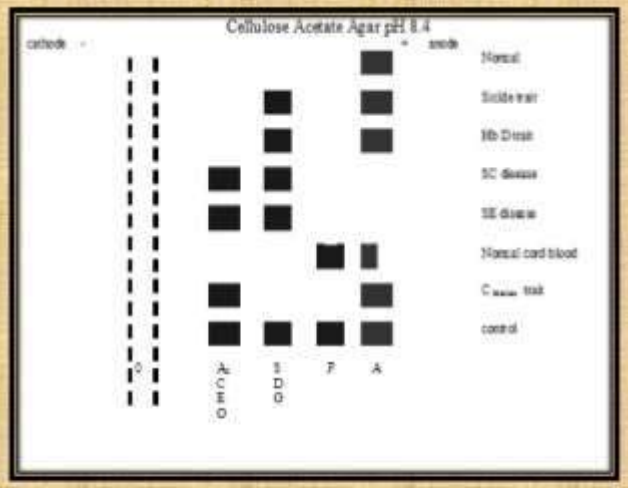
Haematological features.

- Normochromic normocytic RBCs with frequent target forms and occasional sickle cells.
- Reticulocytosis
- Erythroid hyperplasia with increased marrow iron.

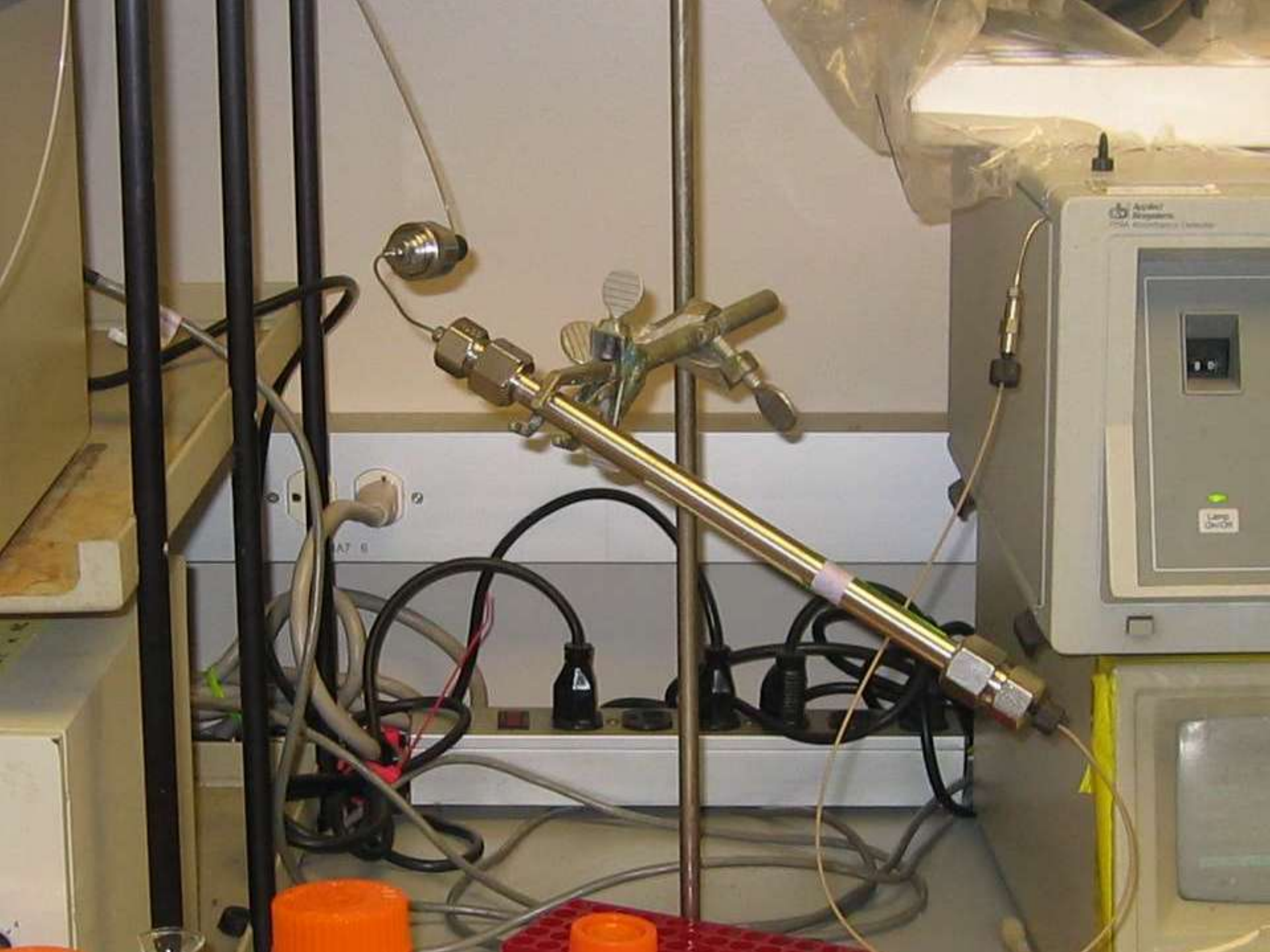


diagnosis

- Clinical features:
  - Chronic haemolytic anaemia + painful crises, autosplenectomy.
  - Family history.
- Haematological features:
  - Normochromic normocytic RBCs with target and sickle forms.
  - Hb-electrophoresis: Hb-S (>80%) and Hb-F (<20%), no Hb-A.
  - Positive sickling and solubility tests.


Cellulose Acetate Agar pH 8.4
cathode -
+ anode
Normal
Sickle trait
Hb Disease
SC disease
SE disease
Normal cord blood
control
A
S
D
F
A


a
b
c
d
e
f
g
control
AS
AS
AS
AC
AC
S
AS
AFSC



Haemolytic Anaemias

Haemolysis:

Shortening of red cell survival with premature red cell death.

When the life span of the red cells is shortened to less than 20 days, Hb drops and anaemia develops (Haemolytic anaemia), with longer life spans the marrow can compensate by hyperactivity &/or expansion keeping Hb within normal limits (Compensated haemolysis)

Classification

- **H. Anaemias due to intrinsic red cell defects (usually inherited)**
  - **Red cell membrane defects (e.g H.Spherocytosis)**
  - **Metabolic defects (Enzymopathies, e.g G6PD deficiency)**
  - **Hb synthesis defects (haemoglobinopathies)**
- **H. Anaemias due to extrinsic defects:**
  - **Immune H. Anaemias**
  - **Mechanical H. Anaemias.**
  - **H. Anaemias due to Infections, Chemical toxins and Physical agents.**

Haemolysis is called intravascular when RBCs are destroyed in the circulation, while it is called extravascular when destruction occurs by the cells of the RES in the spleen, liver & B.M

General Features of Haemolysis

- **Features due to Hb degradation:**
  - **Indirect hyperbilirubinaemia (clinically; Jaundice, gall stones)**
  - **Hyperurobilinogenuria**
  - **RES hyperplasia (clinically; splenomegaly)**
  - **Iron overload**
- **Featurs due to marrow compensation:**
  - **Reticulocytosis**
  - **Skeletal abnormalities due to marrow expansion.**
  - **Folate deficiency.**
- **Features specific of intravascular haemolysis:**
  - **Haemoglobinaemia & hypohaptoglobinaemia.**
  - **Haemoglobin & haemosiderinuria.**

Hereditary Spherocytisis (HS)

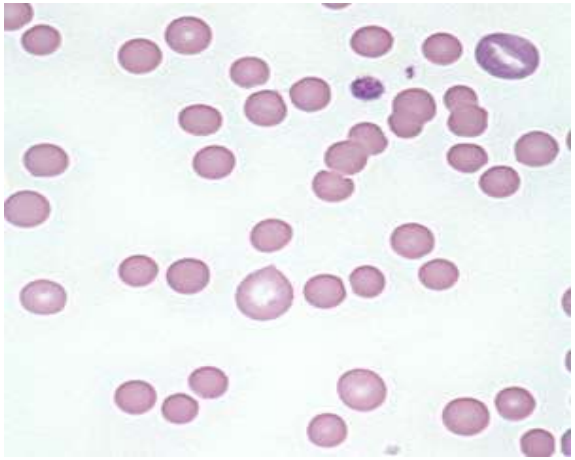
A hereditary haematological disorder characterized by:

- **Autosomal dominant inheritance.**
- **Excessive red cell fragility.**
- **Microspherocytes in the peripheral blood.**
- **Marked improvement (usually cure) of anaemia after splenectomy.**

Molecular defects and pathogenesis

A genetic mutation resulting in abnormality of the cytoskeletal protein; spectrin will cause excessive leakiness of the cell membrane to cat-ions (Na & K) »» Hyperactivity of Na-K pump »» excessine utilization of glucose & O2 (hypermetabolism)….. In the spleen where there is stagnation, hypoxia, hypoglycaemia and acidosis »» Failure of Na-K pump »» Entry to the cell of Na with water »» swelling of the cell »» Spherocytosis »» further stagnation »» loss of cell membrane »» rupture (haemolysis) and microspherocyte formation

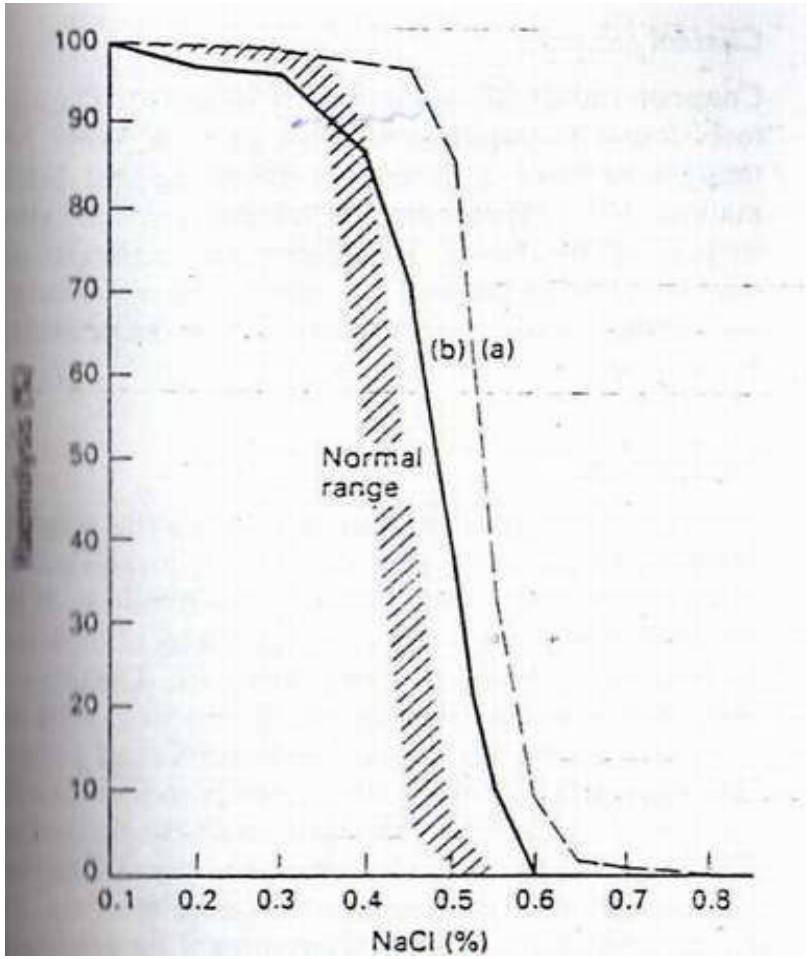
Haematological Featurs

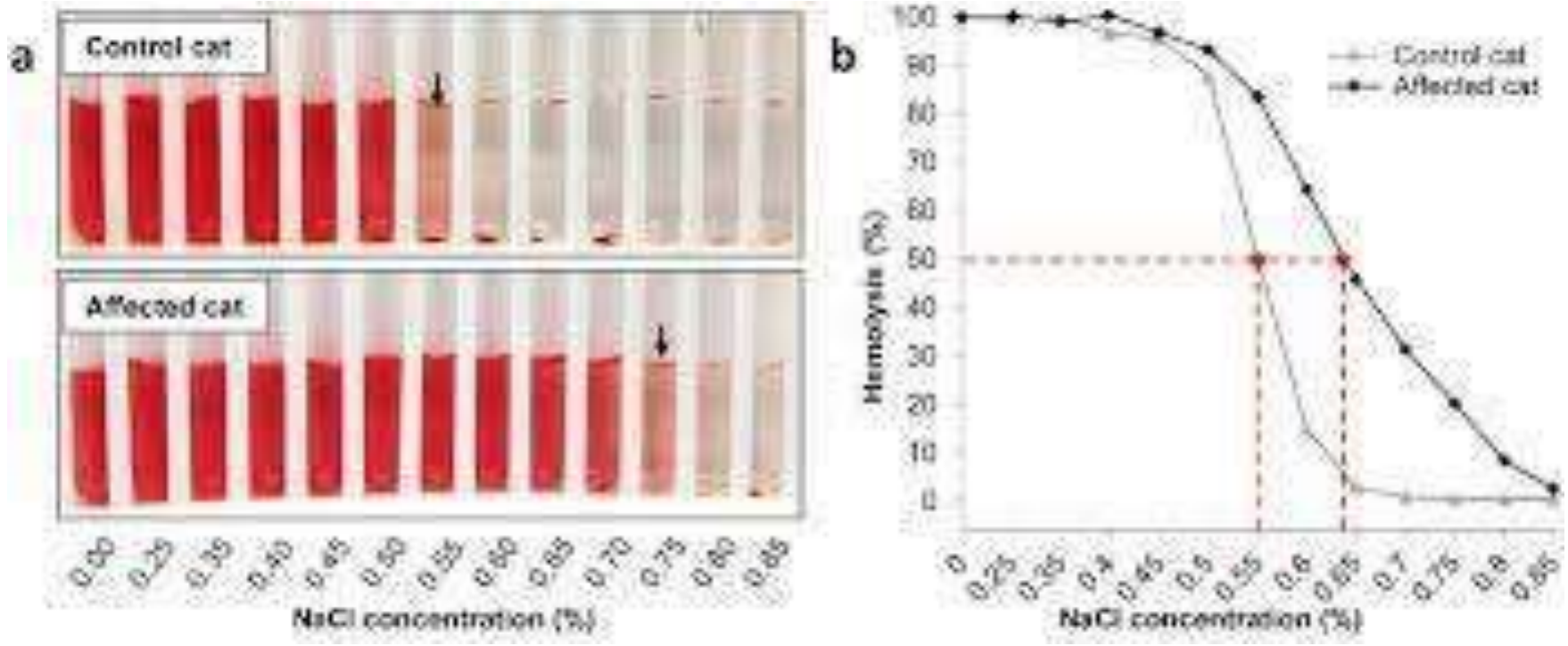


- **Anaemia; normochromic normocytic with spherocytosis**
- **Reticulocytosis**
- **Increased red cell fragility**

Diagnosis

- **Jaundice, anaemia, splenomegaly.**
- **Positive family history, lab evidence among other members.**
- **Spherocytosis with reticulocytosis**
- **Increased red cell fragility**
- **Cure of anaemia after splenectomy**

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Affected cat
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0.25
0.30
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
NaCl concentration (%)
b
Control cat
Affected cat
Hemolysis (%)
100
90
80
70
60
50
40
30
20
10
0
0
0.25
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0.4
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  - **Perroxidation of membrane lipids.**
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- **G6PD deficiency results from point mutations affecting G6PD gene on chromosome – X, mutations will result in isoenzymes that are either:**
  - **Unstable.**
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- **The pathological effects of the deficiency depends on the residual activity of the enzyme:**
  - **Activities of >3-5% normal are sufficient to maintain normal red cell metabolism under normal conditions but will lead to intravascular haemolysis under conditions of extr-oxidant stresses (infections, drugs & ingestion of fava beans).**
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Clinical presentation

- **Neonatal jaundice**
- **Chronic haemolytic anaemia (rare presentation)**
- **Most individuals with G6PD deficiency are asymptomatic unless exposed to oxidant stresses, such as infections, drugs and fava beans ingestion, the latter is called Favisim where there will be a sudden bout of intravascular haemolysis characterized by:**
  - **Abdomenal pain, rigors, back pain and vomiting.**
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  - **Passage of red urine (Hb-uria)**
  - **Jaundice**
  - **Patient may pass into shock and renal failure.**

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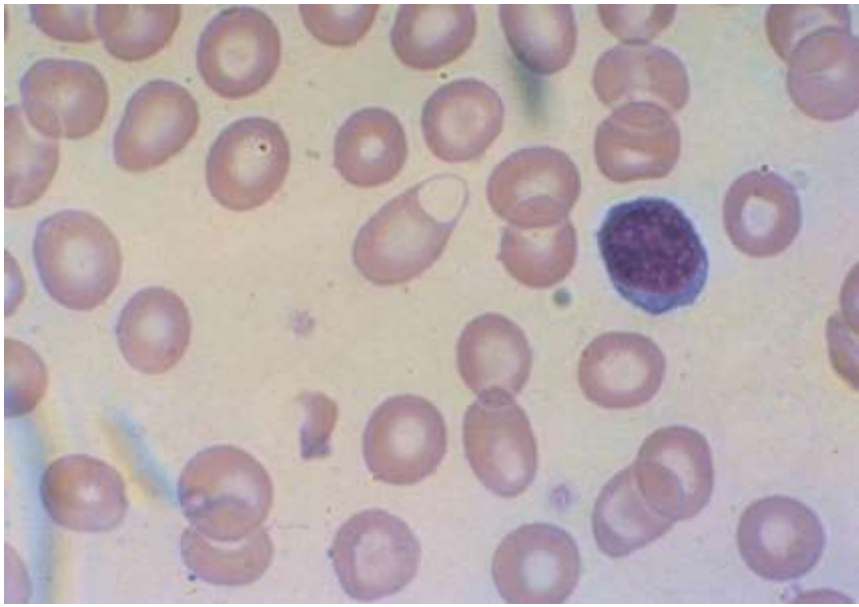

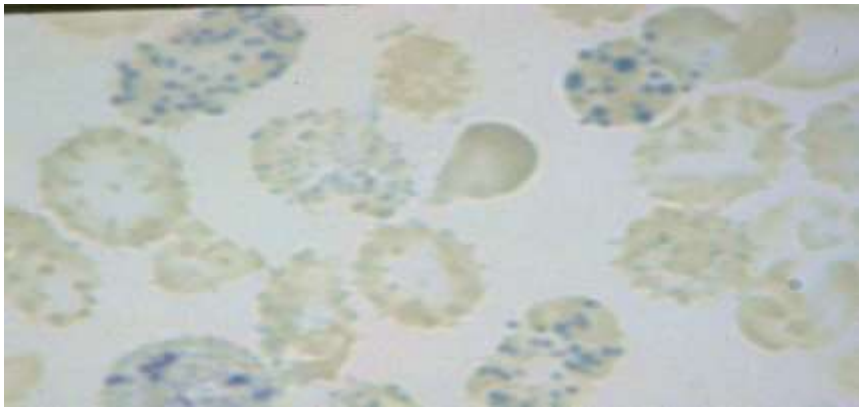

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  - **Screening tests (MRT)**
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Diagnosis of G6PD Deficiency Hemolytic Anemia

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Screening:

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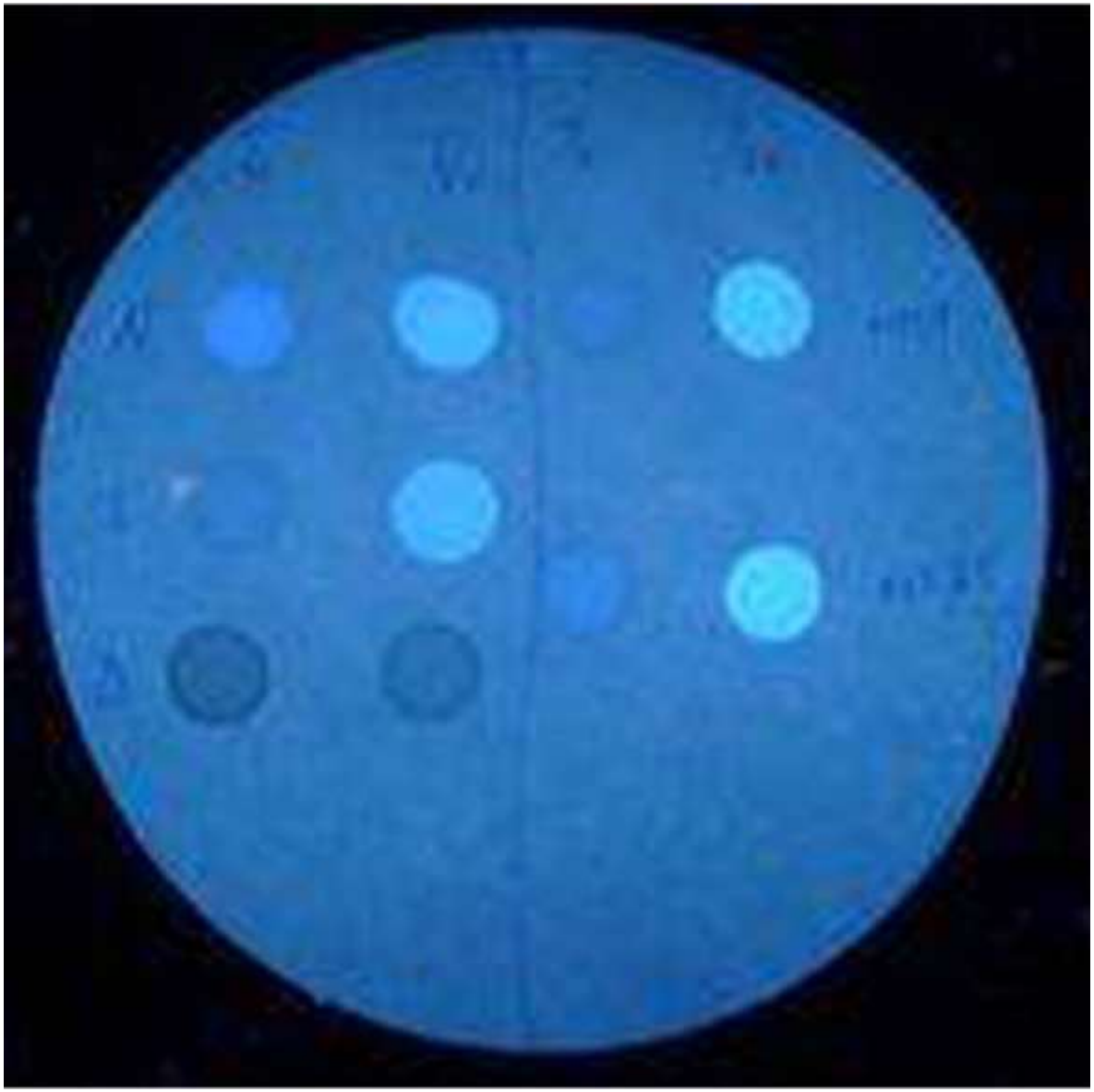
Molecular test:

Detection of G6PD gene mutation



Haemolytic Anaemias due to Extrinsic Factors

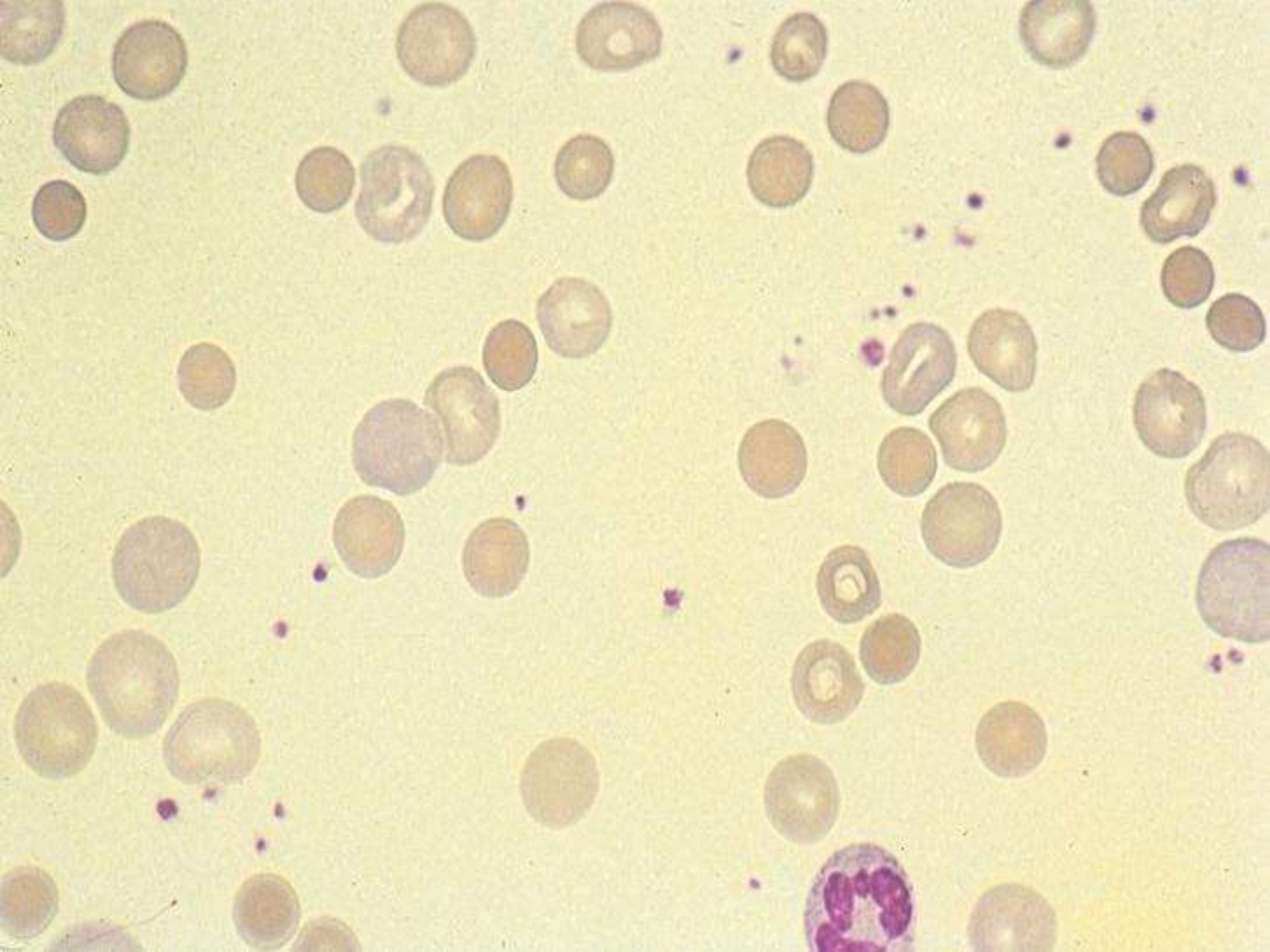
Immune Haemolytic anaemias (IHA)

Definition and Classification

- **Immune haemolysis is defined as red cell destruction brought about by antibody antigen reaction, antibodies are usually directed against red cell antigens. The defining character of all IHA is a positive direct antiglobulin (DAT or Coombs) test.**
- **Classification:**
  - **Autoimmune H. A.: Antibodies produced by the individual himself**
    - **Warm antibody type**
    - **Cold antibody type (cold agglutinin syndromes)**
  - **Alloimmune H.A : antibodies and antigens belong to different individuals:**
    - **Haemolytic disease of the newborn (HDN).**
    - **Incompatible blood transfusion.**
  - **Drug induced IHA:**

Warm AB type AIHA

- **The antibody is IgG and has a maximal activity around 37^0C. Antibody coated RBCs are destroyed extravascularly by the cells of the RE system mainly in the spleen.**
- **The disease affects females more commonly, the onset is usually insidious with jaundice , anaemia and splenomegaly.**
- **Haematologically: RBCs are normochromic, normocytic with spherocytosis, normoblastaemia and marked reticulocytosis.**



Aetiology of warm type AIHA

- **Idiopathic in 30 % of cases.**
- **Secondary to:**
  - **Lymphoproliferative disorders (CLL, HD and NHL)**
  - **Autoimmune disorders (SLE, RA and ulcerative colitis).**
  - **Infections (viral)**
  - **Carcinomas (ovarian ca.)**
  - **Drugs (methyldopa)**

Diagnosis of warm type IHA

- **Diagnosis depends on:**
  - **Clinical findings**
  - **Classical red cell morphology.**
  - **A positive direct Coombs test**

- **If transfusion is needed, these patients present a problem to the blood bank as it is almost impossible to find a compatible blood, usually the least incompatible unit is chosen from a panel of blood units.**

Cold antibodies immune haemolytic anaemia

- 1) Cold haemagglutinin disease
  Can be primary or secondary to lymphoma
- Adenocarcinoma
- Mycoplasma pneumoniae
- Clinically , Acrocyanosis
- 2) Paroxysmal cold haemoglobinuria

Haemolytic Disease of the Newborn (HDN)

- **Destruction of fetal RBCs by maternal AB. Maternal IgG AB can pass the placental barrier and react with fetal red cell antigens, more commonly with antigens in the ABO and Rh systems.**
- **ABO HDN occur in blood group O^+ mothers who have in their sera immune anti-A & anti-B antibodies and carry a blood group A , B or AB fetus, the disease is most commonly mild and presents as NNJ, rarely needs exchange transfusion, it can affect the first pregnancy.**

Rh HDN (Erythroblastosis fetalis)

- **This is more serious than ABO HDN, first born baby is not affected, but at the time of delivery fetal RBCs pass to maternal circulation and the mother may become sensitized (produces anti-D antibodies), the second baby will usually have severe anaemia with severe jaundice (2^{nd} or 3^{rd} day) and may develop kernicterus with severe neurological defects unless promptly treated by exchange transfusion, subsequent deliveries result in still birth, the fetus has gross pallor, oedema , jaundice and gross abdominal distension with a bulky placenta (hydrops fetalis).**
- **Rh HDN affects only about 30% of Rh^{-ve} mothers carrying Rh^{+ve} babies, ABO fetomaternal incompatibility reduces sensitization.**
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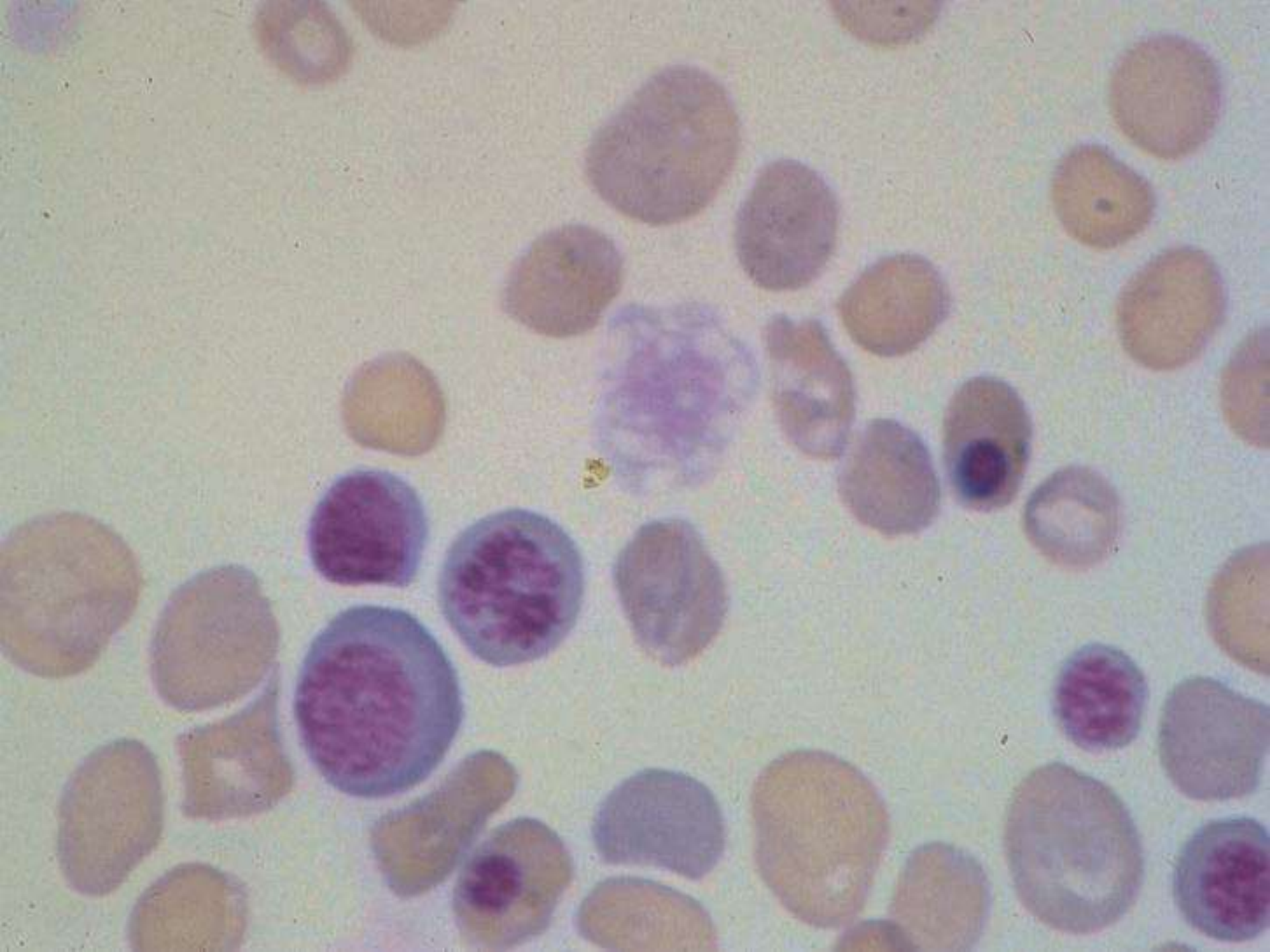


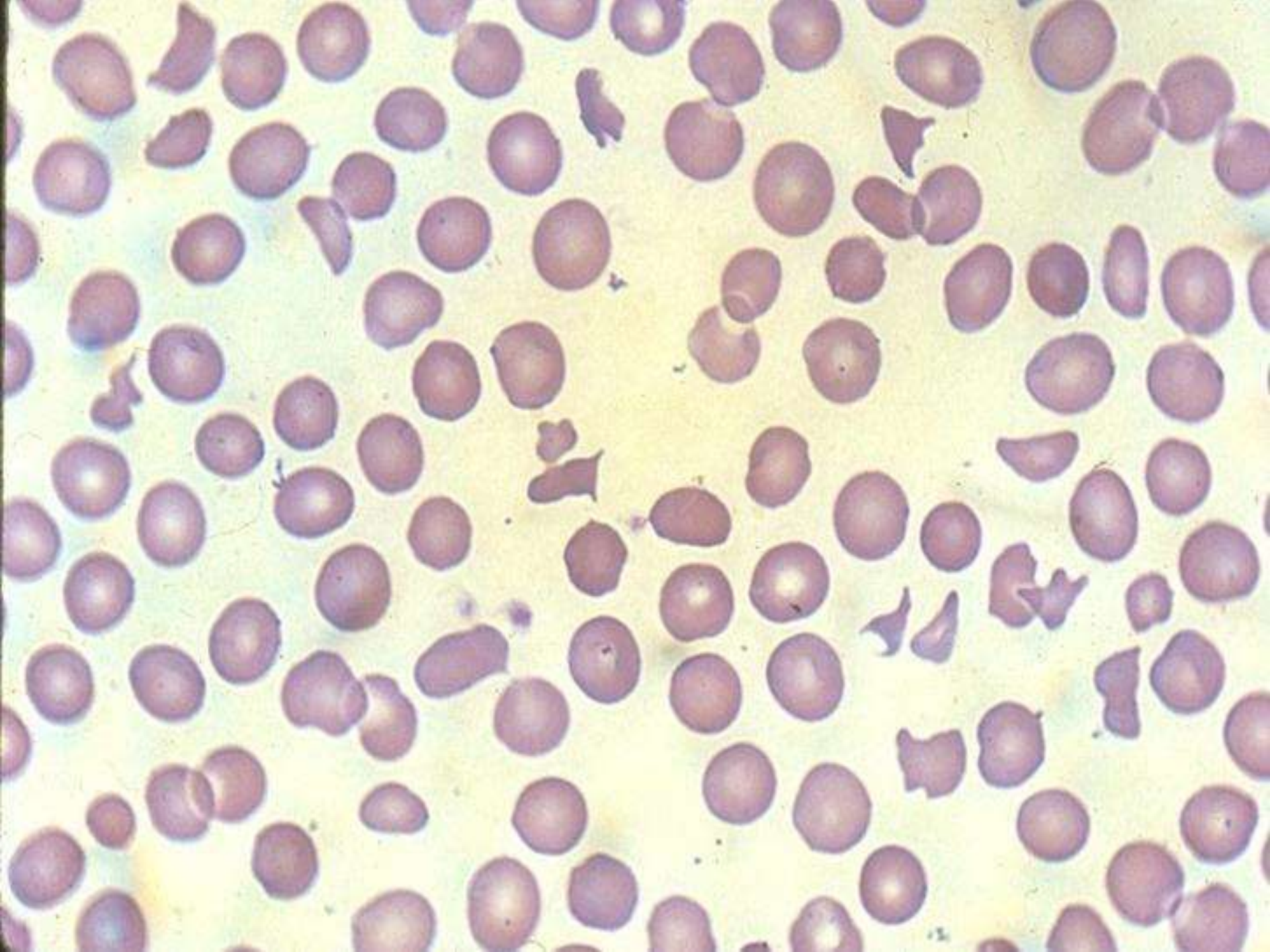
Mechanical anaemias
Fragmentation Anaemias

- **Fragmentation anaemias are group of haemolytic anaemias characterized by presence of fragmented RBCs in the peripheral blood (Schistocytes)and intravascular haemolysis.**
- **Fragmentation anaemias could result from:**
    - **Prosthetic cardiac replacements (valves and patches), associated with turbulent blood flow (cardiac haemolysis)**
    - **Red cell destruction in the small blood vessels " micro-angiopathic haemolytic anaemia (MAHA) " as a result of :**
        - **Wide spread fibrin deposition (DIC)**
        - **Abnormal platelet aggregation (platelet aggregate syndromes ; HUS & TTP).**
        - **Abnormal vascular endothelium (vasculitis)**

MAHA is characterized by thrombocytopenia in addition to schistocytosis & features of intravascular haemolysis.

March haemoglobinuria

- In long marches or marathon running
- Karate sports



Miscellaneous other acquired

- Haemolytic toxins & chemical
- Cl welchii
- Lead poisoning
- Spider & snake venoms
- Black water fever (falciparum malaria)
- Paroxysmal nocturnal haemoglobinuria

Haemolytic Anaemias

Haemolysis:

Shortening of red cell survival with premature red cell death.

When the life span of the red cells is shortened to less than 20 days, Hb drops and anaemia develops (Haemolytic anaemia), with longer life spans the marrow can compensate by hyperactivity &/or expansion keeping Hb within normal limits (Compensated haemolysis)

Classification

- **H. Anaemias due to intrinsic red cell defects (usually inherited)**
  - **Red cell membrane defects (e.g H.Spherocytosis)**
  - **Metabolic defects (Enzymopathies, e.g G6PD deficiency)**
  - **Hb synthesis defects (haemoglobinopathies)**
- **H. Anaemias due to extrinsic defects:**
  - **Immune H. Anaemias**
  - **Mechanical H. Anaemias.**
  - **H. Anaemias due to Infections, Chemical toxins and Physical agents.**

Haemolysis is called intravascular when RBCs are destroyed in the circulation, while it is called extravascular when destruction occurs by the cells of the RES in the spleen, liver & B.M

General Features of Haemolysis

- **Features due to Hb degradation:**
  - **Indirect hyperbilirubinaemia (clinically; Jaundice, gall stones)**
  - **Hyperurobilinogenuria**
  - **RES hyperplasia (clinically; splenomegaly)**
  - **Iron overload**
- **Featurs due to marrow compensation:**
  - **Reticulocytosis**
  - **Skeletal abnormalities due to marrow expansion.**
  - **Folate deficiency.**
- **Features specific of intravascular haemolysis:**
  - **Haemoglobinaemia & hypohaptoglobinaemia.**
  - **Haemoglobin & haemosiderinuria.**

Hereditary Spherocytisis (HS)

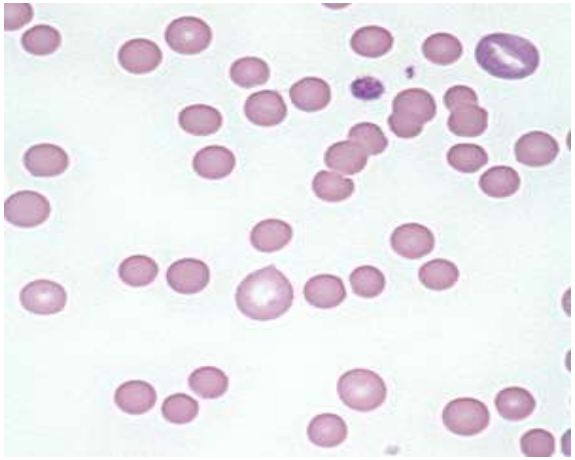
A hereditary haematological disorder characterized by:

- **Autosomal dominant inheritance.**
- **Excessive red cell fragility.**
- **Microspherocytes in the peripheral blood.**
- **Marked improvement (usually cure) of anaemia after splenectomy.**

Molecular defects and pathogenesis

A genetic mutation resulting in abnormality of the cytoskeletal protein; spectrin will cause excessive leakiness of the cell membrane to cat-ions (Na & K) »» Hyperactivity of Na-K pump »» excessine utilization of glucose & O2 (hypermetabolism)….. In the spleen where there is stagnation, hypoxia, hypoglycaemia and acidosis »» Failure of Na-K pump »» Entry to the cell of Na with water »» swelling of the cell »» Spherocytosis »» further stagnation »» loss of cell membrane »» rupture (haemolysis) and microspherocyte formation

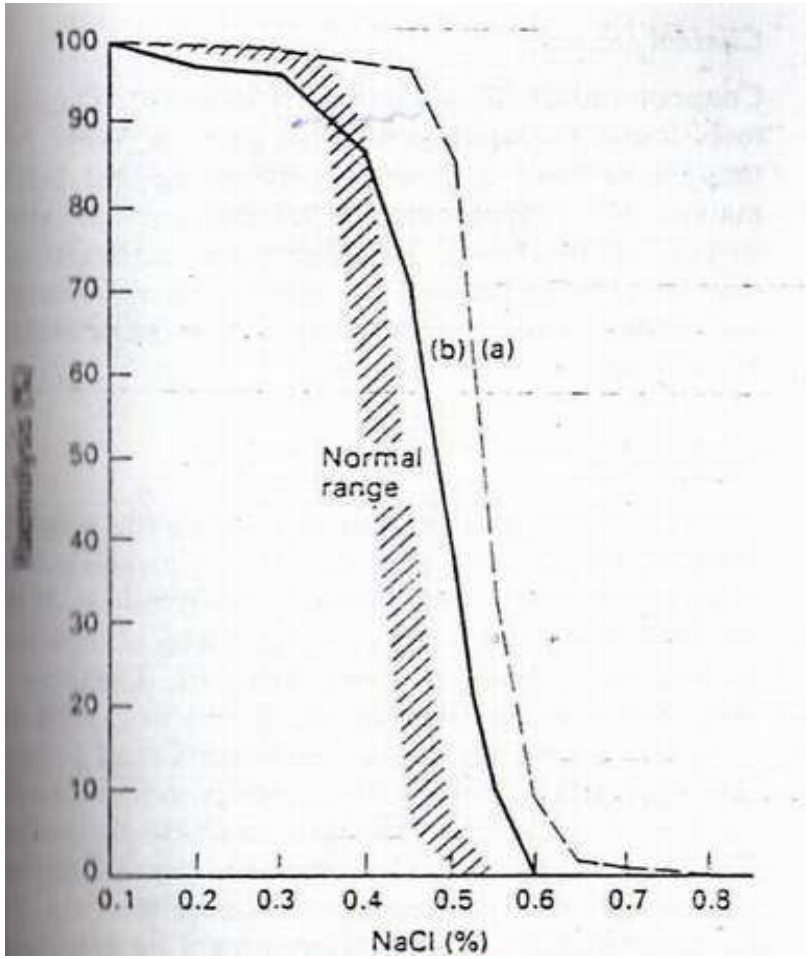
Haematological Featurs

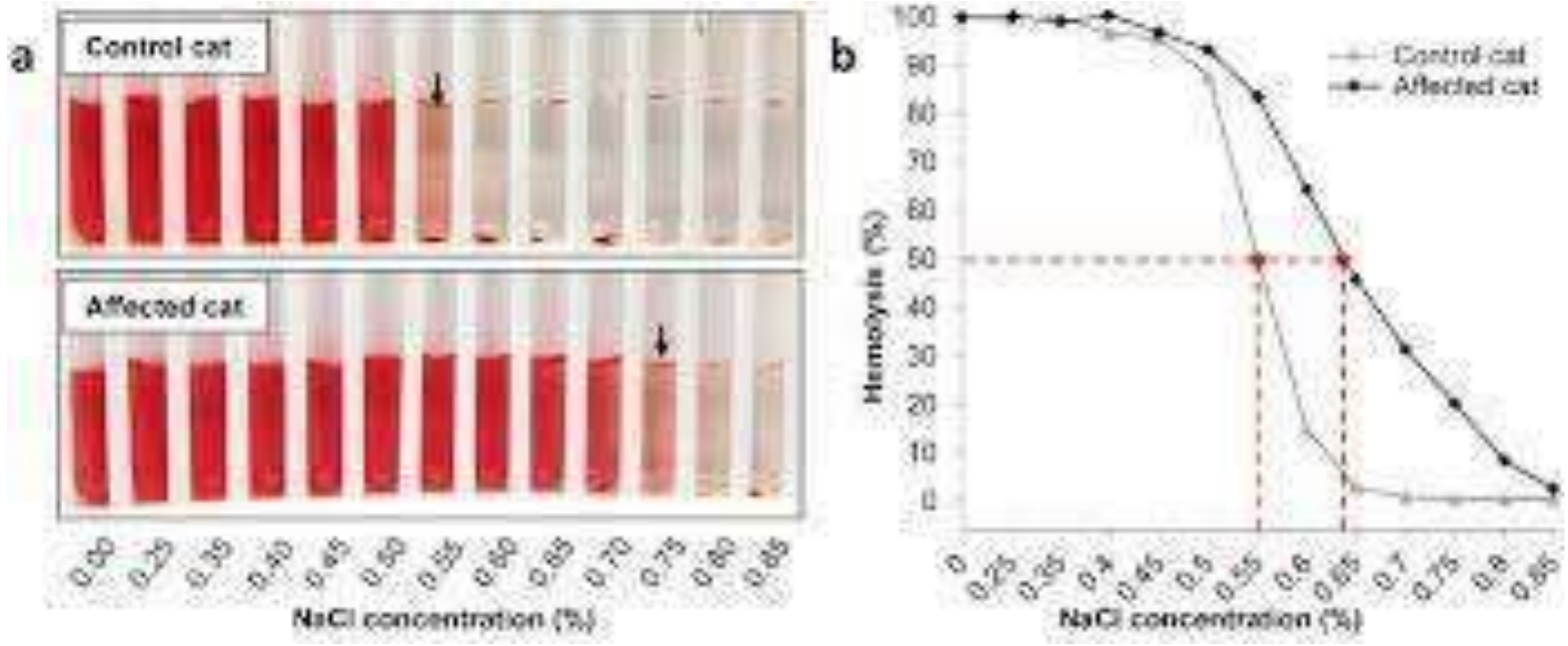


- **Anaemia; normochromic normocytic with spherocytosis**
- **Reticulocytosis**
- **Increased red cell fragility**

Diagnosis

- **Jaundice, anaemia, splenomegaly.**
- **Positive family history, lab evidence among other members.**
- **Spherocytosis with reticulocytosis**
- **Increased red cell fragility**
- **Cure of anaemia after splenectomy**

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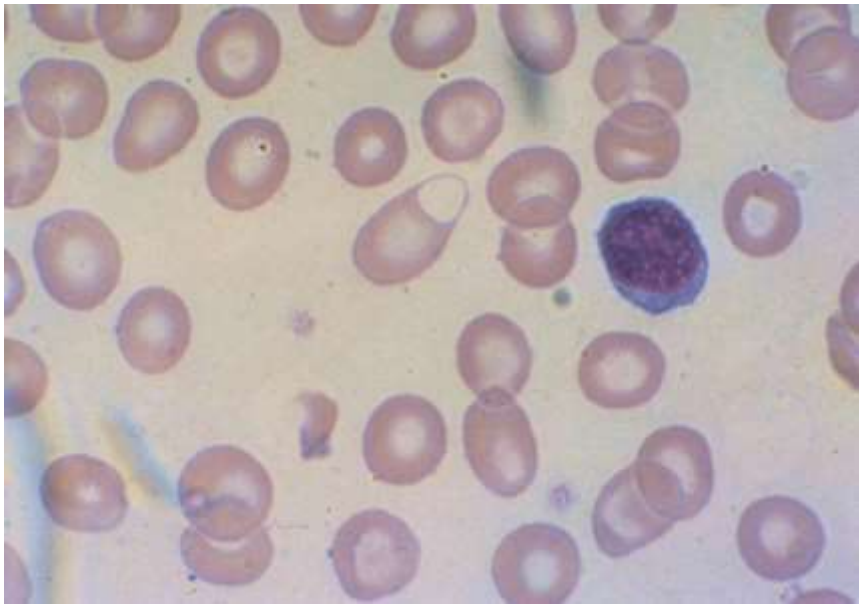

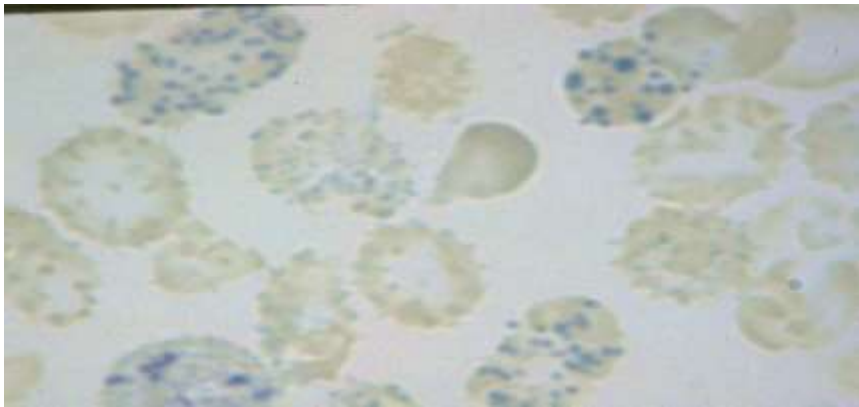

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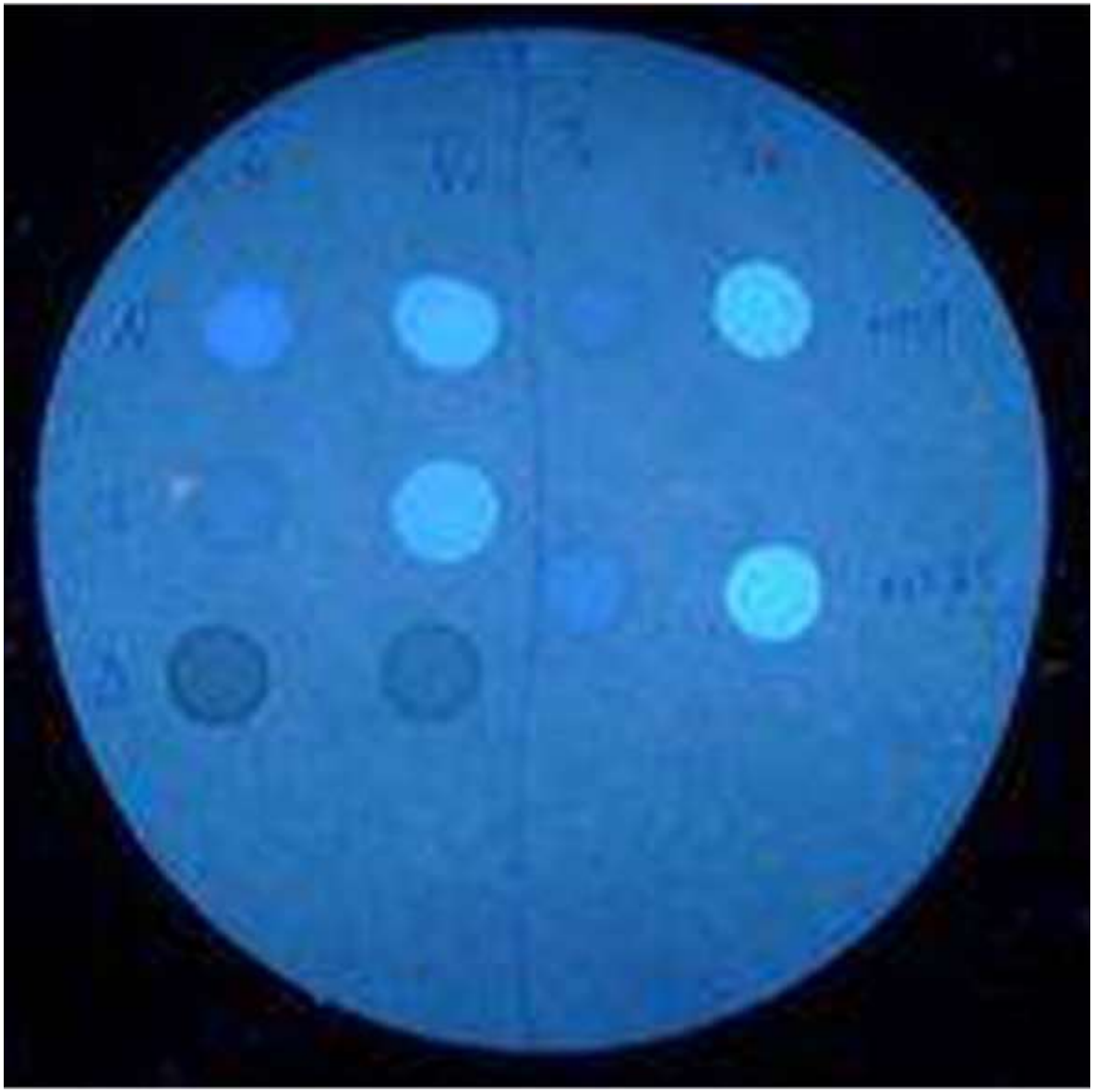

Methemoglobin Reduction Test

- Normal blood → clear red color
- Deficient blood → brown color

Diagnosis of G6PD Deficiency Hemolytic Anemia

Diagnosis of hemolytic anemia

Complete Blood Count (CBC) & reticulocytic count

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Qualitative assessment of G6PD enzymatic activity (UV-based test)

Confirmatory test:

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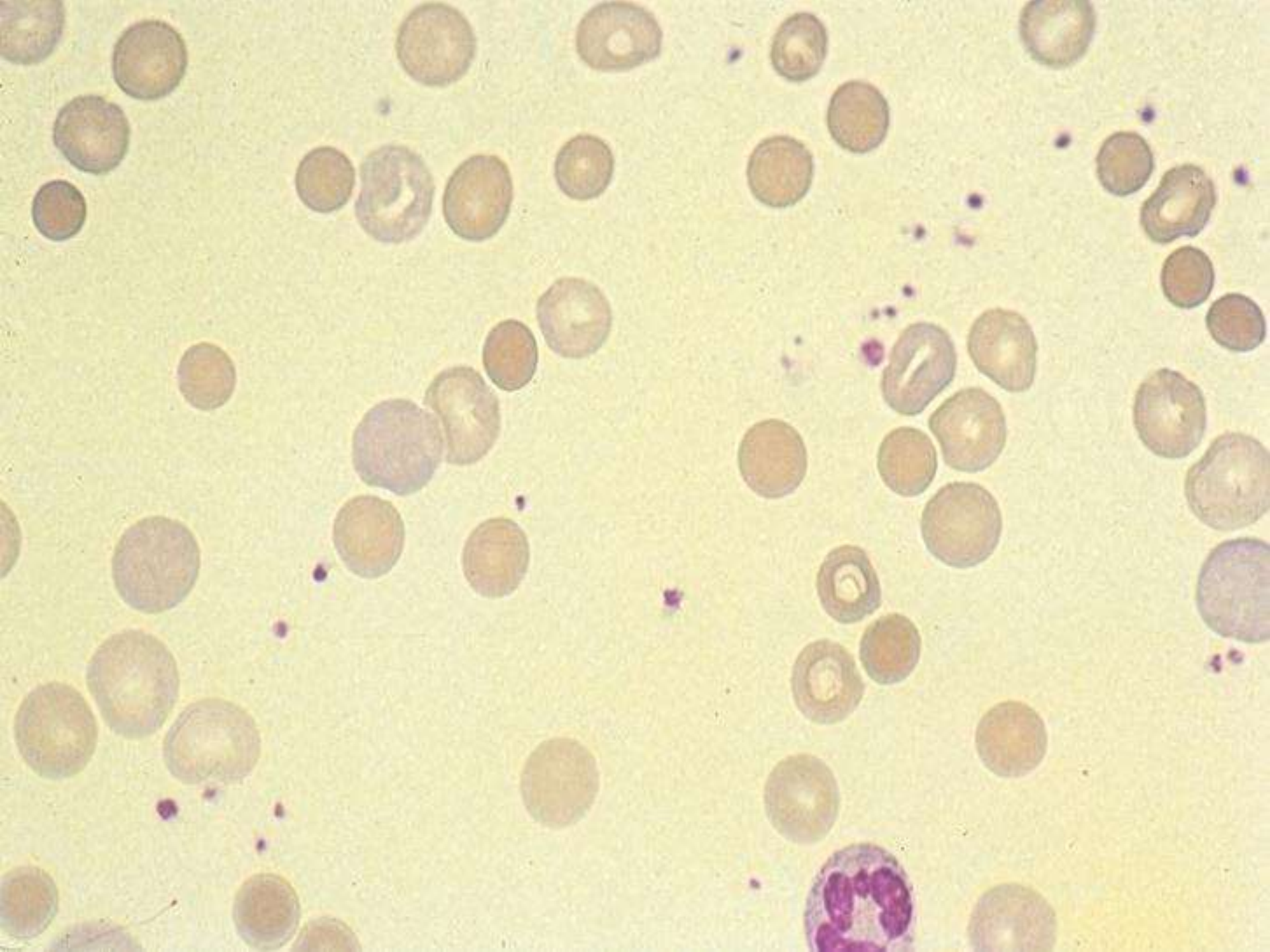

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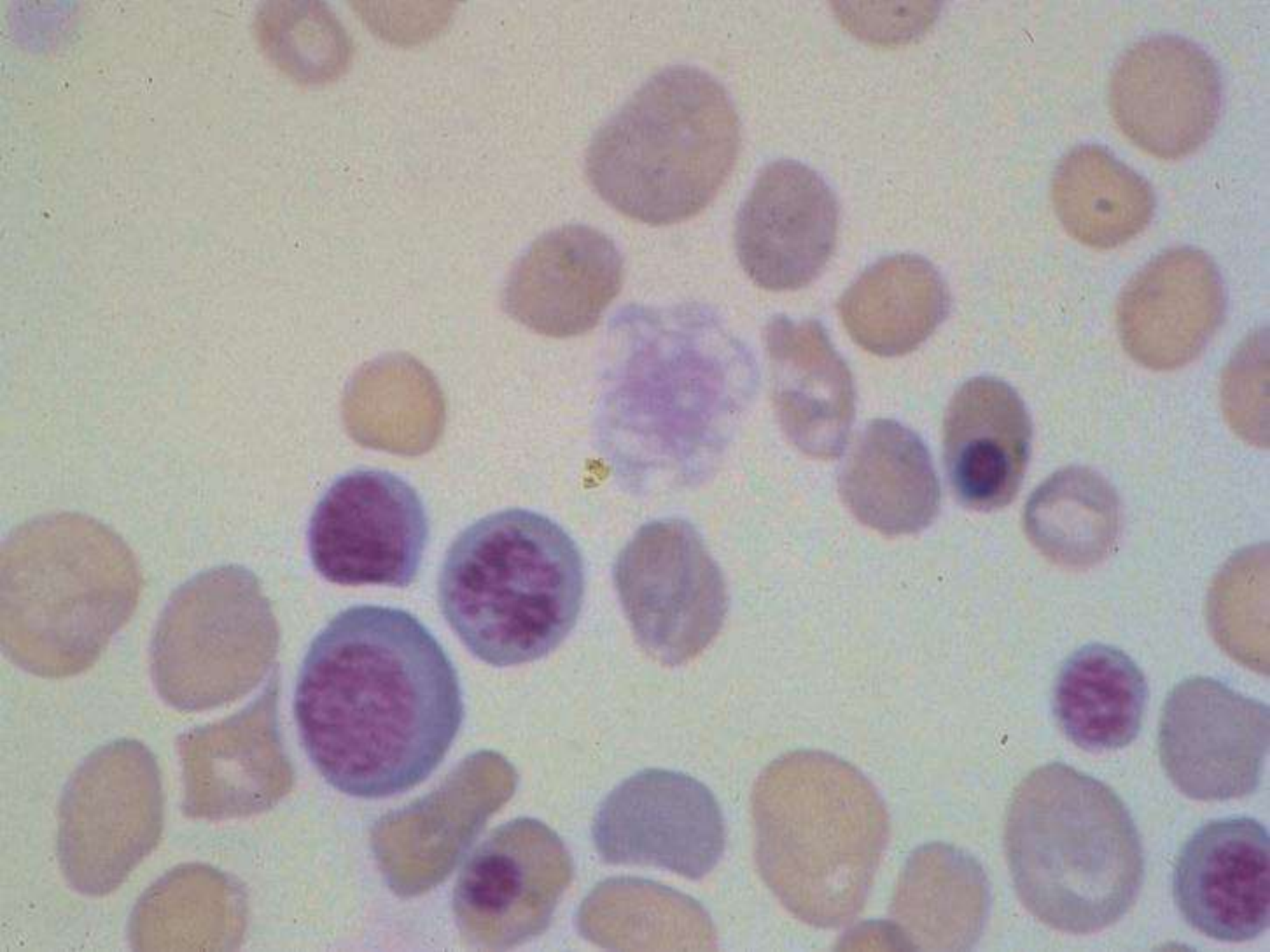

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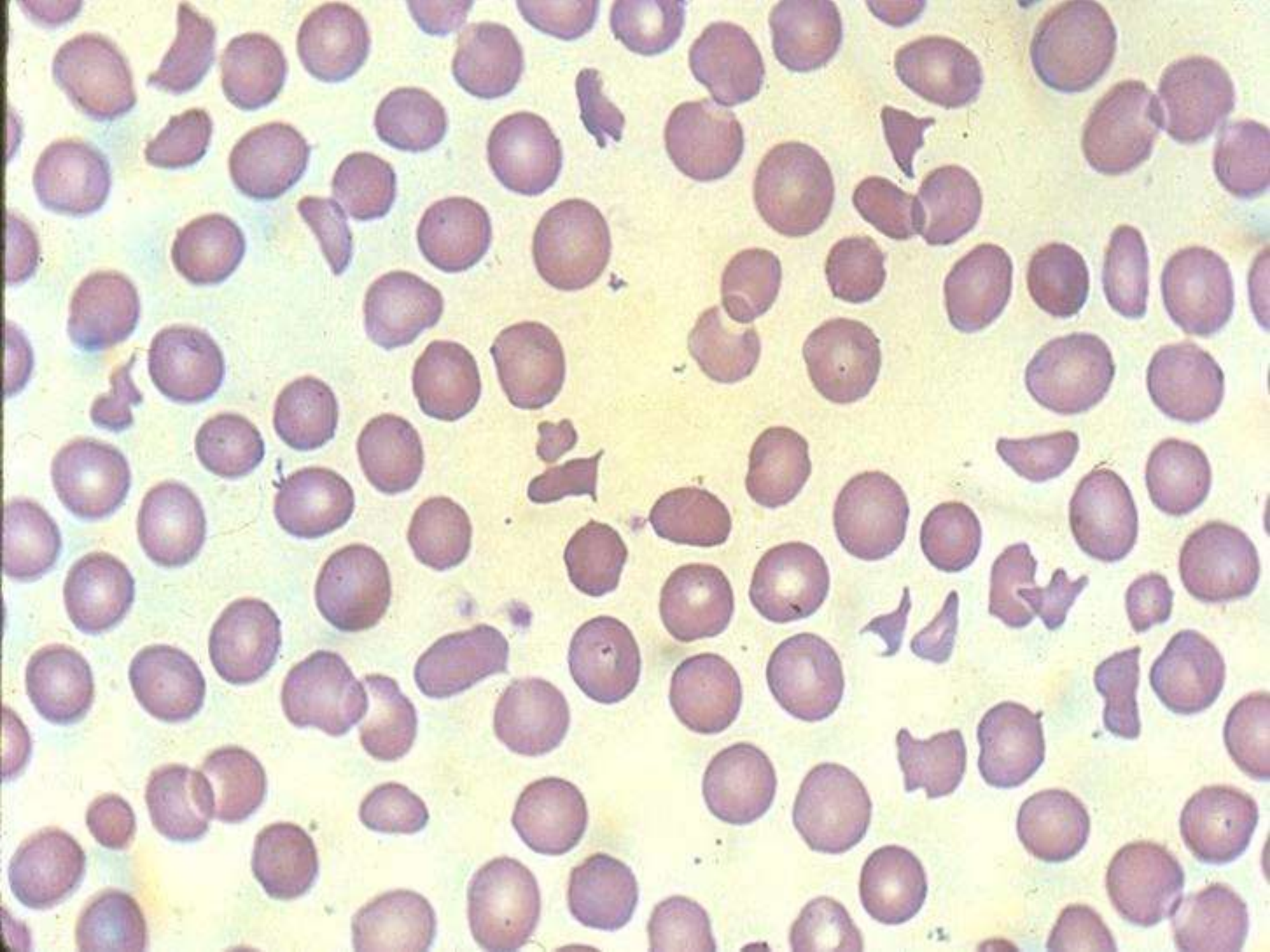

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