Blood Types

Blood Types…

- There are four different types of blood (A, B, AB, O)

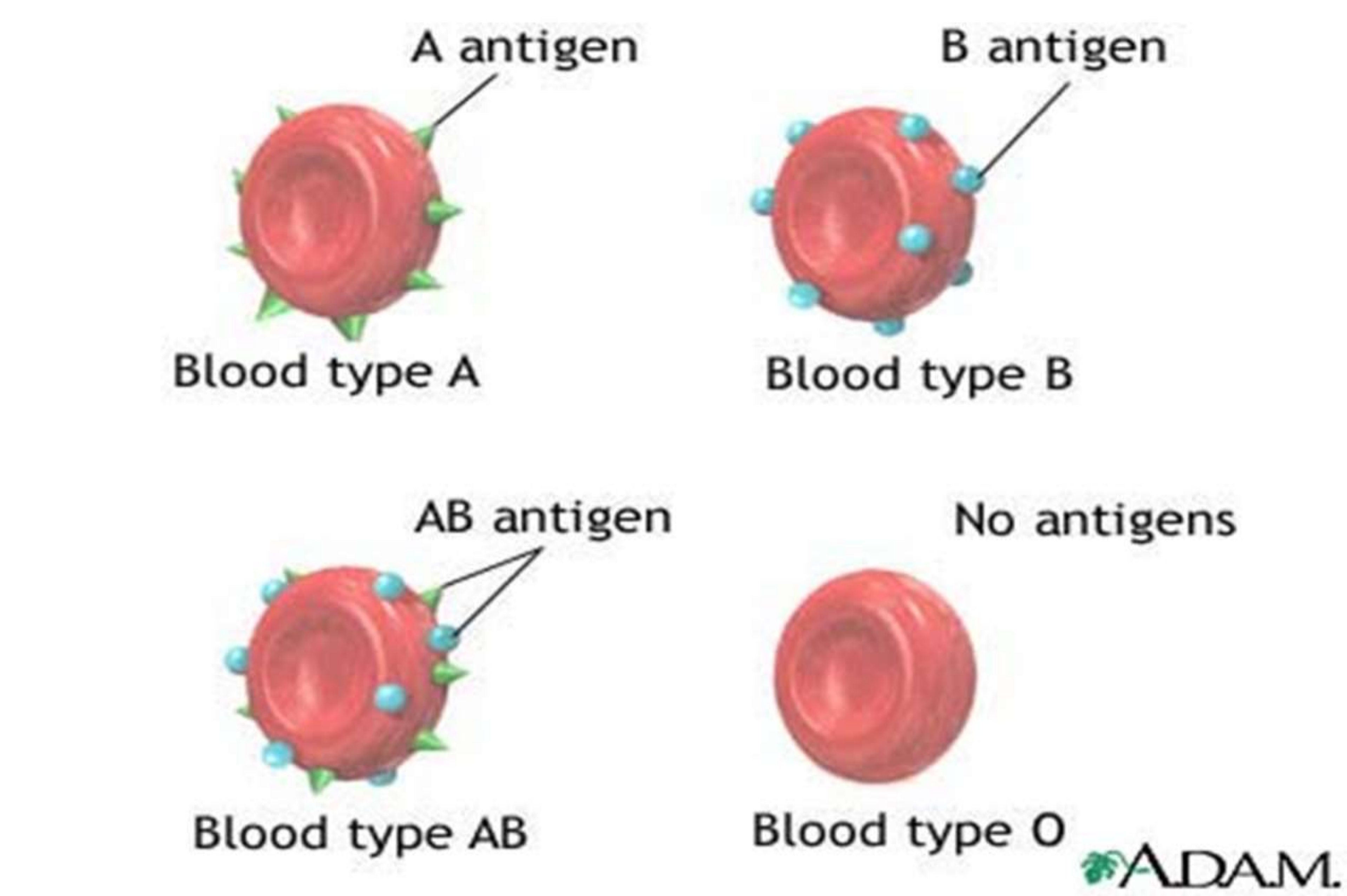
- They are determined by the protein (antigen) found on the RBC surface.

Type A, has protein (antigen) A on the RBC.

Type B, has protein (antigen) B on the RBC.

Type AB, has both protein (antigen) A and B on the RBC.

Type O, has neither protein (antigen) on the RBC.


A antigen
Blood type A
B antigen
Blood type B
AB antigen
Blood type AB
No antigens
Blood type O
A.D.A.M.

Notes

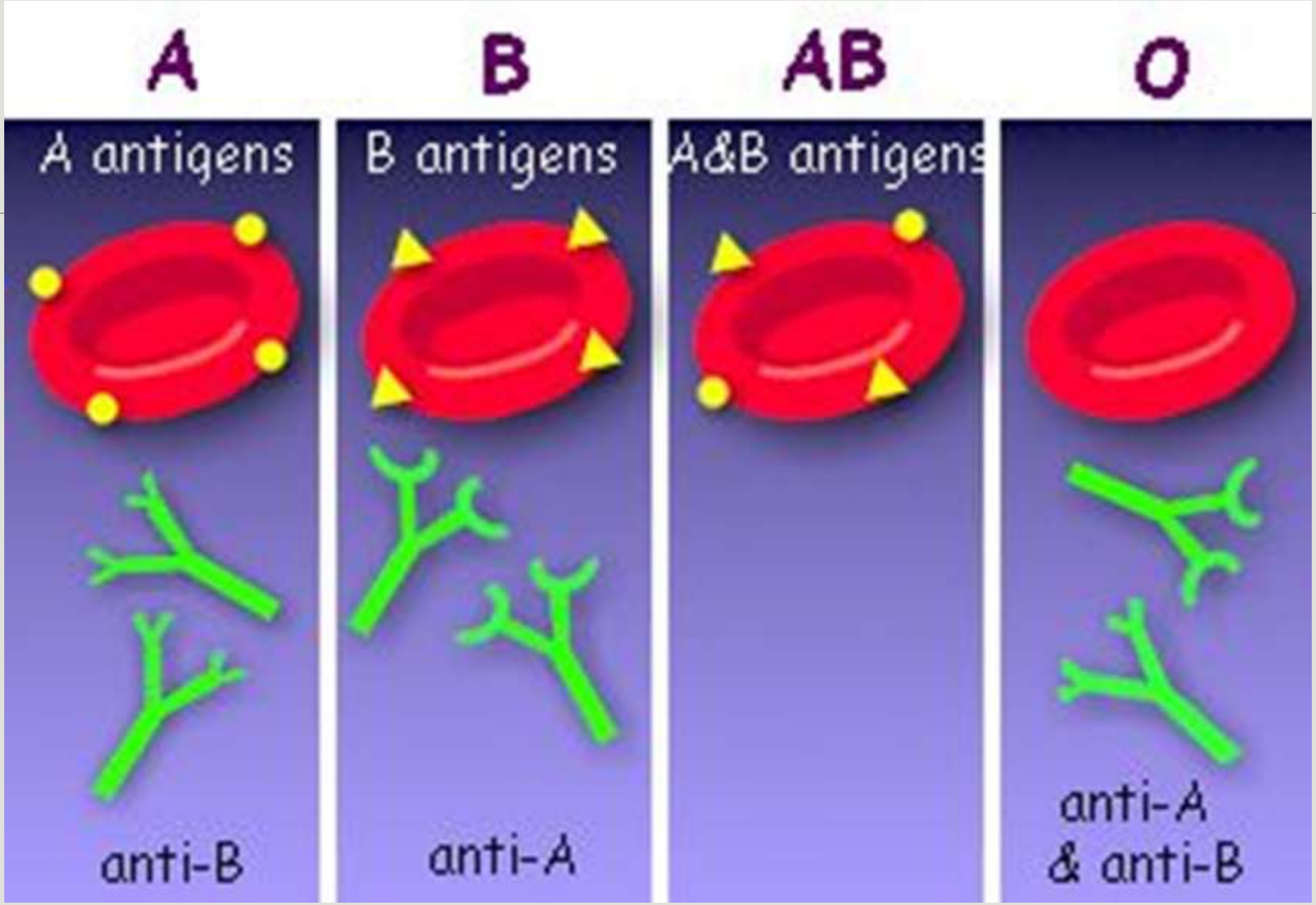
- Blood from the donor to the recipient must be compatible.
- The problem occurs when the protein (antigen) outer layer of the RBC of a donor becomes coagulated or agglutinated (clumped) with the plasma proteins (agglutinins or antibodies) of the recipient.

Type A would have B agglutinins (antibody) in the plasma.

Type B would have A agglutinins (antibody) in the plasma.

Type AB would not have agglutinins (antibody) in the plasma.

Type O would have A and B agglutinins (antibody) in the plasma.


A
B
AB
O
A antigens
B antigens
A&B antigens
anti-B
anti-A
anti-A
& anti-B

- Type O is the universal donor, since it does not have antigens on the surface of the RBC's.
- Type O positive is the most common blood type.
- Type AB is the universal recipient, since it does not have agglutinins (antibody) in its plasma.

Rh positive or negative

- Based on whether it has a antigen D or not.
- Rh+ has this protein, Rh- does not have this protein.

Pregnancy and blood type

Father--Rh+ blood, Mother is Rh– , Child could be Rh +.

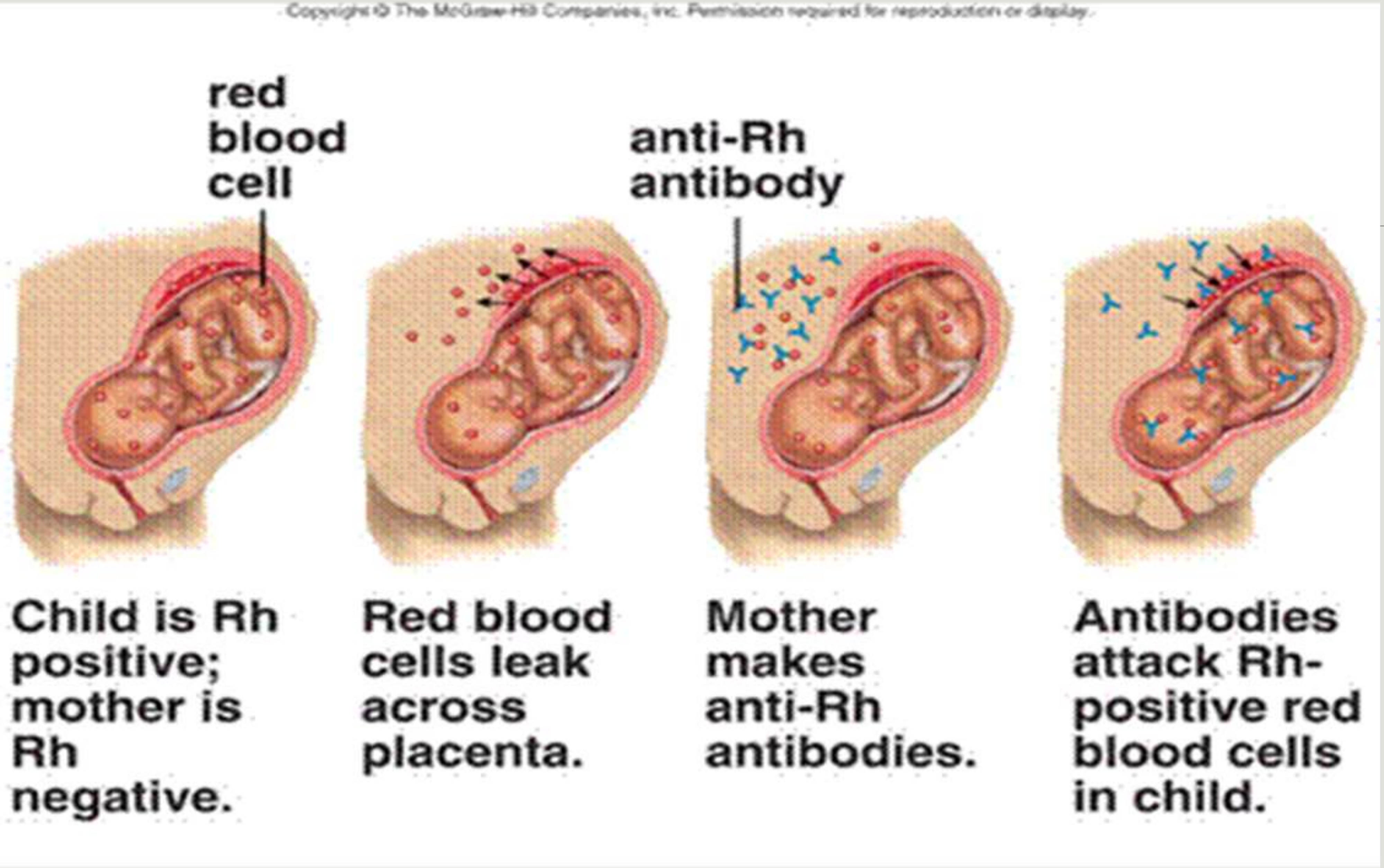
1st pregnancy--if the baby is Rh +, then there are no complications.

However, the mother will start to develop antibodies against the Rh factor

Second pregnancy, if the child is Rh +, the mother's antibodies can cross the placenta and start to attack the fetus' blood cells, causing hemolysis.

Hemolysis--breakdown of RBC and the release of hemoglobin into the plasma which can damage organs.

This is called erythroblastosis fetalis, can cause severe anemia, jaundice possibly death.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red blood cell
anti-Rh antibody
Child is Rh positive; mother is Rh negative.
Red blood cells leak across placenta.
Mother makes anti-Rh antibodies.
Antibodies attack Rh-positive red blood cells in child.

- To prevent this condition, the mother receives anti-Rhesus D immunoglobulin(Rh_0)around the 28 th week of pregnancy and right after delivery.
- The Ig attaches to Rh+ cells from the baby in the mother's blood stream and destroys them, preventing the triggers for the mother's immune system to produce its own anti D antibody.

Antigens and Antibodies

- Clumping of red blood cells following transfusion is called agglutination.
- Agglutination is due to the interaction of antigens on the surfaces of red blood cells with certain antibodies carried in the plasma.
- Only a few of the antigens on red blood cells produce transfusion reactions these include the ABO group and Rh group.

Testing for compatibility involves 2 steps

1- Detrmination of blood type.

2- cross - match test.

- **Cross Matching test:** is a test performed prior to a blood transfusion to determine whether donor blood is compatible (or incompatible) with recipient blood. became part of a series of pre-transfusion test known as compatibility testing.
- Compatibility is determined through matching of different blood group systems, the most important of which are the ABO and Rh system, and/or by directly testing for the presence of antibodies in plasma of recipient against a sample of donor tissues or blood.

Cross-match test

- Major and Minor cross-match tests
- The major crossmatch: involves testing or mixing the patient's serum with donor cells(RBCs) to determine whether the patient has an antibody directed against an antigen on donor′s cells, which may cause a hemolytic transfusion reaction or decreased cell survival of donor cells. This is the most important cross-match.
- The minor crossmatch: involves testing or mixing the patients cells with donor plasma to determine whether there is an antibody in the donor's plasma directed against an antigen on the patient's cells.

The minor cross-match test has been completely eliminated in most blood banks, because donor samples are screened beforehand for the more common Abs.

- The two main functions of the cross-match test can be cited as,

1- It is a final check of ABO compatibility between donor and patient.

2- It may detect the presence of an Ab in the patient's serum that will react with Ags on the donor RBCs.

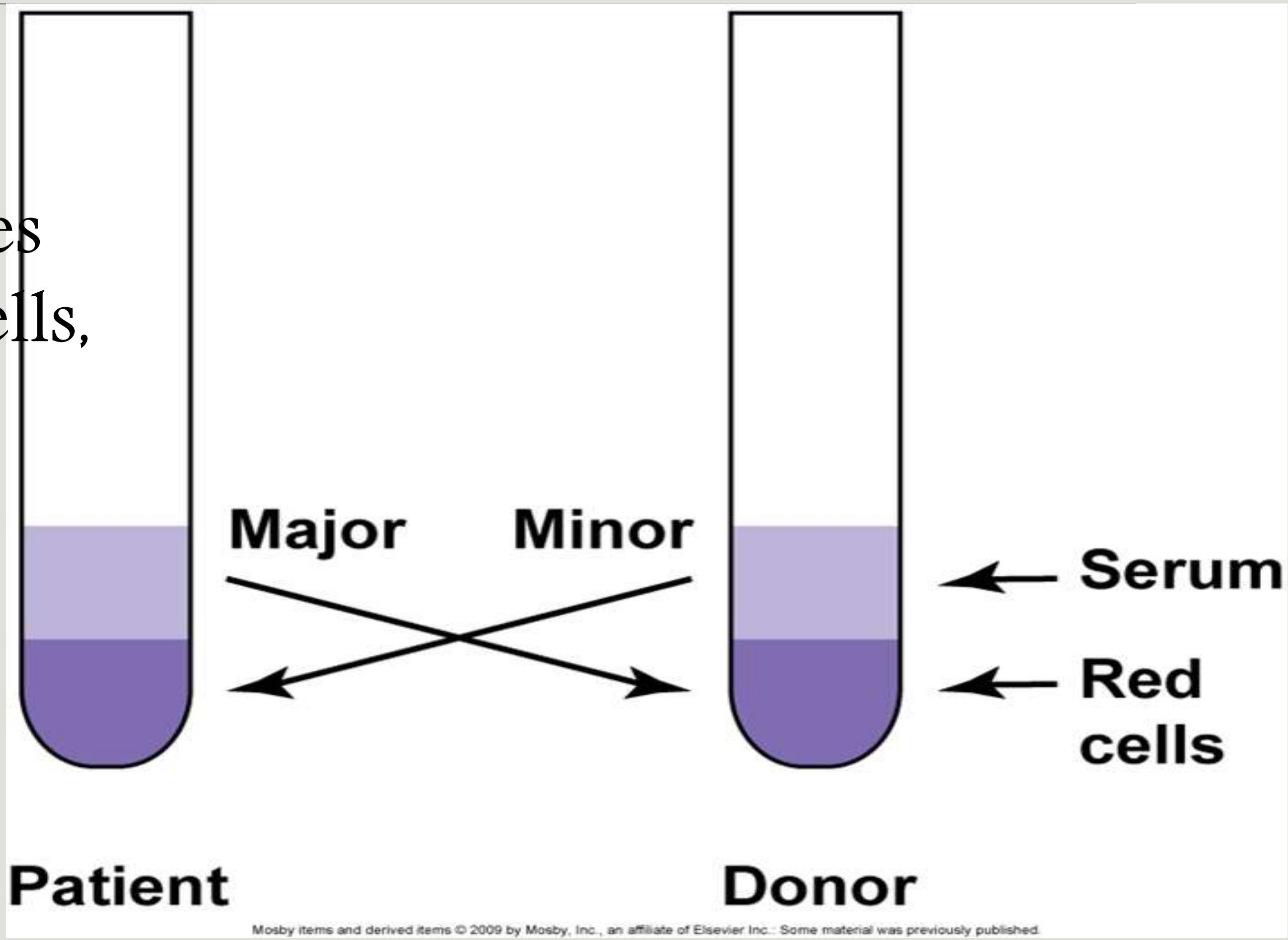
- **Purpose of cross matching:**

1. Prevent transfusion reactions
2. Increase in *vivo* survival of red cells
3. Double checks for ABO errors
4. Another method of detecting antibodies

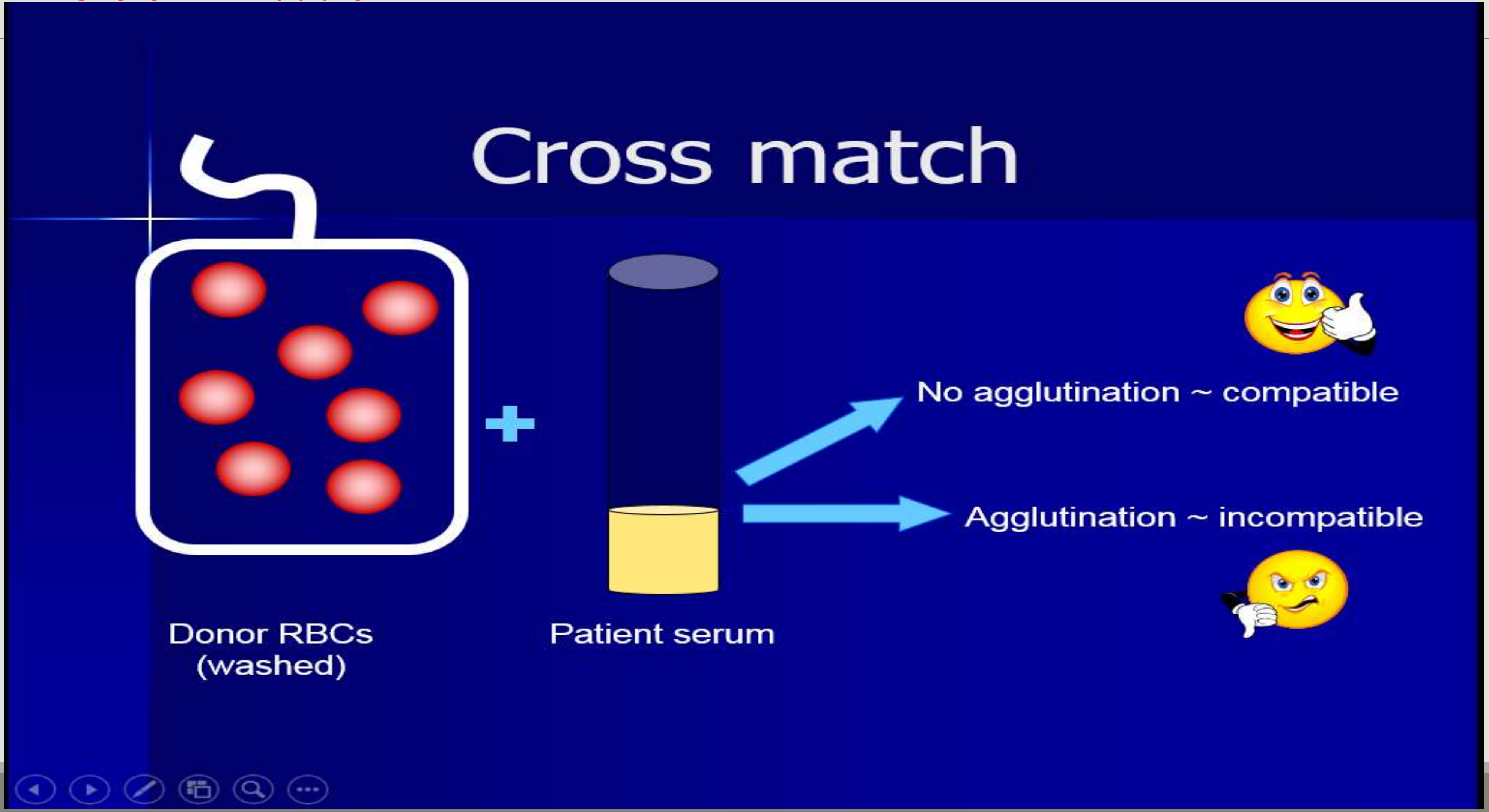
Major vs. Minor Cross match

- Why is the minor cross match unnecessary?

Donated units are tested for antibodies
Most blood is transfused as packed cells, having little antibodies



Cross match



Cross match
No agglutination ~ compatible
Agglutination ~ incompatible
Donor RBCs
(washed)
Patient serum

Cross matches…

Will

- Verify donor cell ABO compatibility
- Detect most antibodies against donor cells

Will Not

- Guarantee normal survival of RBCs
- Prevent patient from developing an antibody
- Detect all antibodies
- Prevent delayed transfusion reactions
- Detect ABO/Rh errors


thank
you

Body temperature

- **Body temperature :**

  is a measure of the body's ability to generate and get rid of heat.

  The body is very good at keeping its temperature within a narrow, safe range in spite of large variations in temperatures outside the body.

Body temperature is checked to:

- Detect fever.
- Detect abnormally low body temperature (hypothermia) in people who have been exposed to cold.
- Detect abnormally high body temperature (hyperthermia) in people who have been exposed to heat.
- Help monitor the effectiveness of a fever-reducing medicine.
- Help plan for pregnancy by determining if a woman is ovulating.

Route of body temperature measurment

- body temperature can be measured in many locations on your body.

1- oral route

2-Axillary route

3- Rectal route

4-tympanic membrane(ear)

- A rectal or ear (tympanic membrane) temperature reading is slightly higher than an oral temperature reading.
- A temperature taken in the armpit is slightly lower than an oral temperature reading.
- The most accurate way to measure body temperature is to take a rectal temperature.

- Most people think of a "normal" body temperature as an oral temperature of 98.6 °F (37 °C). This is an average of normal body temperatures. But "normal" varies from person to person. Your temperature may actually be 1°F (0.6°C) or more above or below 98.6 °F (37 °C). Also, your normal body temperature changes by as much as 1°F (0.6°C) throughout the day, depending on
- how active you are.
- and the time of day usually being lowest in the early morning and rising as much as 1°F (0.6°C) in the early evening.
- Body temperature is very sensitive to hormone levels and may be higher or lower when a woman is ovulating or having her menstrual period.
- Type of drinks, food…

- In most adults, an oral temperature above 100.4 °F (38 °C) or a rectal or ear temperature above 101 °F (38.3 °C) is considered a fever.
- A child has a fever when his or her rectal temperature is 100.4 °F (38 °C) or higher

Abnormal:

Oral, ear (tympanic), rectal, or temporal artery temperature

- Fever: 100.4 °F (38 °C) to 103.9 °F (39.9 °C)
- High fever: 104 °F (40 °C) and higher

Armpit (axillary) temperature

- Fever: 99.4 °F (37.4 °C) to 102.9 °F (39.4 °C)
- High fever: 103 °F (39.5 °C) and higher

A rectal or ear temperature of less than 97 °F (36.1 °C)means a low body temperature (hypothermia).

How To Prepare

- Take your temperature several times when you are feeling well to find out what is normal for you. Check your temperature in both the morning and evening, since body temperature can vary by as much as 1°F (0.6°C) throughout the day.
- Wait at least 20 to 30 minutes after smoking, eating, or drinking a hot or cold liquid before taking your temperature. Also, wait at least an hour after vigorous exercise or a hot bath.

- What Affects the Test
- Inaccurate temperature readings can be caused by:

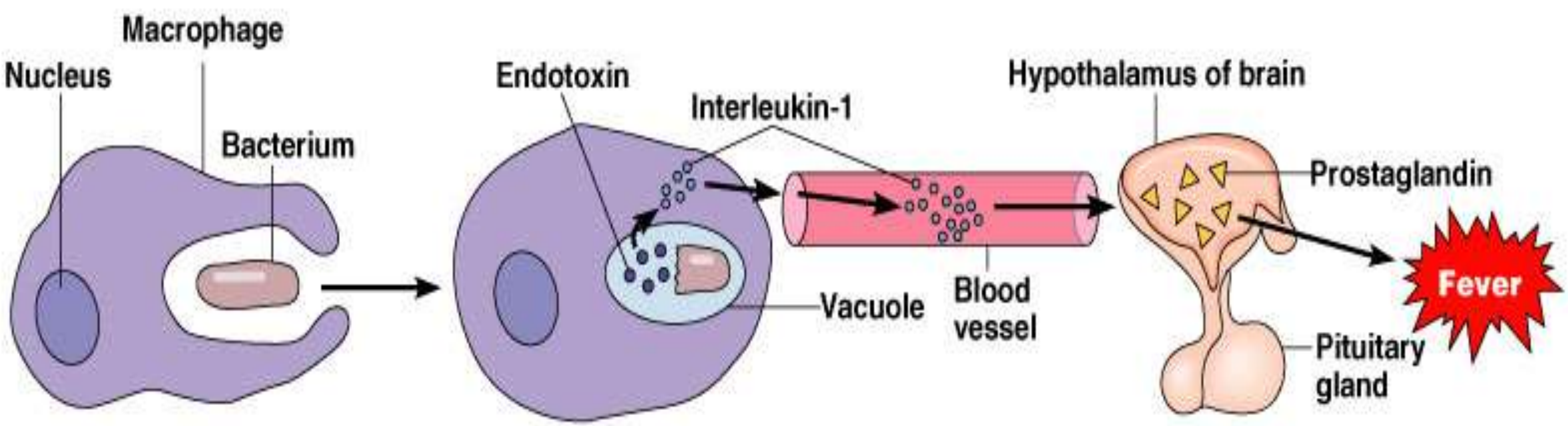
- 1. Not keeping your mouth closed around the thermometer when taking an oral temperature.
- 2. Not leaving a thermometer in place long enough before reading it.
- 3. Not putting the proper thermometer in the right place.
- 4. Not following the instructions for proper use that come with the thermometer.
- 5. A weak or dead thermometer battery.
- 6. Taking an oral temperature within 20 minutes after smoking or drinking a hot or cold liquid.
- 7. Taking a temperature by any method within an hour of exercising vigorously or taking a hot bath.

Fever

- A temperature setpoint (Thermoregulation):is the level at which the body attempts to maintain or keep its temperature within certain boundaries, even when the surrounding temperature is very different.
- When the setpoint is raised, the result is a fever.
- Most fevers are caused by infectious disease and can be lowered, if desired, with antipyretic medications.

A fever may occur as a reaction to:

- Infection.
- Medicines, such as antibiotics, narcotics, barbiturates,antihistamines, and many others. These are called drug fevers.
- Some medicines, such as antibiotics, raise the body temperature directly. Other medicines interfere with the body's ability to readjust its temperature when other factors cause the temperature to rise.
- Severe trauma or injury, such as a heart attack, stroke, heat exhaustion or heatstroke, or burns.
- Other medical conditions, such as arthritis, hyperthyroidism, and even some cancers, such as leukemia, Hodgkin's lymphoma and liver and lung cancer.



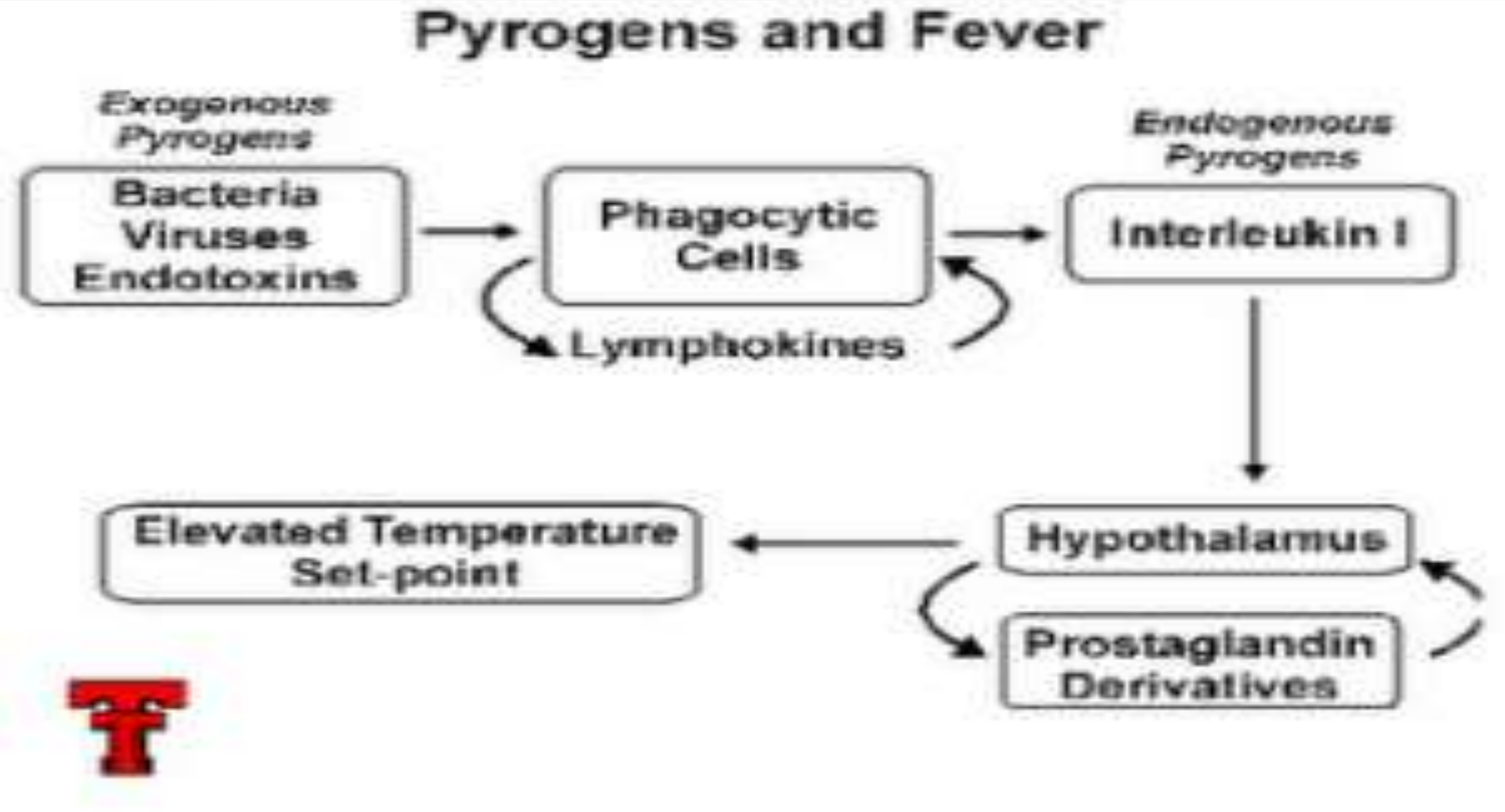
1 A macrophage ingests a gram-negative bacterium

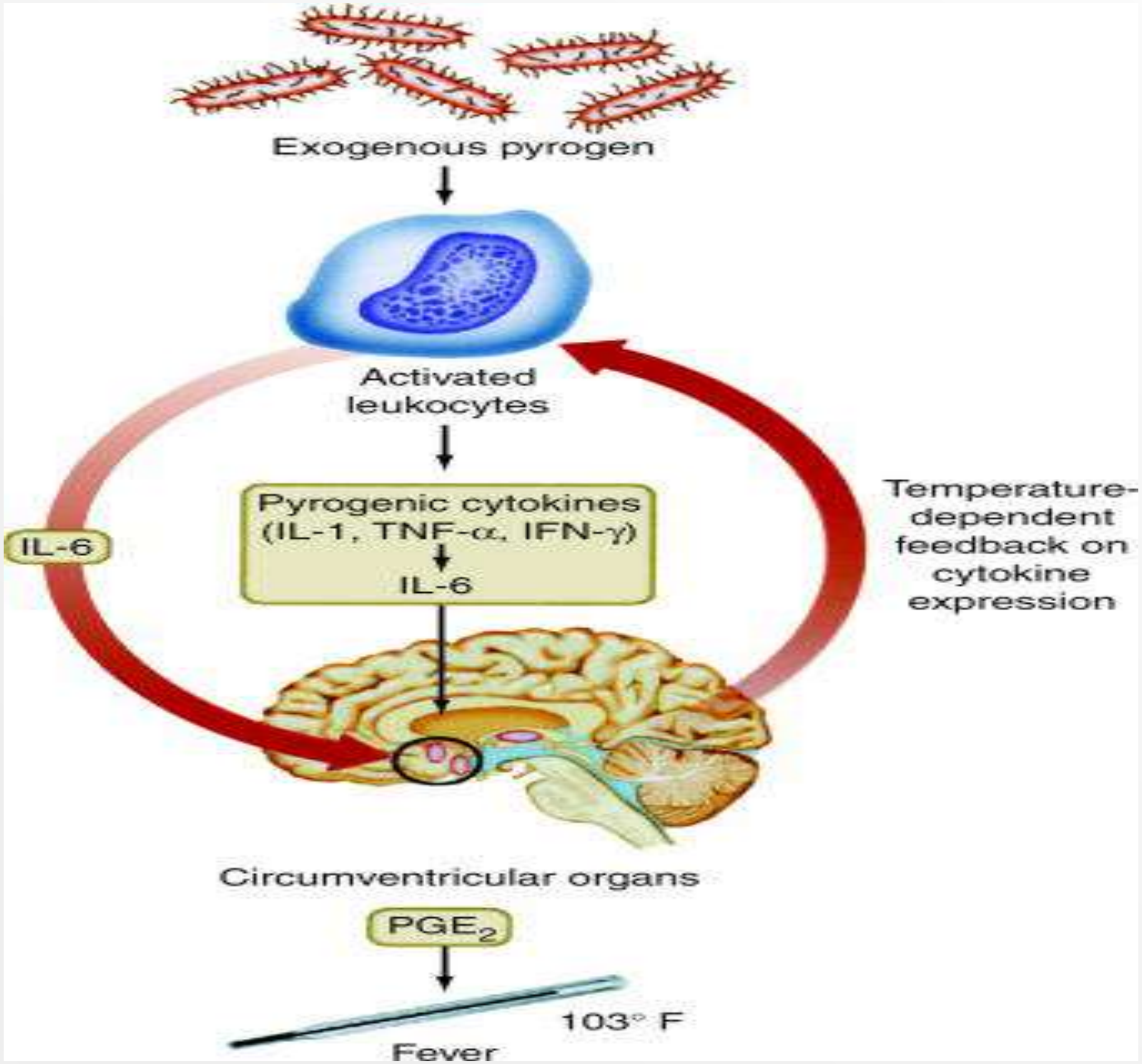
2 The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce interleukin-1(IL-1)

3 IL-1 is released by the macrophage into the bloodstream, through which it travels to the hypothalamus of the brain

4 IL-1 induces the hypothalamus to produce prostaglandins, which reset the body's "thermostat" to a higher temperature, producing fever

- Pyrogen: any substance that can cause fever ex:bacterial toxin




Exogenous pyrogen
Activated leukocytes
Pyrogenic cytokines (IL-1, TNF-α, IFN-γ)
IL-6
IL-6
Temperature-dependent feedback on cytokine expression
Circumventricular organs
PGE2
103° F
Fever

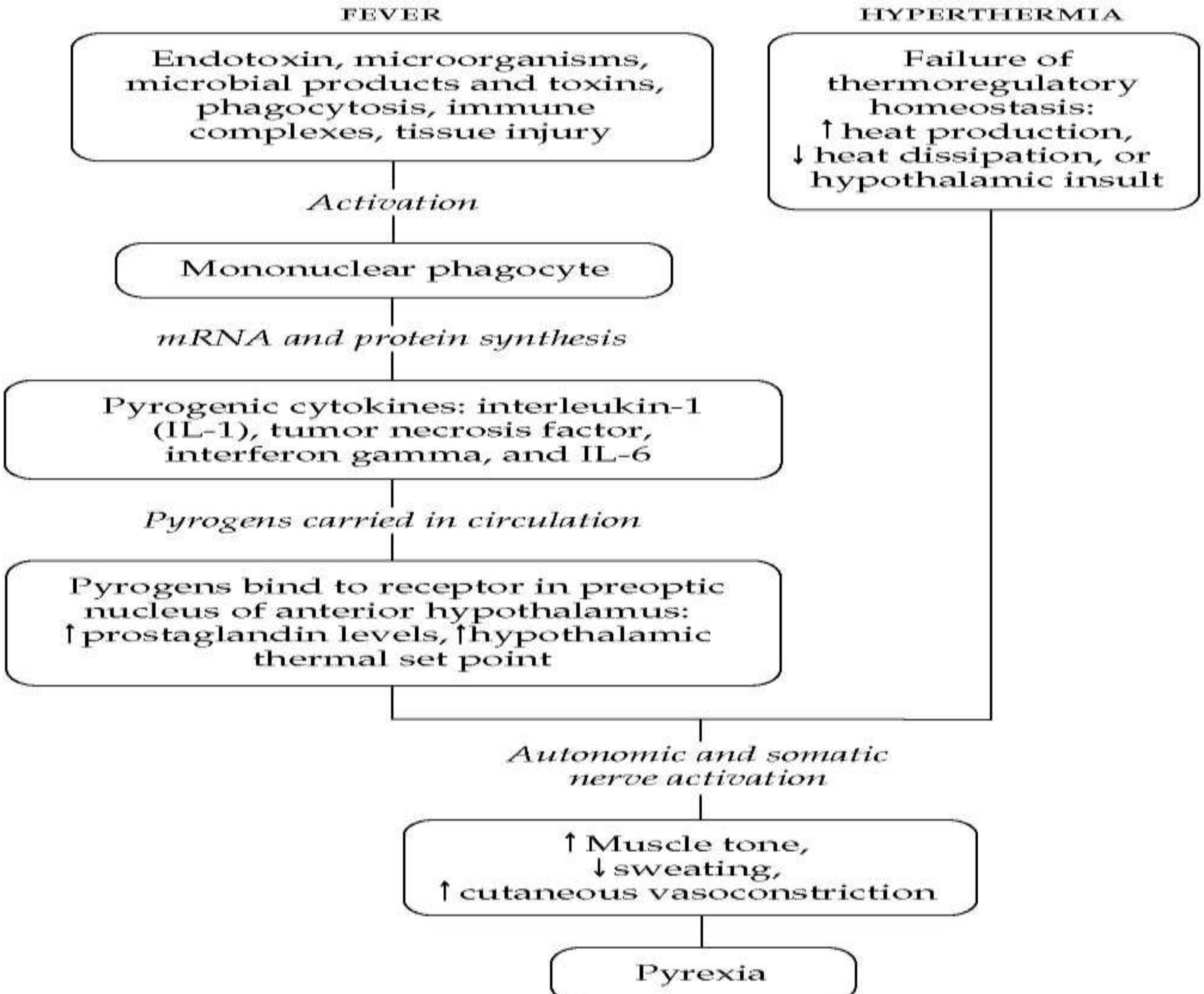
Hyperthermia

- Hyperthermia is elevated body temperature due to failed thermoregulation that occurs when a body produces or absorbs more heat than it dissipates.
- . This is due to an imbalance between heat production and heat loss
- Extreme temperature elevation then becomes a medical emergency requiring immediate treatment to prevent disability or death.

- The most common causes include heat stroke and adverse reactions to drugs.
- The former(heat strok) is an acute temperature elevation caused by exposure to excessive heat, or combination of heat and humidity, that overwhelms the heat-regulating mechanisms.

- The latter is a relatively rare side effect of many drugs, particularly those that affect the central nervous system.
- Malignant hyperthermia is a rare complication of some types of general anesthesia.

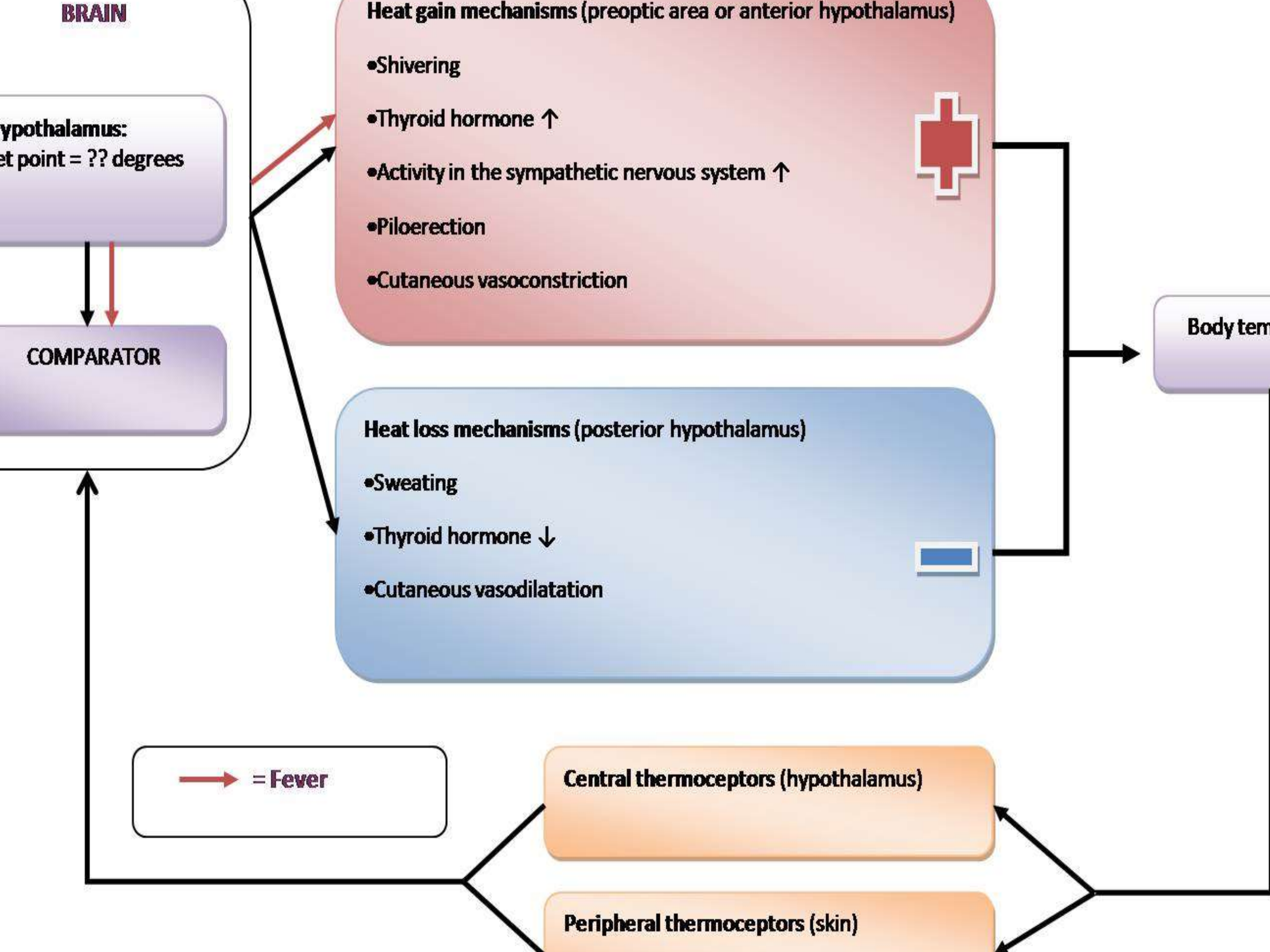
- Hyperthermia differs from fever in that the body's temperature setpoint (hypothalamus)remains unchanged.
- The opposite is hypothermia, which occurs when the temperature drops below that required to maintain normal metabolism.


FEVER
HYPERTHERMIA
Endotoxin, microorganisms, microbial products and toxins, phagocytosis, immune complexes, tissue injury
Failure of thermoregulatory homeostasis: ↑ heat production, ↓ heat dissipation, or hypothalamic insult
Activation
Mononuclear phagocyte
mRNA and protein synthesis
Pyrogenic cytokines: interleukin-1 (IL-1), tumor necrosis factor, interferon gamma, and IL-6
Pyrogens carried in circulation
Pyrogens bind to receptor in preoptic nucleus of anterior hypothalamus: ↑ prostaglandin levels, ↑ hypothalamic thermal set point
Autonomic and somatic nerve activation
↑ Muscle tone, ↓ sweating, ↑ cutaneous vasoconstriction
Pyrexia

Hypothermia

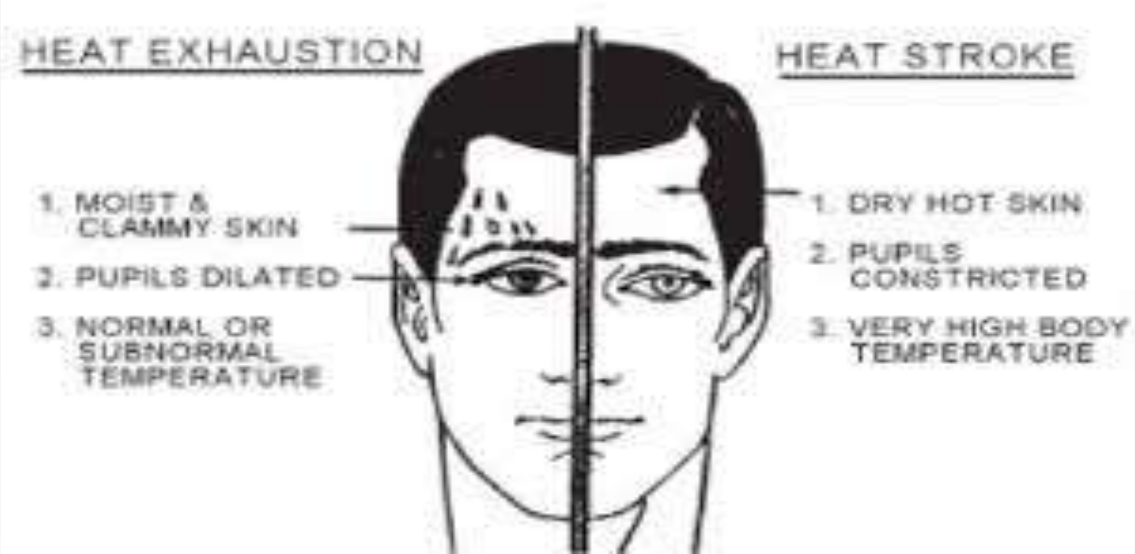
- In hypothermia, body temperature drops below that required for normal metabolism and bodily functions.
- In humans, this is usually due to excessive exposure to cold air or water, but it can be deliberately induced as a medical treatment. Symptoms usually appear when the body's core temperature drops by 1-2 °C (1.8-3.6 °F) below normal temperature

- An abnormally low body temperature (hypothermia) can be serious, even life-threatening.
- Low body temperature may occur from cold exposure, shock, alcohol or drug use, or certain metabolic disorders, such as diabetes or hypothyroidism.


BRAIN
ypothalamus:
et point = ?? degrees
COMPARATOR
Heat gain mechanisms (preoptic area or anterior hypothalamus)
•Shivering
•Thyroid hormone ↑
•Activity in the sympathetic nervous system ↑
•Piloerection
•Cutaneous vasoconstriction
Heat loss mechanisms (posterior hypothalamus)
•Sweating
•Thyroid hormone ↓
•Cutaneous vasodilatation
Body tem
= Fever
Central thermoceptors (hypothalamus)
Peripheral thermoceptors (skin)

Heatstroke

- **is a life-threatening** emergency characterized by a body temperature of 40° C (104° F) or higher and neurologic dysfunction that may include delirium, seizures, or coma.



heatstroke

- occurs when the body fails to regulate its own temperature and body temperature continues to rise.
- Symptoms of include mental changes (such as confusion, delirium, or unconsciousness) and skin that is red, hot, and dry, even under the armpits.

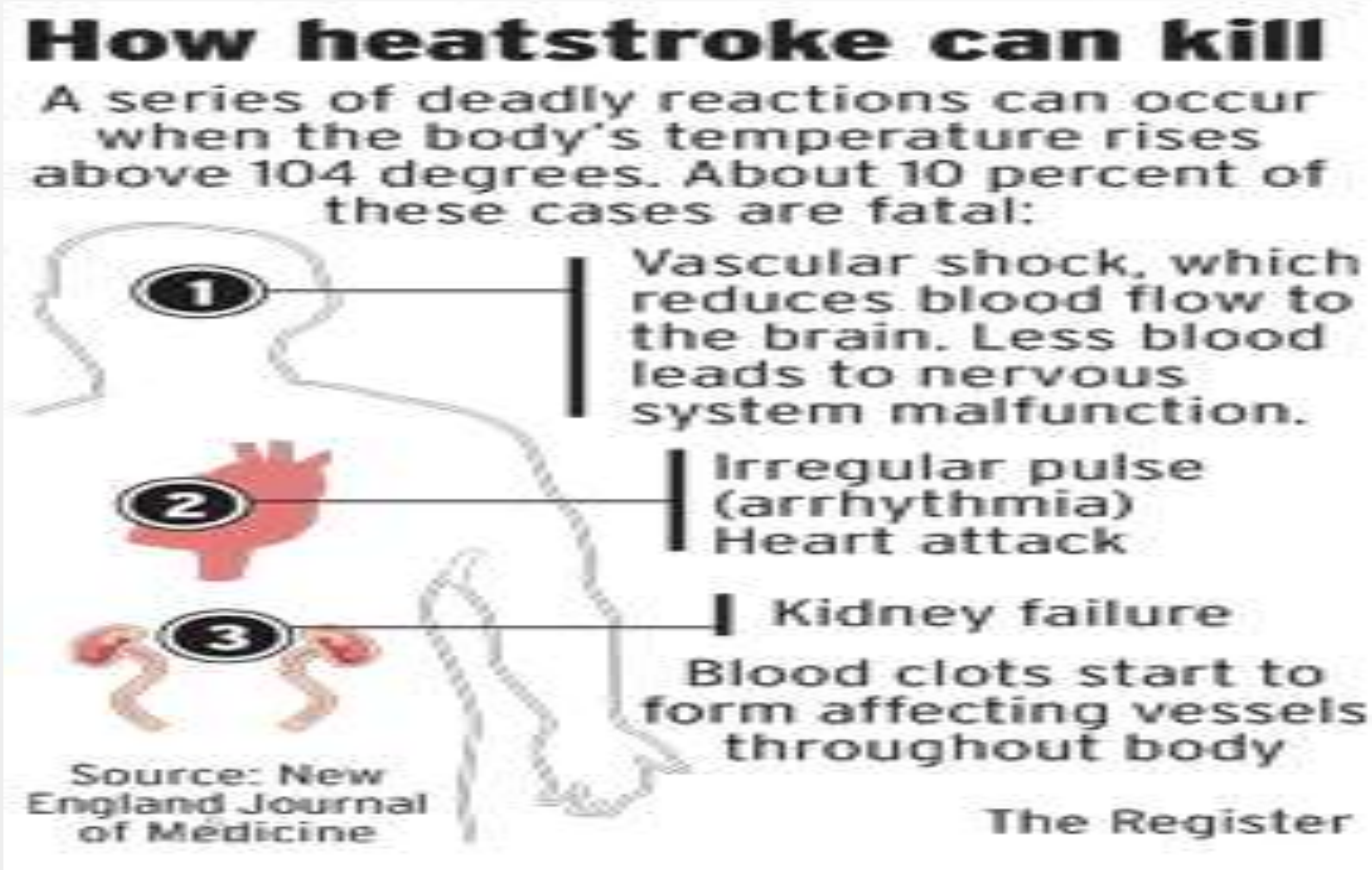
- Classic heatstroke can develop without exertion when a person is exposed to a hot environment and the body is unable to cool itself effectively. In this type of heatstroke, the body's ability to sweat and transfer the heat to the environment is reduced.
- A person with heatstroke may stop sweating. Classic heatstroke may develop over several days.
- Babies, older adults, and people who have chronic health problems have the greatest risk of this type of heatstroke.

- Exertional heatstroke may develop when a person is working or exercising in a hot environment. A person with heatstroke from exertion may sweat profusely, but the body still produces more heat than it can lose. This causes the body's temperature to rise to high levels.
- Both types of heatstroke cause severe dehydration and can cause body organs to stop functioning. **Heatstroke is a life-threatening medical emergency** requiring emergency medical treatment.

Sunstroke - First Aid

* Move the person to shade
* Give cold water to drink
* Pour water over the skin
  (mainly head & neck)
* Place ice packs on : Neck
  Armpit & Groin

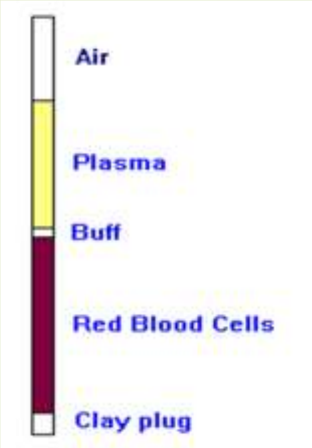
Heat Exhaustion:	Heat Stroke
• Heavy sweating • Heavy thirst • Panting/rapid breathing • Rapid pulse • Headache • Blurred vision • Exhaustion, weakness • Clumsiness • Confusion • Dizziness or fainting • Cramps	• No sweating • Red or flushed, hot dry skin • Any symptom of heat exhaustion but more severe • Difficult breathing • Pinpoint pupils • Bizarre behavior • Convulsions • Confusion • Collapse


How heatstroke can kill
A series of deadly reactions can occur when the body's temperature rises above 104 degrees. About 10 percent of these cases are fatal:
1
Vascular shock, which reduces blood flow to the brain. Less blood leads to nervous system malfunction.
2
Irregular pulse (arrhythmia)
Heart attack
3
Kidney failure
Blood clots start to form affecting vessels throughout body
Source: New England Journal of Medicine
The Register

Warning Signs:
Heat Exhaustion
vs.
HEAT STROKE
Heat- related illness is
Preventable!
Stay somewhere cool
Drink plenty of water
Avoid sugar, alcohol & caffeine
Wear light clothing
Paleness
Tiredness
Weakness
Dizziness
Headache
Fainting
Muscle cramps
Heavy sweating
Nausea or vomiting
Extremely high body temperature (103°F+)
Red, hot, dry skin (with no sweating)
Rapid, strong pulse
Throbbing headache
Dizziness
Nausea
Confusion
Unconsciousness
Watch out!
If left untreated, heat exhaustion can progress to
STOP
If you recognize symptoms of heat stroke, it is LIFE THREATENING.
Get the person somewhere cool and seek medical attention IMMEDIATELY.
Michelle L. Holshue, 2012
Data on heat-related illness via CDC:
http://emergency.cdc.gov/disasters/extremeheat/

Hematocrit Determination

Hematocrit (Ht or HCT) or Packed cell volume (PCV) or Erythrocyte volume fraction(EVF)



- Hematocrit or PCV is measurement of the ratio of the volume occupied by the RBCs to the volume of whole blood in a sample of capillary or venous blood.
- It is the volume percentage (vol%) of red blood cells in blood. It is normally 45% for men and 40% for women.
- determines the percentage of red blood cells (RBCs) in whole blood.
- This ratio is usually measured and expressed either as a percentage or as a decimal fraction (e.g. 41% or 0.41).

Clinically , the hematocrit is used to:

- 1- PCV is used to detect for anemia, polycythemia, hemodilution or hemoconcentration and other red cell volume alterations.
- 2- To calculate mean corpuscular volume **or** mean cell volume (MCV) in conjugation with an RBCs count. [Is a measure of the size of the average of RBCs volume]
- To calculate MCV, the hematocrit (Hct) is divided by the concentration of RBCs (RBC)
- MCV=Hct / RBCs count.

- 3- To calculate mean corpuscular hemoglobin concentration (MCHC) (a measure of the concentration of haemoglobin in a given volume of packed red blood cells) in conjugation with an hemoglobin concentrations.
- It is calculated by dividing the haemoglobin by the hematocrit.
- MCHC=Hb /Hct

Reagents, supplies, and equipment:

- Capillary tubes (75 mm long), blue banded tubes (blue tip) contain no anticoagulant and are used with EDTA-anticoagulated blood. Red banded tubes (red tip) are heparinized for use capillary blood or finger sticks.
- Clay-type tube sealant
- Micro hematocrit centrifuge
- Micro hematocrit reader
- Cotton
- lancet

Capillary Haematocrit Tube

Principle:

- The hematocrit is usually determined by spinning a blood-filled capillary tube in a centrifuge.
- **Specimen:**
- Venous blood anticoagulated with EDTA or capillary blood collected directly into heparinized capillary tubes can be used.
- Specimens should be centrifuged within 6 hours of collection.
- Hemolyzed samples cannot be used for testing.

Methods for measurement

- 1-Spun microhematocrit method.
- 2- Automated hematocrit.

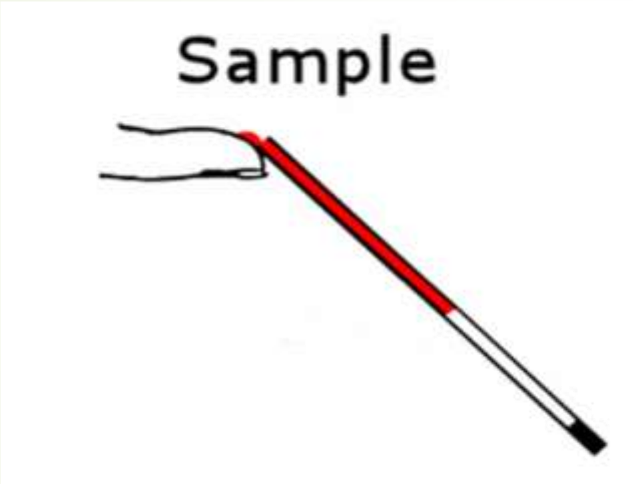
- Can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes ,this separates the blood into layers.
- The volume of packed red blood cells, divided by the total volume of the blood sample gives the PCV
- Because a tube is used this can be calculated by measuring the lengths of the layers.

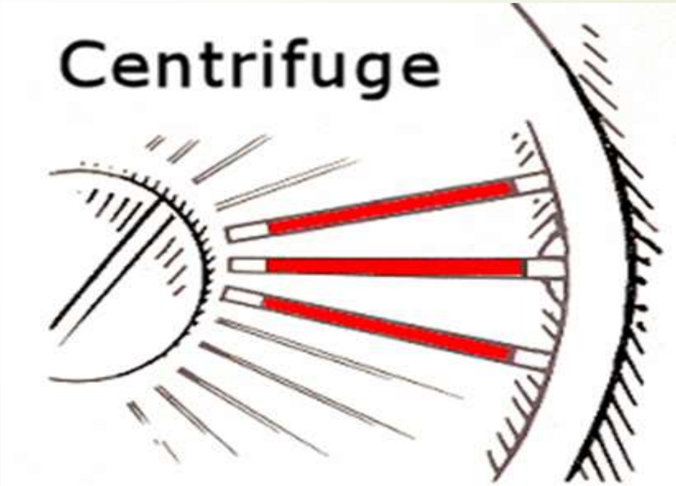
- With modern lab equipment, the hematocrit is not directly measured by an automated analyzer and It is determined by multiplying the red cell count by the mean cell volume(PCV= RBC counts χMCV)
- The results arrived by this way, rather than by direct measurement of PCV, is slightly more accurate because the direct method includes small amounts of blood plasma trapped between the red cells.

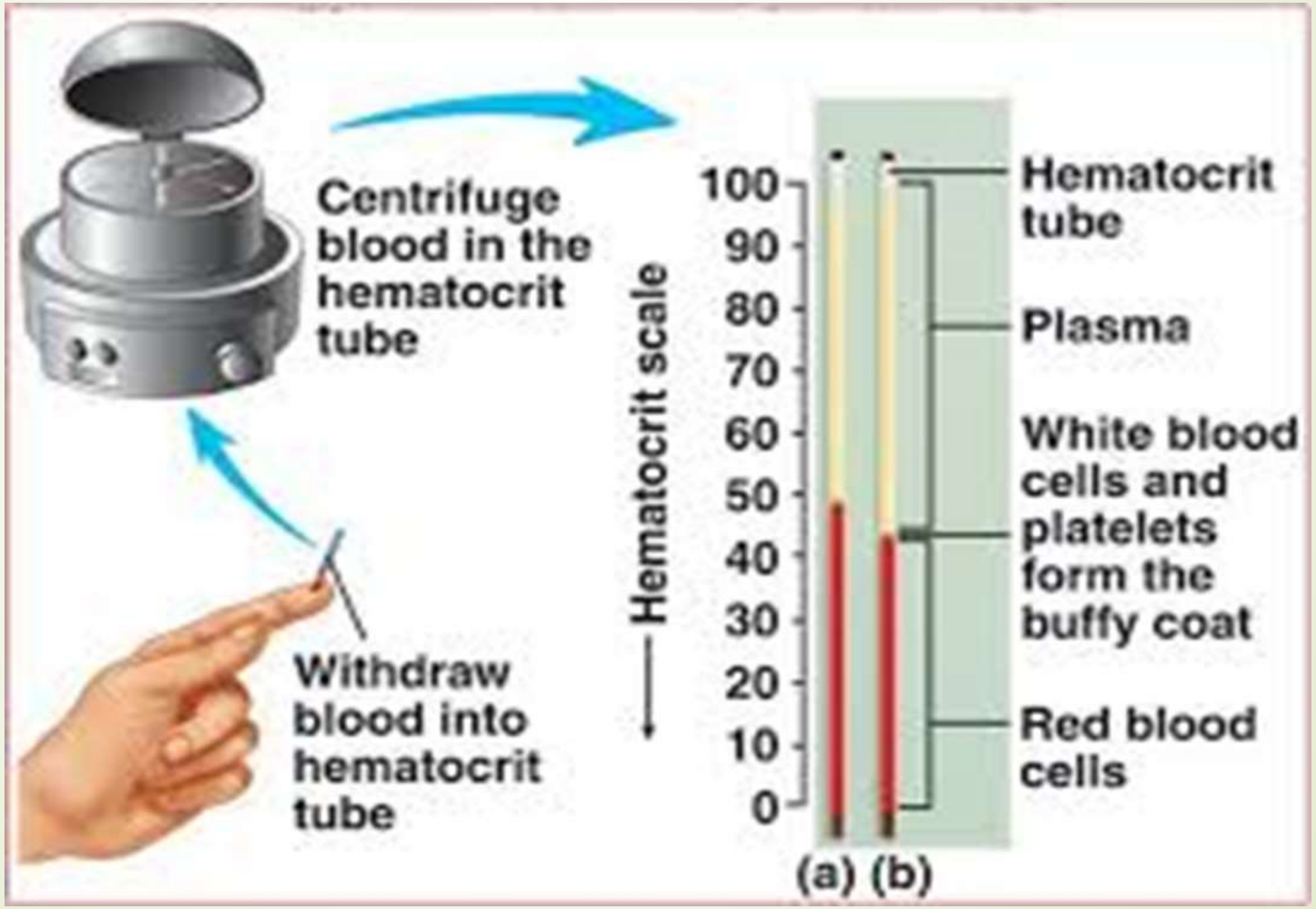
Procedure:

- 1. Puncture the skin of the finger and collect blood from the capillary directly into heparinized microhaematocrit tube; fill three forths of their length.
- 2. Seal one end of the tube with clay or a sealant. Avoid trapping air between the blood and plug.
- 3. Place the tube into a calibrated microhaematocrit centrifuge, sealed ends out against a rubber ring. Place firmly the lid over the centrifuge head. Close the cover. Set the timer (most instruments require 5 minutes centrifugation time). Centrifuge the tube (usually at 10,000 to 15,000 RPM).

- 4. The tube should be removed and read within a minute or two after the centrifuge has stopped to avoid re-dispersion of cells. Hemolysis should be noted, since this may lower the hematocrit results in relation to the hemoglobin (the hematocrit is 3 times the value of the hemoglobin, if the cells are normocytic).
- 5. Use a lined card to determine the hematocrit value., by measuring the height of the total blood column and the height of the red cell layer.

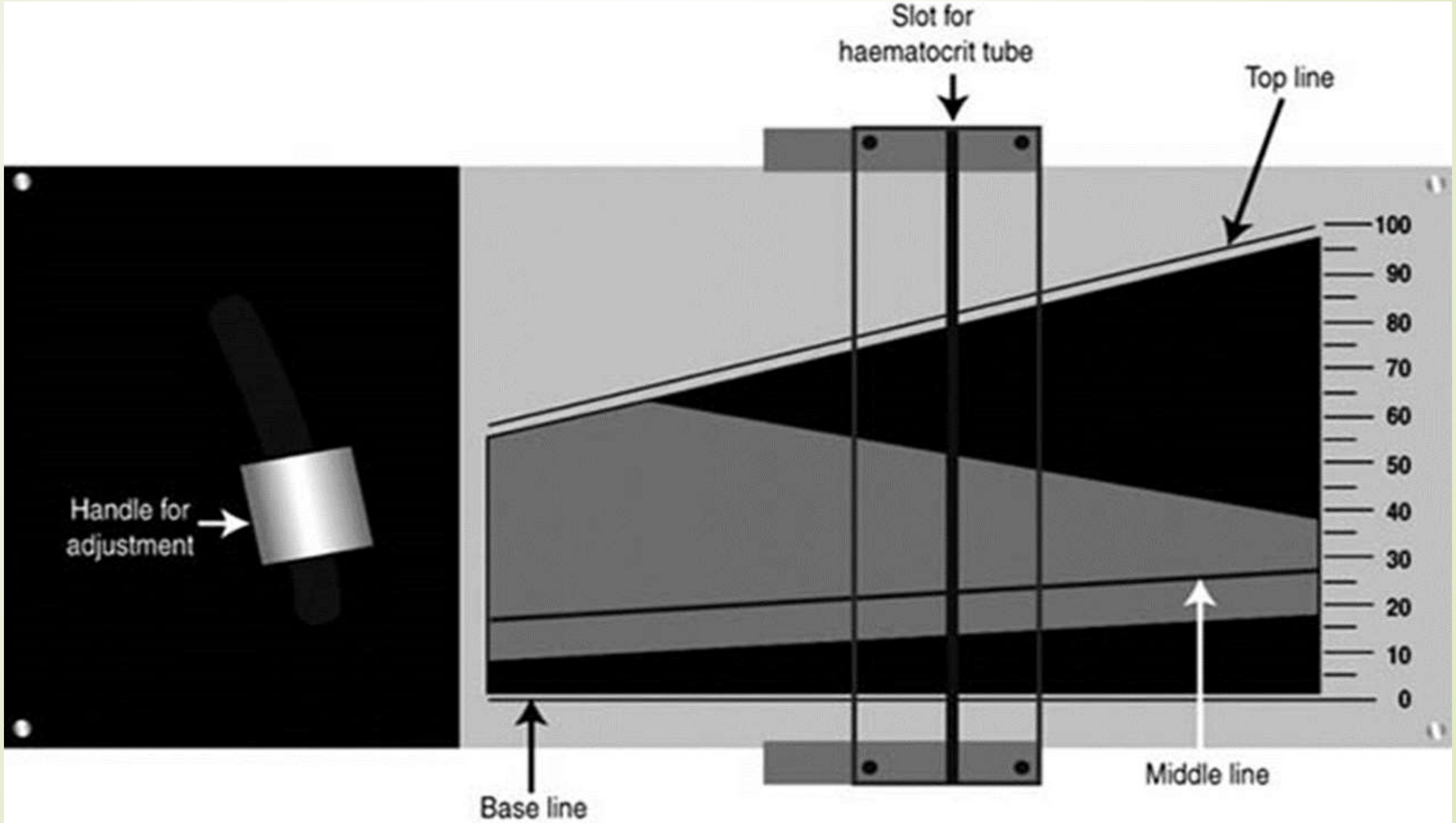

Sample


Centrifuge



Centrifuge blood in the hematocrit tube
Withdraw blood into hematocrit tube
Hematocrit scale
100
90
80
70
60
50
40
30
20
10
0
(a) (b)
Hematocrit tube
Plasma
White blood cells and platelets form the buffy coat
Red blood cells

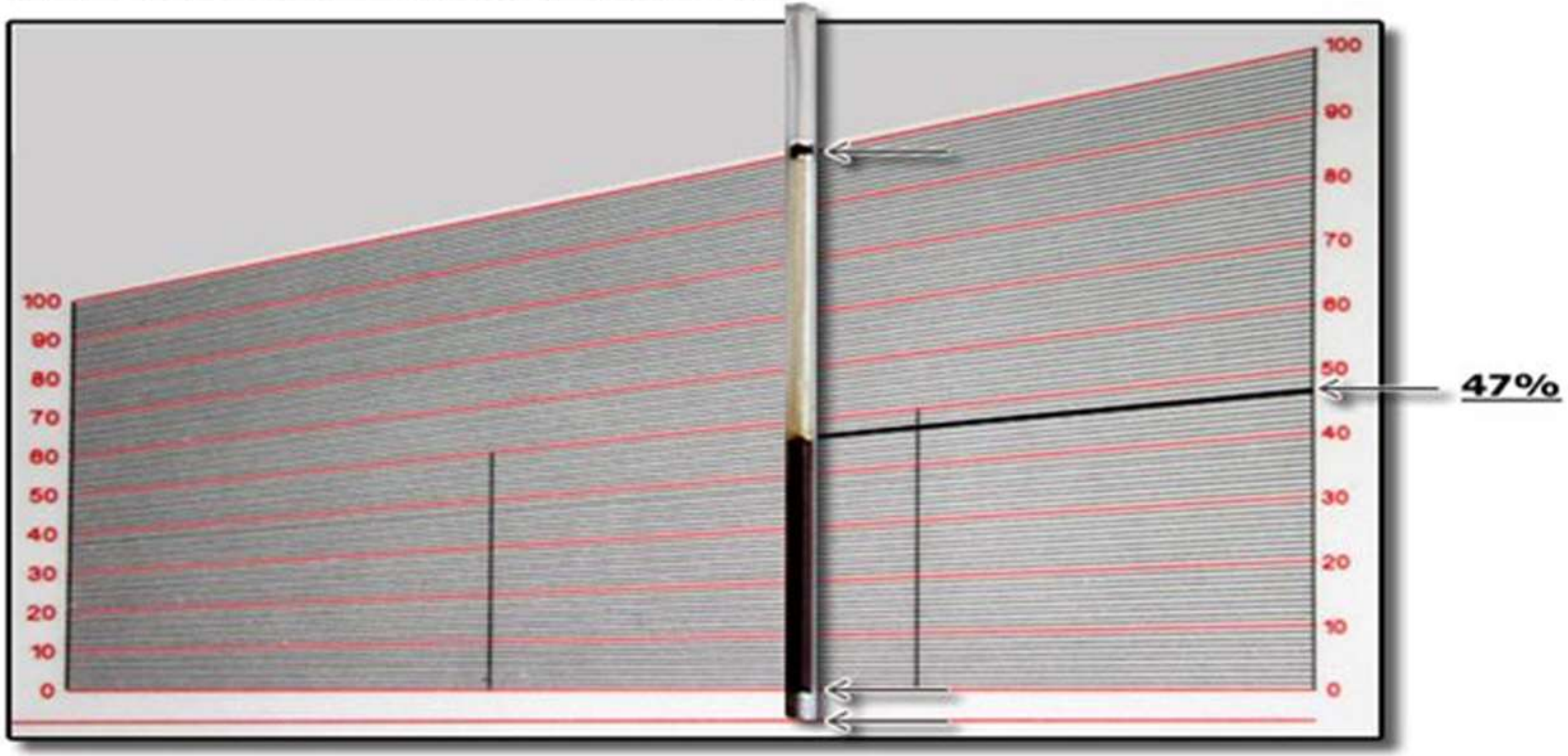
Microhematocrit reader card



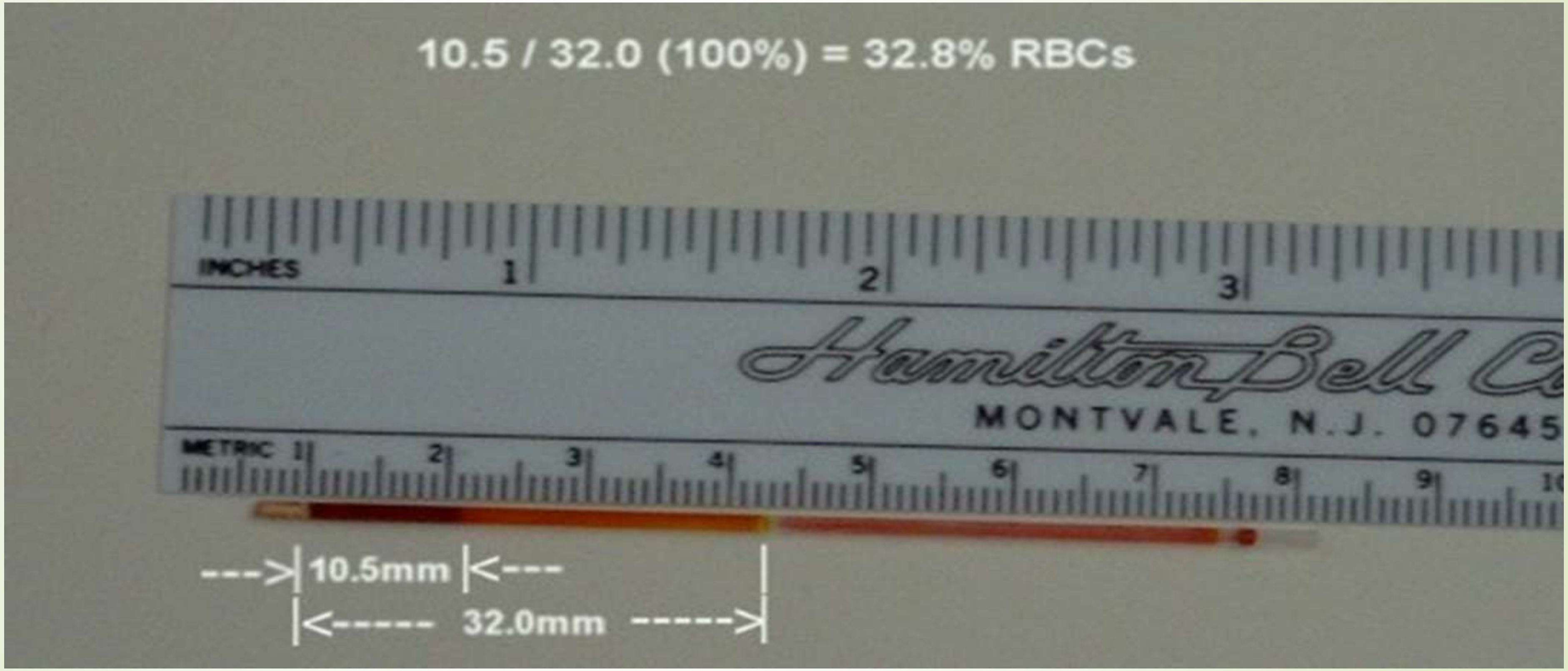
Blood

Hematocrit - Normal

Micro-hematocrit Capillary Tube Reader



Place the hematocrit tube on the chart. The end of the tube with white clay should touch the bottom red line. The bottom of the blood column should touch the 0% line. Slide the tube along the chart till the top of the blood column reaches the 100% line. Now read the top of the red column on the scale on the right.


10.5 / 32.0 (100%) = 32.8% RBCs
INCHES
1
2
3
Hamilton Bell Co
MONTVALE, N.J. 07645
METRIC
---> 10.5mm <---
<----- 32.0mm ----->

Hematocrit reader

- **Reference ranges:**
- Newborn (53-65%).
- Infant/child 30—43%
- men 42-52%
- women 37-47%

- When hematocrit determinations are below normal, medical conditions such as anemia and leukemia may be present.
- Above-normal hematocrit determinations indicate medical conditions like dehydration, such as occur in severe burn cases.

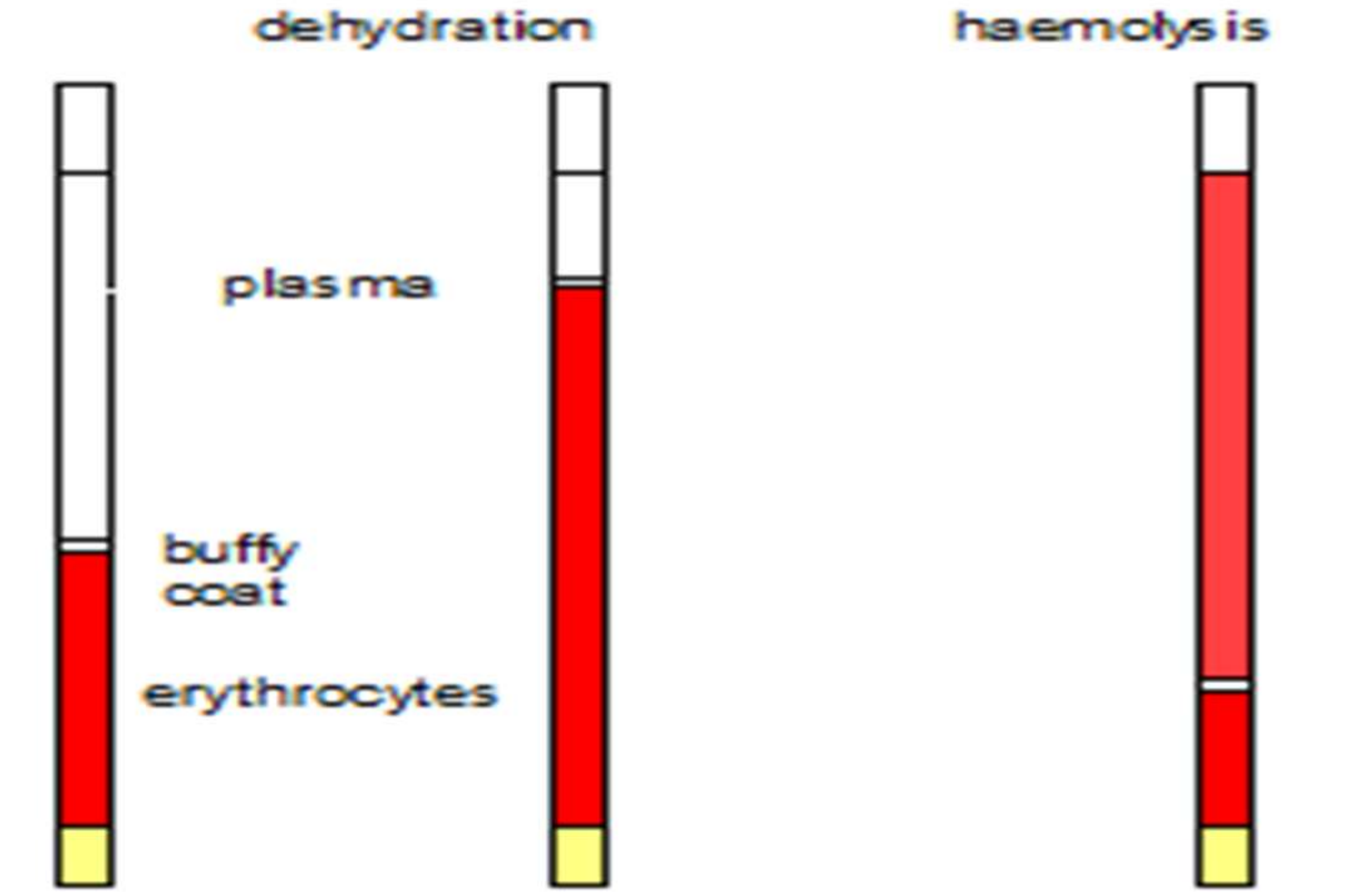
Some causes of a low hematocrit include:

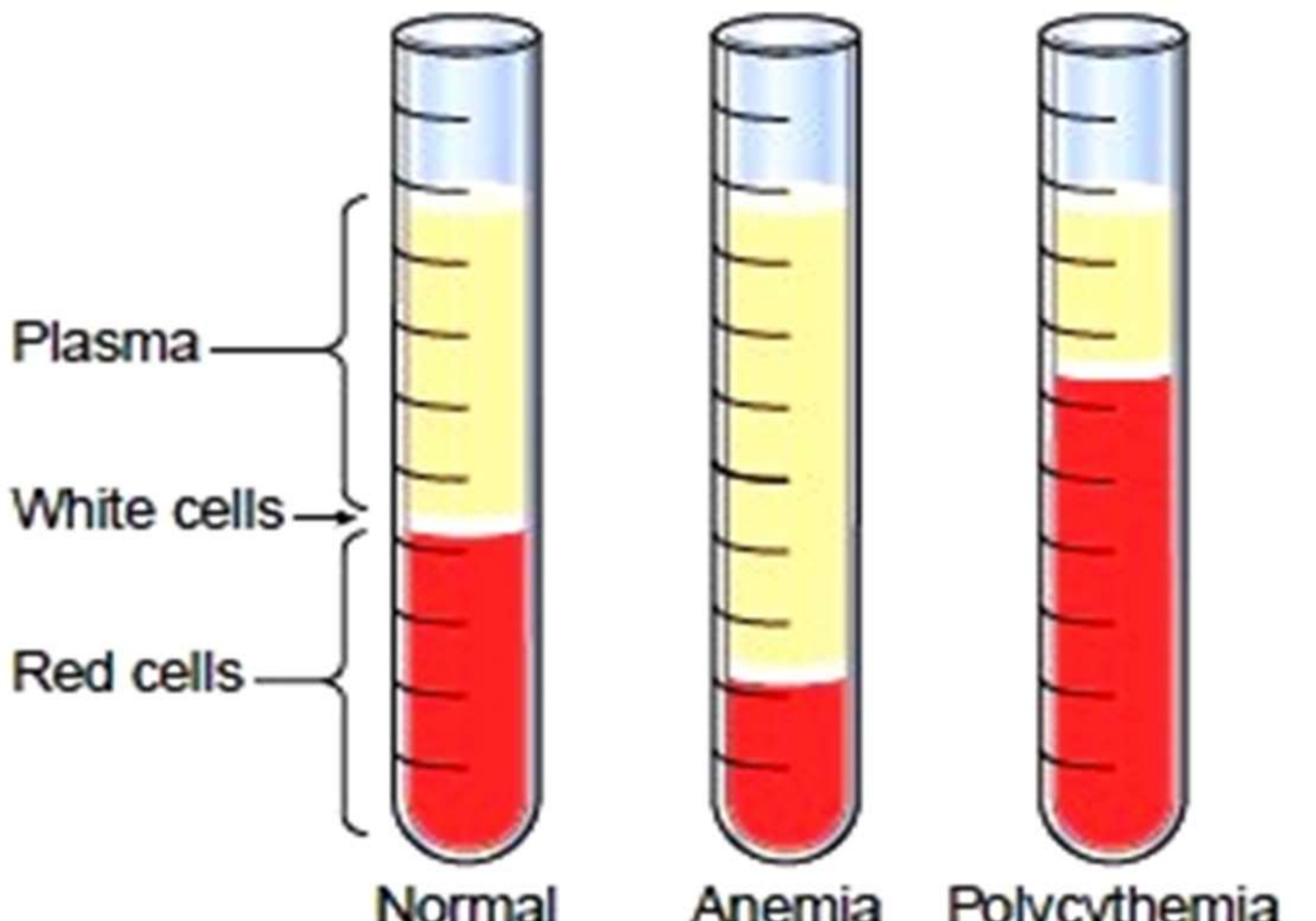
- Excessive destruction of red blood cells, for example, hemolytic anemia
- Decreased production of hemoglobin (e.g., thalassemia)
- Acute or chronic bleeding from the digestive tract (e.g., ulcers)
- Nutritional deficiencies such as iron, folate or B12 deficiency.
- Damage to the bone marrow from, for example, a toxin, radiation or chemotherapy, infection or drugs.
- Kidney failure.

Some causes of a high hematocrit include:

- Dehydration—this is the most common cause of a high hematocrit. As the volume of fluid in the blood drops, the RBCs per volume of fluid artificially rises; with adequate fluid intake, the hematocrit returns to normal.
- Polycythemia
- Kidney tumor that produces excess erythropoietin hormone.
- Smoking
- Living at high altitudes

CAPILLARY TUBE SECTIONS




Plasma
White cells
Red cells
Normal
Anemia
Polycythemia

- The hematocrit is a ratio of the packed cells to total volume.
- Example: If the column of packed red cells measures 20 mm and the whole blood column measures 50 mm, the hematocrit is 20/50 = 0.4 or (0.4 × 100%) = 40%.

Sources of error and comments

- Improper sealing of the capillary tube causes a decreased Hct reading as a result of loss of blood during centrifugation. a higher number of erythrocytes are lost in relation to the plasma.
- An increased amount of anti-coagulant decreases the Hct reading as a result of erythrocyte shrinking.
- A decreased or increased result may occur if the specimen was not properly mixed.

Sources of error and comments

- The buffy coat of the specimen should not be included in the Hct reading, because its inclusion falsely elevates the result.
- A decrease or increase in the readings may be seen if the microhematocrit reader is not used properly.
- If the centrifugation too short or the speed too low an increase in trapped plasma (1%–3%) will occur in normal blood.

- Increased amounts of trapped plasma in red cell column can produce errors in patients with an erythrocyte abnormality such as sickle cell anemia.
- Do allow the tubes to remain in the centrifuge for more than 10 min after the end of centrifugation???
- No, the red cells can begin to settle out and cause a false reading of the hematocrit.

- The trapping of the plasma causes the microhematocrit to be 1–3% (0.01–0.03) higher than that obtained on automated instruments, which calculate the Hct and are unaffected the trapped plasma.
- A temporarily low Hct reading may result immediately after a blood loss, because plasma is replaced faster than erythrocytes.
- Proper specimen collection is an important consideration. The introduction of interstitial fluid from a skin puncture causes decreased Hct readings.

- A number of disorders such as
- Sickle cell anemia, Macrocytic anemia and thalassemia---may cause plasma to be trapped in the erythrocytes even if the procedure was performed properly.
- The mirohematocrit centrifuge should never be forced to stop by applying pressure to the metal cover plate.This will cause the RBCs layer to sling forward and results in a falsely elevated value.

The rule of three

- The hematocrit is approximately 3X the hemoglobin value when RBCs are normal size and contain normal amounts of hemoglobin.
- (by comparing the hemoglobin with the hematocrit results (in % units) using the following formula:

  * Hct ± 3 units = Hgb x 3

- Example:

*The following results are obtained from a patient:

Hb = 12.0g/dL Hb (12)x3=36; Hct=0.36

Hct = 0.36

Acceptable range for the Hct would be 0.33-0.39.

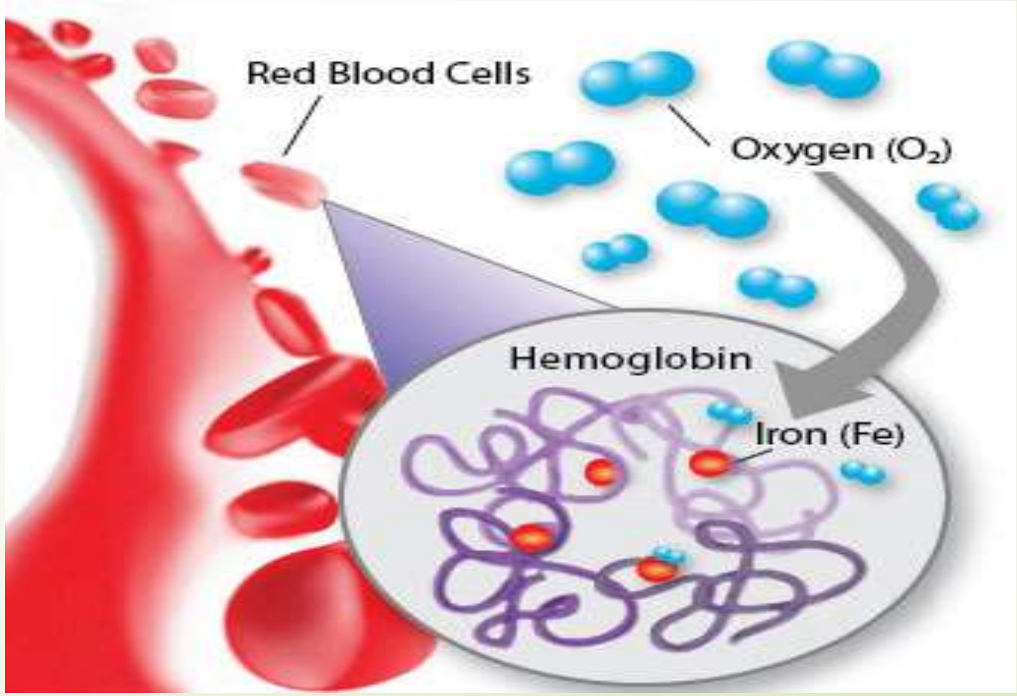

thank you

Hemoglobin

(Hgb or Hb)

What is hemoglobin?

- **Hemoglobin:** is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues back to the lungs.



Structure of Hb

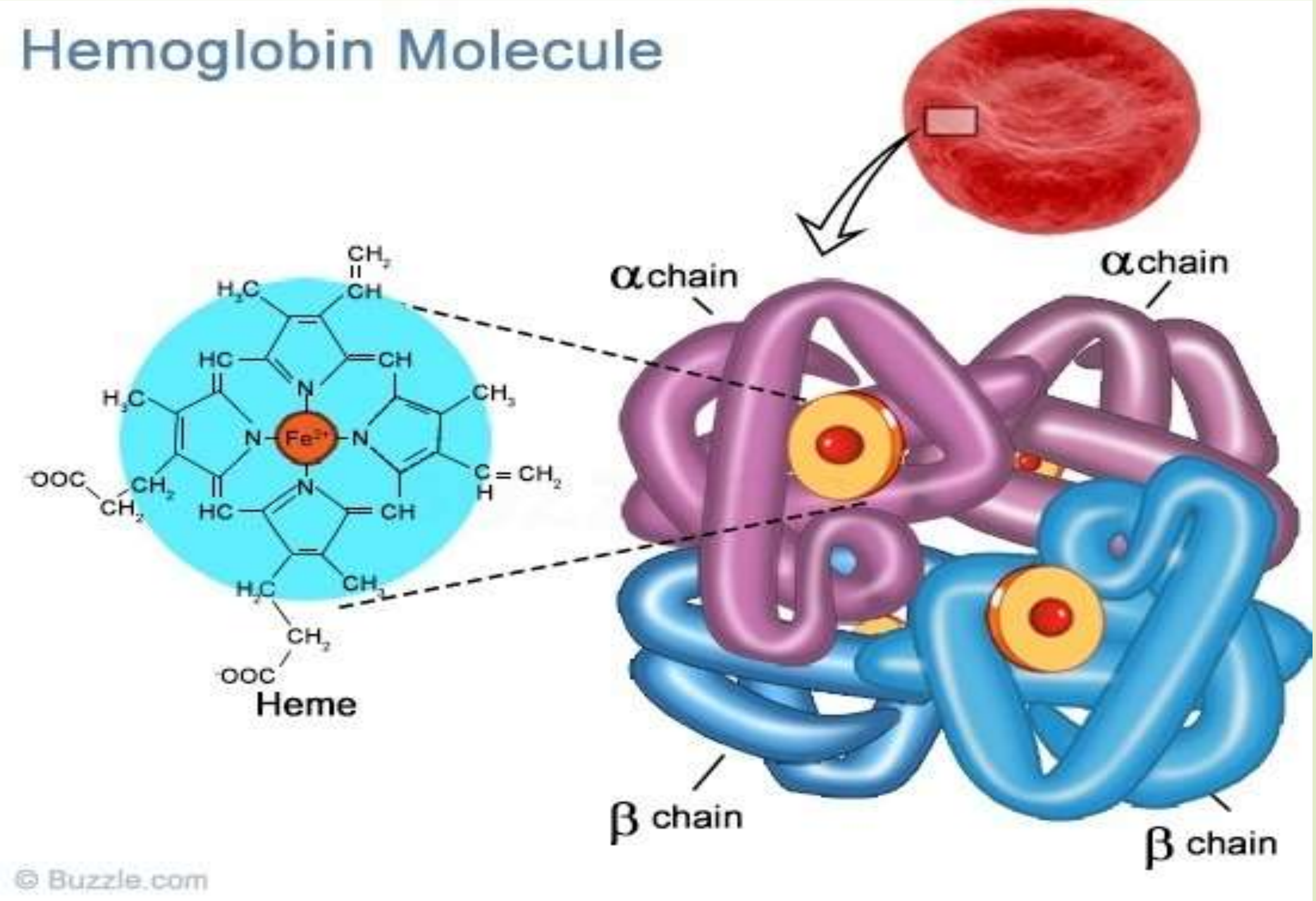
- Hemoglobin is made up of four protein molecules (**globulin chains**) that are connected together ,each one contain **heme** group.
- Hemoglobin =4globins+4 heme groups.
- The normal adult hemoglobin molecule contains two alpha-globulin chains and two beta-globulin chains.
- In fetuses and infants, beta chains are not common and the hemoglobin molecule is made up of two alpha chains and two gamma chains.
- As the infant grows, the gamma chains are gradually replaced by beta chains, forming the adult hemoglobin structure.
- Alpha , beta chains with gamma and delta being less often.

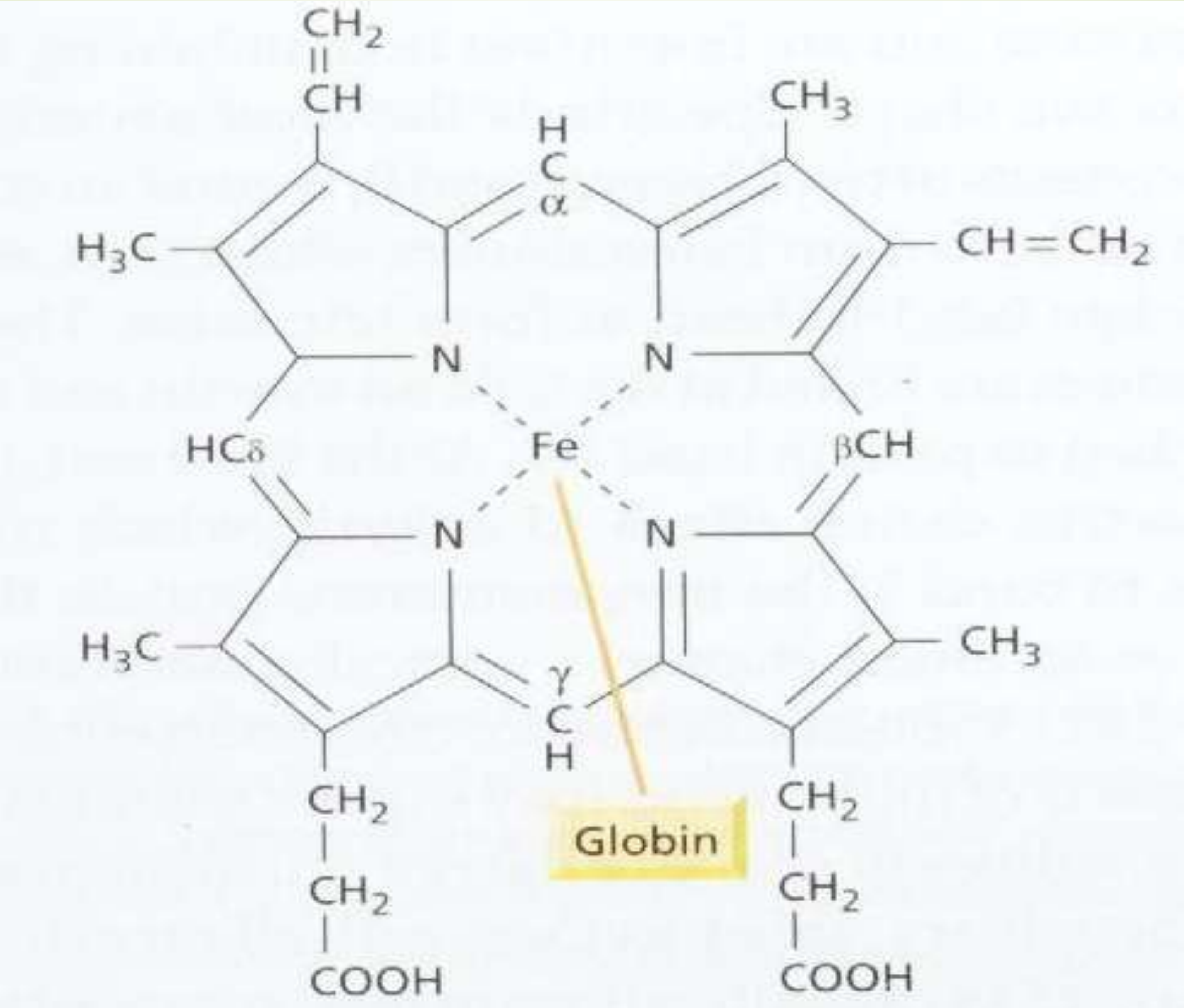
4 globins polypeptide chain composed of amino acids (a.a).

Each polypeptide chain is composed of 141-146 amino acids.

- The absence, replacement or addition of only one a.a modifies the property of the hemoglobin.

- Heme consist of iron atom contained in the center of a large heterocyclic organic ring called a porphyrin.
- Heme consists of a protoporphyrin ring with an iron atom at its center.
- The protoporphyrin ring consists of four pyrrole groups which are united by methane bridges (=C–).
- The hydrogen atoms in the pyrrole groups are replaced by four methylene (CH3–), two vinyl (–C=CH2) and two propionic acid (–CH2–CH2–COOH) groups


Hemoglobin Molecule
α chain
α chain
β chain
β chain
Fe2+
Heme
© Buzzle.com



CH2
CH
CH3
H
C
α
H3C
CH=CH2
N
N
HCδ
Fe
βCH
N
N
H3C
CH3
γ
C
H
CH2
CH2
COOH
Globin
CH2
CH2
COOH

They are two pairs of polypeptide in Hb molecule.

- Hb A($\alpha_2\beta_2$) : the two types of polypeptide chain are called (alpha) chains each of which contains 141 amino acid residues and the beta chains each of which contains 146 amino acid resides.
- * Hb A2($\alpha_2\delta_2$) : consists of two alpha and two delta chains and is found at low levels in normal human blood. not all hemoglobin in the blood of normal adult is hemoglobin A.

Adult haemoblobin

	Hb A	Hb A_2	Hb F
structure	$\alpha_2\beta_2$	$\alpha_2\delta_2$	$\alpha_2\gamma_2$
Normal %	96-98 %	1.5-3.2 %	0.5-0.8 %

- About 2.5% of the hemoglobin is hemoglobin A2.
- In which beta chain are replaced by delta chain.
- the delta chains also contain 146 amino acids residues but 10 individual residues differ from those in the beta chains.
- Hemoglobin A2 may be increased in beta thalassemia and in people with Sickle-cell disease.

Hemoglobin A1c(HbA1c)

- glycated hemoglobin test, or glycohemoglobin, is an important blood test that provides an average of blood sugar control over the past 2 to 3 months.
- When diabetes is not controlled (meaning that sugar is too high), sugar builds up in blood and combines with hemoglobin, becoming "glycated. Has a glucose attached to terminal valine in each beta chain and is of special interest because the quantity in the blood increases in poorly controlled diabetes mellitus, hemoglobin A1c test will be higher.

- Normal range for the hemoglobin A1c test is between 4% and 5.6%.
- Hemoglobin A1c levels between 5.7% and 6.4% indicate increased risk of diabetes.
- Levels of 6.5% or higher indicate diabetes.

Fetal hemoglobin Hb F($\alpha_2\gamma_2$)

- Its structure is similar to that of hemoglobin A except that beta chains are replaced by gamma chains.
- The gamma chains also contain 146 a.a residues but have 37 that differ from those in the beta chain.
- Fetal hemoglobin is normally replaced by adult hemoglobin soon after birth.

Hemoglobin S

- The alpha chain are normal but the beta chains are abnormal because among the 146 a.a residues in each beta polypeptide chain ,one glutamic acid residue has been replaced by a valine residue.

Reactions of hemoglobin

- Oxyhemoglobin that is carrying oxygen ,it is bright red.
- * Hemoglobin binds to oxygen to form oxy hemoglobin ,oxygen bind to Fe^{+2} in the heme.
- When Hb with Co_2 give carbamino hemoglobin.
- Co reacts with Hb to form carboxy hemoglobin (Hbco).

- The affinity of hemoglobin for O_2 is much lower than its affinity for Co, which consequently displaces O_2 on Hb and reducing oxygen carrying capacity of blood.

Carbone monoxide(Co) poisoning

- Is often listed as form of anemic hypoxia because the amount of Hb that carry oxygen is reduced but the total Hb content of the blood is unaffected by Co.
- The affinity of Hb for Co is 210 times its affinity for O2 and Carboxy hemoglobin (CoHb) liberate Co very slowly.

- The chery-red color of CoHb is visible in skin , nail and mucous membrane.

methemoglobin

- Can be genetic or when blood is exposed to various drugs and other oxidizing agents in *vitro* and in *vivo.*
- The ferrous iron Fe^{+2} that is normally in the molecule is converted to ferric Fe^{+3} forming methemoglobin.
- methemoglobin is dark bluish colored and when it is present in large quantities in the circulation it cause a dusky discoloration of the skin resembling cyanosis.

The normal concentration of Hb in the blood

- 12-16 gm / dL in women.
- 13.5-18 gm/ dL in men

Decreased levels of haemoglobin are found in:

- 1- Anemia.
- 2- After severe hemorrhage.
- 3- Hemolysis due to transfusion of incompatible blood, reactions to chemicals and drugs, bacteraemia, and artificial heart valves.
- 4- Variety of systemic diseases e.g. leukemia, lymphoma, uremia, cirrhosis, hyperthyroidism, carcinomatosis and systemic lupus erythematosis.

Increased levels of haemoglobin are found in:

- 1- Haemo concentration states of blood (an increase in the proportion of red blood cells relative to the plasma, brought about by a decrease in the volume of plasma or an increase in the concentration of circulating red blood cells) e.g. sever burns
- 2- Polycythaemia .
- 3- Chronic obstructive pulmonary disease (COPD)
- 4- Congestive cardiac failure (CCF).
- 5- High altitudes.

Increased Hemoglobin	Decreased Hemoglobin
•High altitude due to low Oxygen tension. •Obstructive lung disease. •Congestive Heart disease due to hypoxia. •Polycythemia (increased RBCs). •Smoking.	•Anemia. •Parasitic infestations. •Drugs. •Lead poisoning. •Iron deficiency. •Copper deficiency.. because copper is necessary for the formation of protein-ceruloplasmin which converts ferric to ferrous. •Kidney disease.. because formation of erythropoietin is decreased by kidney.

1-Sahli method

- This test requires dilution of blood and visual color match.
- **Principle:**
- Haemoglobin is converted into acid haematin by addition of 0.1 N HCl. The resultant solution is then matched against a reference solution in a colorimeter or colored strip (SAHLI'S Haemoglobinometer).

Reagents and Equipment

- 1- Sahli's haemoglobinometer.
- 2- Sahli's pipette.
- 3- 0.1 N HCl.
- 4- Dropping,
- Stirring rod.

Procedure:

- 1- Prepared plastic tube and labeled , Add 100 micro liter of N HCl by micropipette. Or by dropper .
- 2- Shake the sample of blood 5 times gently , Aspirate 20 micro-litre by micropipette and add to the plastic tube that contain N HCL and mixed .
- 3- Transfer the solution into the graduated tube of Sahli's haemoglobinometer by plastic pipette.
- 4- Allow it to stand for 10 minutes, so that haemoglobin gets converted into acid haematin.

- 5- Compare the color of the solution in the graduated tube with that of reference strip on either side of haemoglobinometer.
- 6- If the color of the graduated tube is darker, add drop by drop either 0.1N HCl or distilled water by the dropping pipette and mix with glass rod, until the color matches with the reference strips.
- 7- Note the reading on the graduated tube. This is the haemoglobin level in g/dl. Some tubes also give level in percentage. To convert into g/dl, multiply the percentage with 0.146(Example:10% X 0.146=14.6 g/dL)

advantages

- 1-Instruments and reagents are inexpensive
- 2- Test is easy to perform
- 3- Electricity is not required.

Disadvantages

- 1-Color matching is subjective.
- 2- The color of the glass standard is not a true match for the color of diluted blood.
- 3-Graduated tubes must be cleaned before use.
- 4-Acid hematin is not a stable compound and readings must be taken within the recommended time interval.

- 5-sample can only be measured one at a time.
- 6- after prolonged use the numbers on the graduated cylinder fade and are difficult to read.

2-Cyanmethaemoglobin method:

- This method is the most accurate and most commonly used method
- **Principle:**
- The blood is diluted in a solution containing Potassium Cyanide and Potassium Ferricyanide (Drabkin's solution).
- * It converts haemoglobin (Hb) and methaemoglobin (Hi) to cyanmethaemoglobin (HiCN), which is a stable compound. The absorbance of the solution is measured in photoelectric calori-meter at a wavelength of 540nm.

3-Filter paper method (tallqvist) is a direct method

- **This method does not require lysis or dilution of blood.**
- **Principle**
- The red color of blood corresponds to the amount of hemoglobin present. The degree of anemia can be visually assessed by matching the color of a drop of blood on filter paper against a standardized color chart.
- The color chart has been developed to represent the color range of normal to anemic blood on filter paper.

Equipment and Supplies

- Required: Filter/blotting paper that allows absorption and rapid drying of blood drop
- A standardized color comparison chart that represents ranges of hemoglobin levels
- Sterile lancets
- 70% alcohol and cotton wool

Maintenance and Storage

- Do not leave the color charts in the sun for extended periods of time as they may fade.
- • Do not leave filter paper in the sun or in high humidity because the heat and humidity will damage them.
- • Store color charts and filter paper at room temperature.

Interpretation

- Some charts provide readings in grams per liter, while others provide
- readings in ranges of hemoglobin that indicate normal (>12.0 g/dl),
- mild anemia (<11.0 g/dl to 9.0 g/dl), moderate anemia (<9.0 g/dl to 7.0 g/dl), or severe anemia (<7.0 g/dl).
- If a result indicates moderate to severe anemia, follow up with a more accurate test where possible.

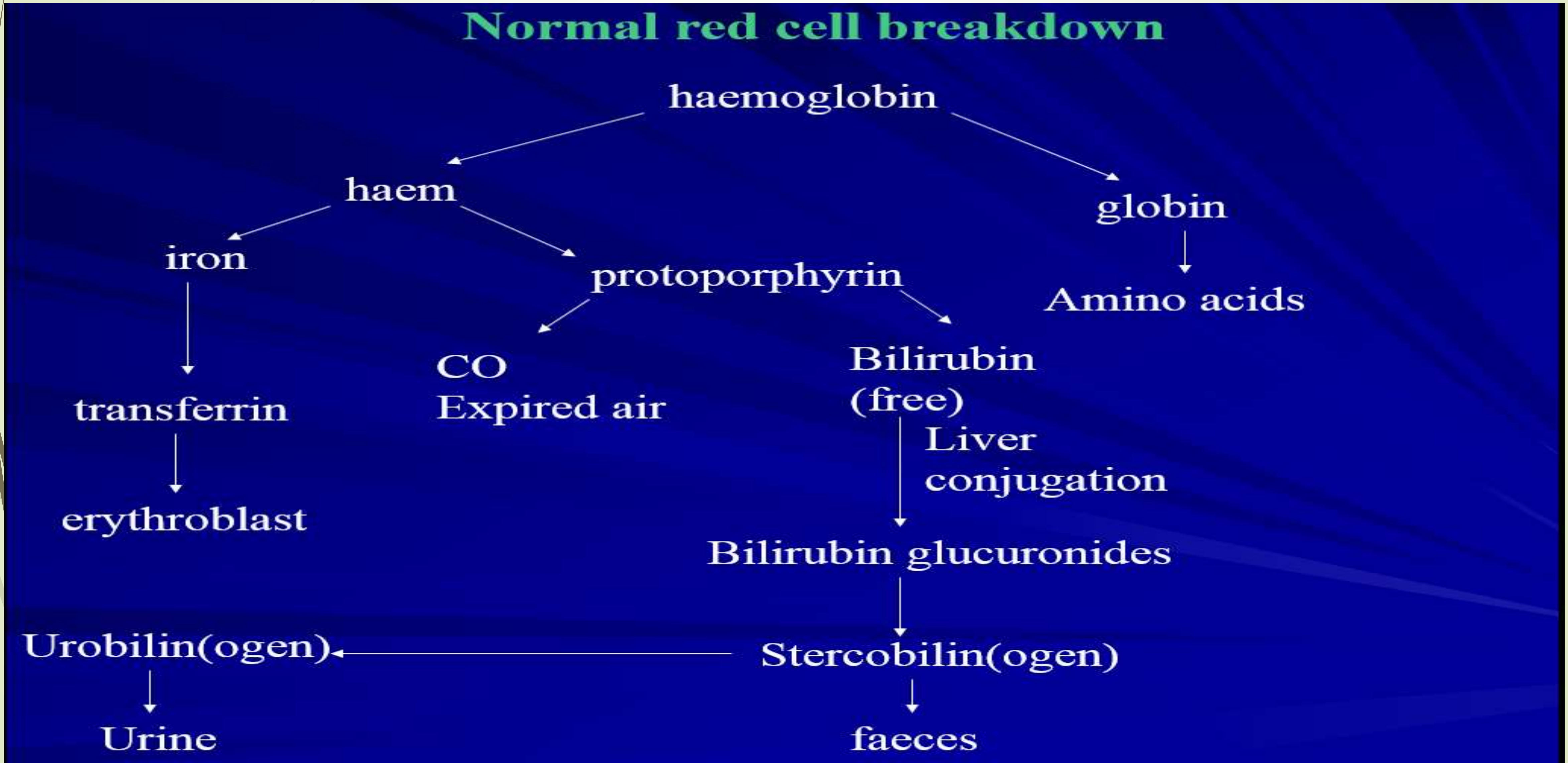
Advantages

- • Inexpensive
- • Rapid
- • Simple
- • No reagents required
- Portable
- • Electricity not required

Disadvantages

- The chart is supplied with a limited quantity of filter paper. Other types of paper can not be substituted because they are not calibrated with the chart.
- • The scale may become contaminated with blood over time.
- • Lighting conditions influence interpretation of the result.
- • Size and thickness of blood spot, temperature, and humidity all affect drying time, which in turn affects color.

Normal red cell breakdown

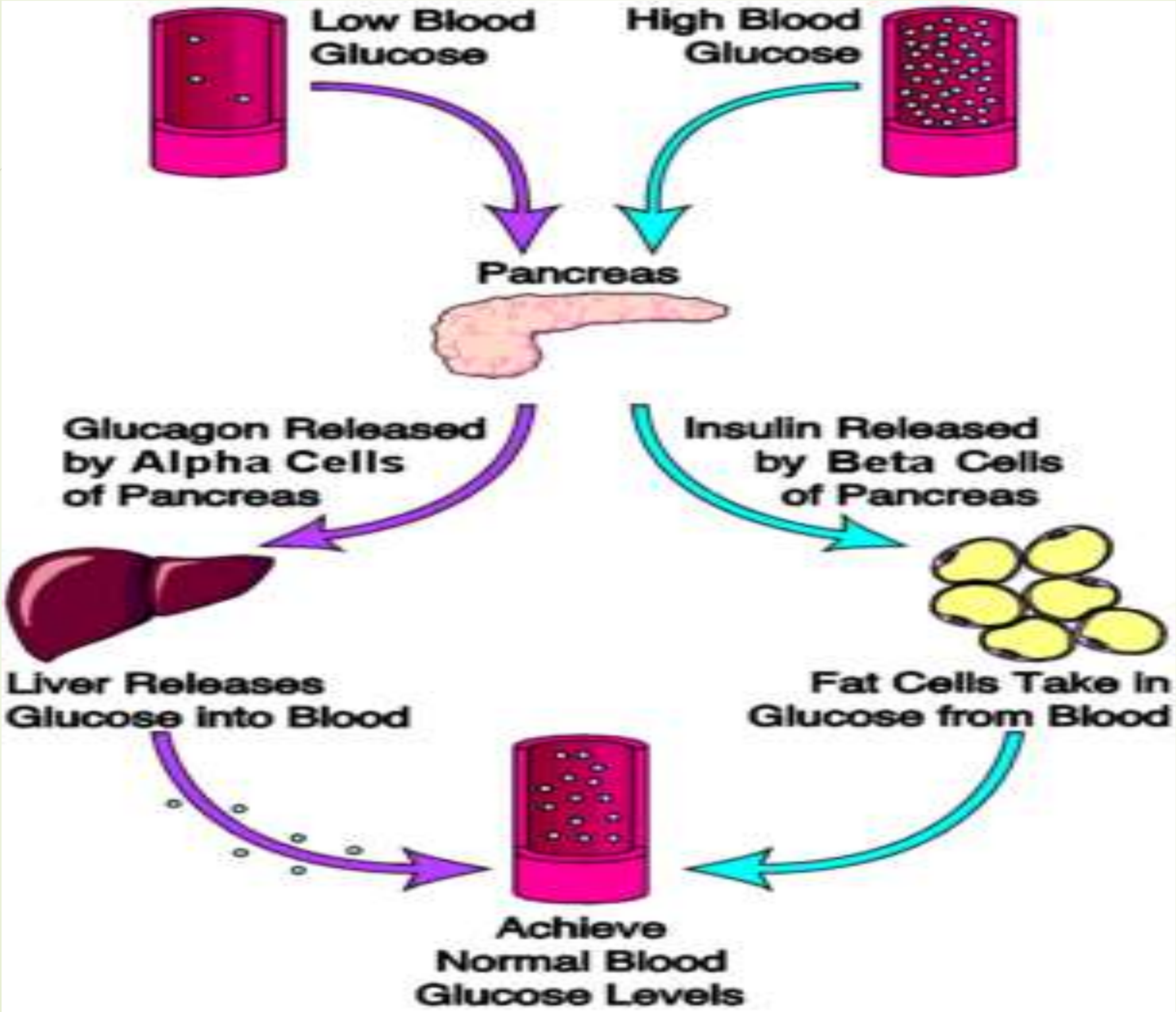


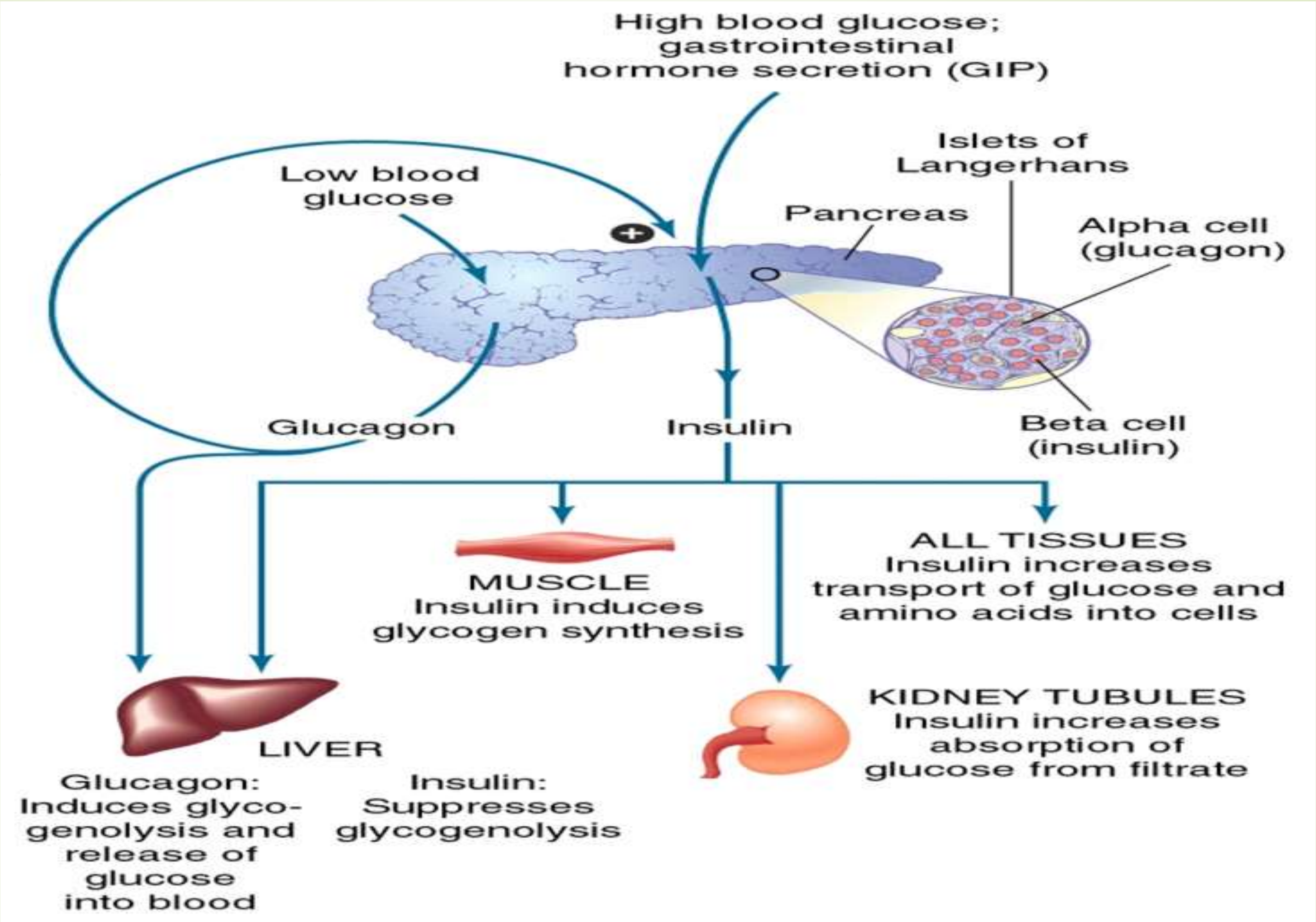
- THANK YOU.

Oral Glucose Tolerance Test
(OGTT)

Pancreatic axis

- Insulin
  - β cells secrete due to **high blood glucose levels**
  - Glucose uptake into tissues increases.
- Glucagon
  - α cells secrete when **blood glucose is low**
  - Glucose is released from tissues back into blood.


Low Blood Glucose
High Blood Glucose
Pancreas
Glucagon Released by Alpha Cells of Pancreas
Insulin Released by Beta Cells of Pancreas
Liver Releases Glucose into Blood
Fat Cells Take In Glucose from Blood
Achieve Normal Blood Glucose Levels


High blood glucose; gastrointestinal hormone secretion (GIP)
Low blood glucose
Islets of Langerhans
Pancreas
Alpha cell (glucagon)
Beta cell (insulin)
Glucagon
Insulin
MUSCLE
Insulin induces glycogen synthesis
ALL TISSUES
Insulin increases transport of glucose and amino acids into cells
LIVER
Glucagon: Induces glyco-genolysis and release of glucose into blood
Insulin: Suppresses glycogenolysis
KIDNEY TUBULES
Insulin increases absorption of glucose from filtrate

Glucose homeostasis: Is the balance of insulin and glucagon to maintain blood glucose.

- Insulin: secreted by the pancreas in response to elevated blood glucose following a meal.
- Insulin lowers blood glucose by increasing glucose uptake in muscle and adipose tissue and by promoting **glycolysis** and **glycogenesis** in liver and muscle.
- Glucagon: a fall in blood glucose increases the release of glucagon from the pancreas to promote glucose production and serves to keep blood glucose levels high enough for the body to function well.

Glucagon

- Glucagon generally elevates the concentration of glucose in the blood by promoting gluconeogenesis **(the conversion of amino acids into glucose)**. and glycogenolysis **(break down glycogen in liver to be released into the blood as glucose).**
- As these stores become depleted, glucagon then encourages the liver and kidney to synthesize additional glucose by gluconeogenesis. Glucagon turns off glycolysis in the liver.

- Glucose homeostasis

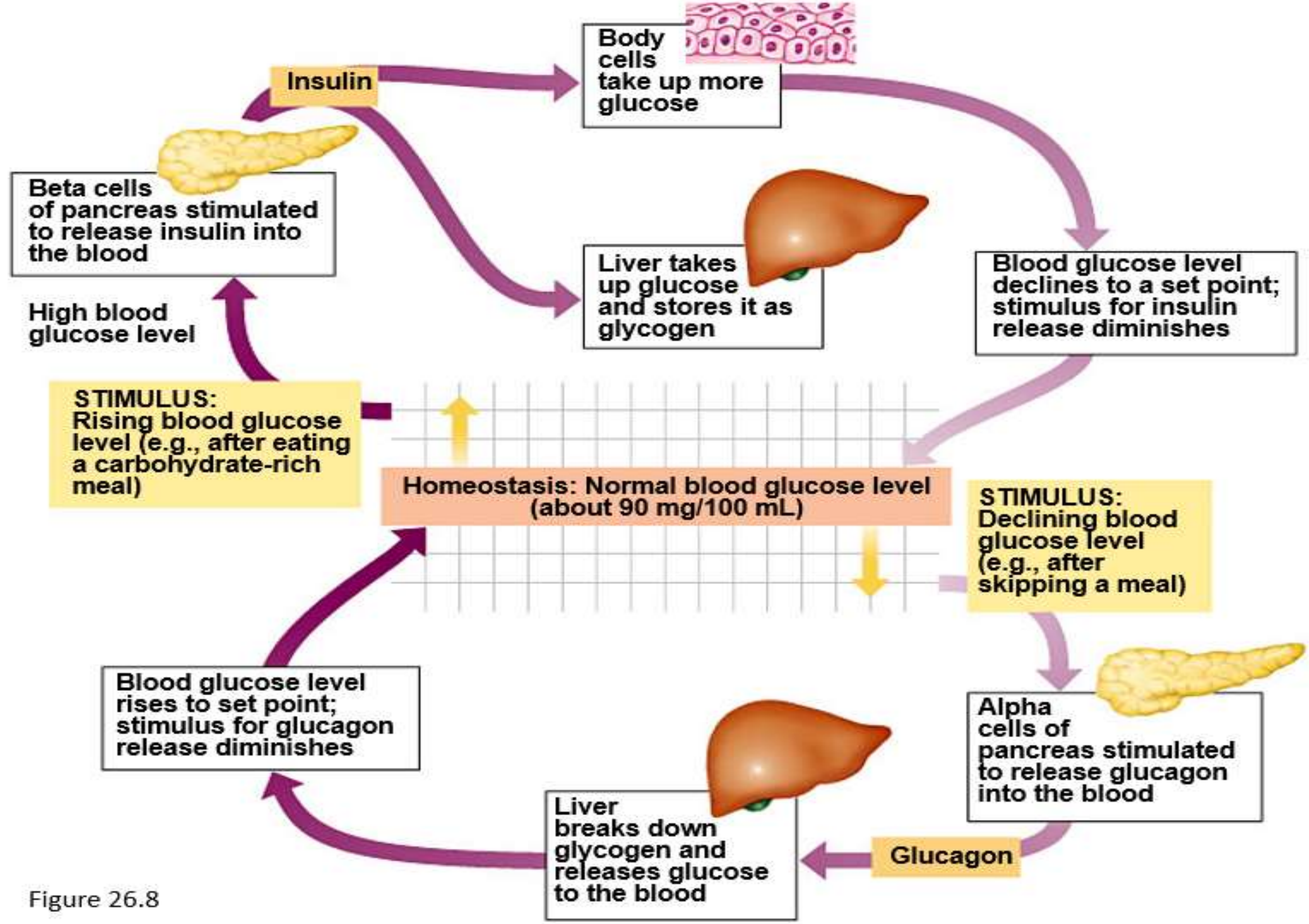


Figure 26.8

- A high or too much glucose in the blood is also called high blood sugar or **hyperglycemia**.
- A low blood sugar level is called **hypoglycemia** .
- **Insulin:Glucagon Ratio**: everything that happens to glucose, amino acids and fat in the well fed state depends upon a high insulin to glucagon ratio.

There are 2 types of diabetes mellitus:

- **Type 1**: Insulin-dependent diabetes mellitus (IDDM)
- (5-10%) of cases, usually developed in childhood or early adulthood.
- It is the result of an autoimmune process-mediated destruction of pancreatic beta cells.
- Resulting in absolute deficiency of insulin.

- Type 2: Non-insulin dependent diabetes mellitus (NIDDM).
- 90% of cases.
- Results from a combination of **insulin resistance** and altered insulin secretion or **relative insulin deficiency**.


INSULIN

Criteria for diagnosis of DM include any one of the following:

- 1. A1C of 6.5% or more.
- 2. Fasting(no caloric intake for at least 8 hrs),plasma glucose of 126 mg/dl (7.0 mmol/L).
- 3. Random plasma glucose concentration of 200mg/dl (11.1mmol/L), with classic symptoms of hyperglycemia or hyperglycemic crisis.(**polyuria, polydipsia, polyphagia, weight loss and lethargy).**
- 4.Two-hour plasma glucose of 200 mg/dl(11.1mmol/L) or more during an oral glucose tolerance test(OGTT), using glucose load by dissolving 75 g anhydrous glucose in water.

Normal blood glucose levels

- Fasting (70–100 mg/dl)
- HbA1c (Less than 5.7%)
- 2 hours after meals (Less than 140 mg/dl)

Oral Glucose tolerance testing (GTT):

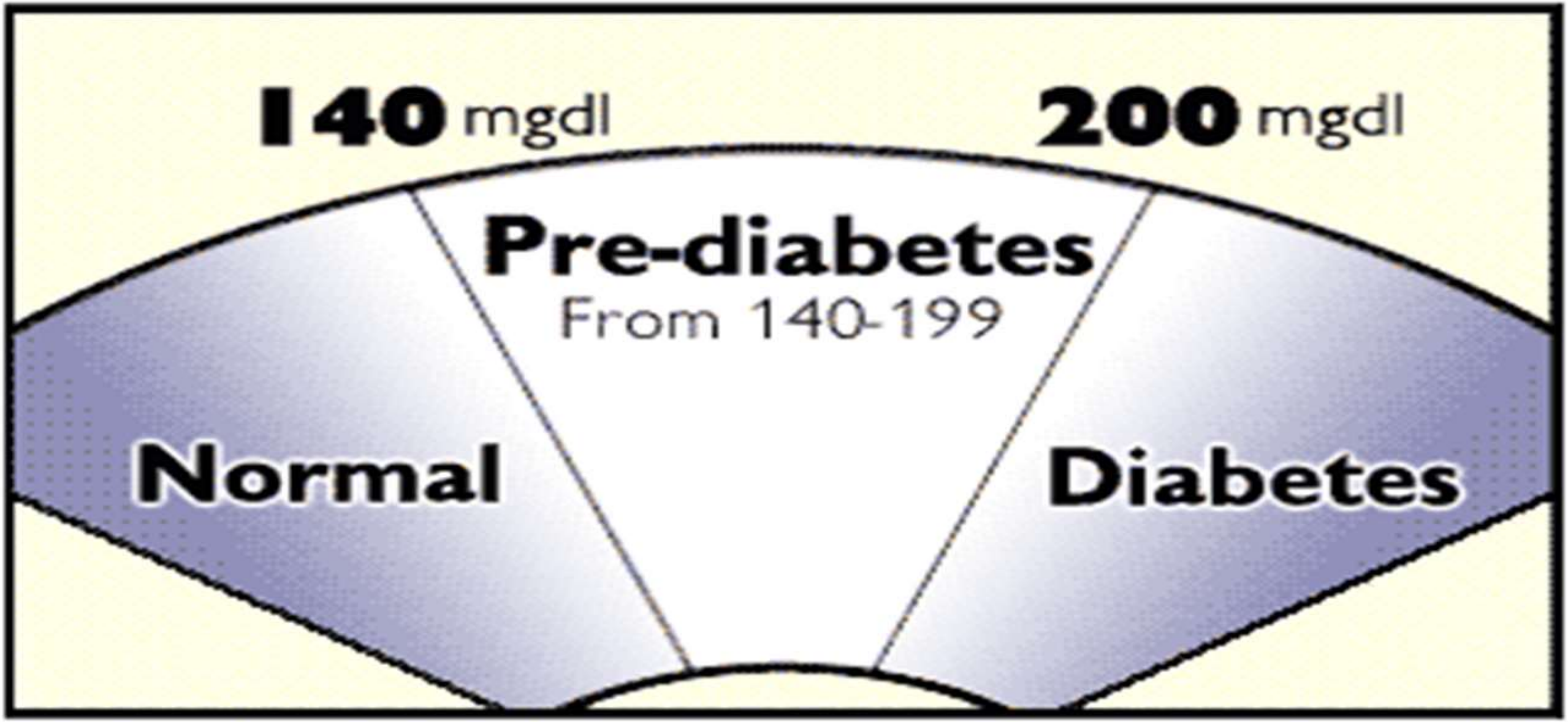
- **Glucose Tolerance Test**: evaluates how quickly an individual can restore their blood glucose to normal following ingestion of a large amount of glucose, i.e. measures an individuals ability to maintain glucose homeostasis.
- is used to evaluate the ability to regulate glucose metabolism.
- The reference range of serum or plasma glucose is less than 140 mg/dL at 2 hours after a 75-g glucose load.

- After baseline fasting plasma glucose (FPG) testing, a glucose load is administered—either **intravenously** or, more commonly, **orally**—and plasma glucose is measured at specified intervals thereafter.
- In the standard OGTT, plasma glucose concentration is measured 2 hours after a 75-g oral glucose load; for GDM, an additional measurement may be made at **1 hour**.

- The OGTT is seldom used as a confirmatory test in the diagnosis of DM, but it may be helpful when fasting or random glucose results are equivocal. It is required for diagnosing **IGT (Impaired glucose tolerance (IGT) is a pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of cardiovascular pathology.)**, which is associated with an increased risk of developing **DM type 2** by many years. However, the OGTT is increasingly reserved for research purposes. OGTT using **a 100-g** glucose load or **a 50-g** load (the latter to screen for gestational diabetes) is no longer recommended by the ADA .

The oral glucose tolerance test (OGTT) :

- The OGTT usually requires that you have the fasting glucose test first. Then you take a dose of high-sugar (glucose) solution to challenge your body to clear the glucose from your blood. After two hours, another blood glucose test is done. The final test results indicate whether you have a normal level of blood glucose or may have prediabetes or diabetes:
- Normal: Normal blood sugar levels measure less than 140 mg/dl after the oral glucose tolerance test.
- Prediabetes: Blood glucose levels of 140-199 mg/dl after the OGTT is diagnosed as prediabetes. People with these results are considered to have impaired glucose tolerance (IGT).
- Diabetes: Diabetes is diagnosed with blood glucose of 200 mg/dl or above.



Oral Glucose Tolerance Test

OGTT

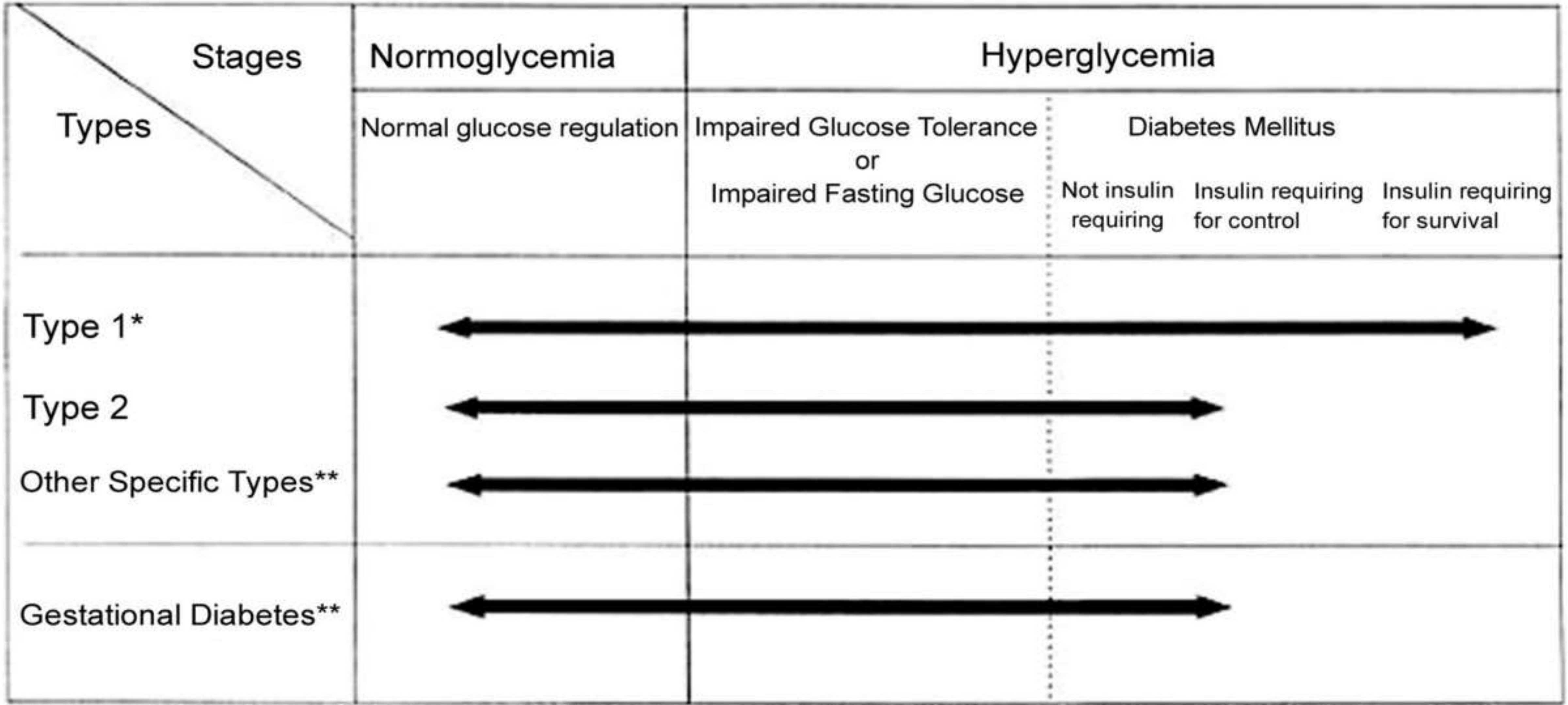
- **The test is usually used to test for:**
- diabetes .
- insulin resistance and pre-diabete state.
- and sometimes reactive hypoglycemia or (postprandial hypoglycemia), is a medical term describing recurrent episodes of symptomatic hypoglycemia occurring within 4 hours after a high carbohydrate meal in people who do not have diabetes
- and acromegaly, or rarer disorders of carbohydrate metabolism.

Indications/Applications

- Indications for the OGTT include the following:
- 1. Equivocal FPG or random plasma glucose results
- 2. To screen for GDM at 24–28 weeks of gestation in all pregnant women not known to have diabetes
- 3. To screen for DM at 6–12 weeks postpartum in women with a history of GDM, using non pregnant OGTT criteria.

Factors may affect the result of test

- Previous diet
- Time of day ???
- Drug steroids, oral contraceptive and thiazide diuretics may impair glucose tolerance.
- And others as, smoking, alcohol, recent surgery, illnesses, and infectious diseases, weight loss through dieting and long periods of bed rest (such as from a hospitalization or illness).



Disorders of glycemia: etiologic types and stages. *Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous therapy (i.e., "honeymoon" remission). **In rare instances, patients in these categories (e.g.,Vacor toxicity, type 1 diabetes presenting in pregnancy) may require insulin for survival.

- The American Diabetes Association (ADA) diagnostic criteria for impaired glucose tolerance (IGT) are discussed
- Impaired fasting serum or plasma glucose (FG) is defined as an FG level of 100-125 mg/dL.
- Criteria for diabetes mellitus (DM) are as follows:
- FG level of ≥126 mg/dL and a random plasma glucose level of ≥200 mg/dL or
- Random serum or plasma glucose levels ≥200 mg/dL on two occasions or
- Classic DM symptoms (eg, **polydipsia**, **polyuria**) plus serum or plasma glucose levels ≥200 mg/dL at 2 hours in an oral glucose tolerance test (OGTT)

Impaired glucose tolerance (IGT):

- People with glucose levels between normal and diabetic levels have so-called impaired glucose tolerance (IGT). It is a pre diabetic state of dysglycemia which is associated with insulin resistance and there is increased risk of cardiovascular disease.
- It may precede type 2 diabetes mellitus.
- People with impaired glucose tolerance do not have diabetes.
- **Weight loss** and **exercise** may help people with impaired glucose tolerance return their glucose levels to normal. In addition, some physicians advocate the use of medications, such as metformin (Glucophage), to help prevent/delay the onset of overt diabetes.

The preparation for a glucose tolerance test

- preparation for the oral glucose tolerance test involves:
- fasting overnight (from 8 to 16 hours) and participating normally in activities of daily living.
- The individual should eat and drink as they normally do prior to the test.
- The morning of the test, the person should not consume caffeine or smoke.

Prediabetes

- People with prediabetes have glucose levels that are higher than normal but not high enough yet to indicate diabetes .
- The condition used to be called borderline diabetes.
- Most people with prediabetes don't have symptoms, but they are considered to be at high risk of developing heart disease and stroke than who does not have prediabetes. 50% high risk of developing type 2 diabetes.

The fasting plasma glucose test (FPG):

- The fasting plasma glucose test can be done after an overnight fast or after an 8-hour fast during the day. It is a relatively easy, inexpensive test. After the fast, a simple blood test measures glucose levels before you eat again. The test results indicate whether your blood glucose level is normal or whether you have prediabetes or diabetes:
- Normal: Normal blood sugar levels measure less than 100 mg/dl (milligrams per deciliter) after the fasting glucose test.
- Prediabetes: Blood glucose levels of 100-125 mg/dl after an overnight or eight-hour fast may indicate prediabetes. People with these results are considered to have impaired fasting glucose (IFG).

- Diabetes: Diabetes is diagnosed when the blood glucose is 126 mg/dl or above.
- In most cases, your doctor will repeat any abnormal test before confirming the diagnosis.

What is the hemoglobin A1C Test?

- The hemoglobin A1C test is a simple blood test that reflects the average blood sugar for the past **2 to 3** months. It can be used to diagnose prediabetes or diabetes. It can also be used to check if your diabetes is under control:
- Normal: 5.6% or less
- Prediabetes: 5.7 to 6.4%
- Diabetes: 6.5 % or above

Interpretation of OGTT results

- Fasting plasma glucose (measured before the OGTT begins) should be below **110 mg/dL** (6.1 mmol/L). Fasting levels between **110 and 125 mg/dL** are borderline ("impaired fasting glycaemia"), and fasting levels repeatedly at or above **126 mg/dL** (7.0 mmol/L) are diagnostic of diabetes.
- A 2- hour OGTT glucose level below **140 mg/dL** is normal, whereas higher glucose levels indicate hyperglycemia. Blood plasma glucose between **140 –200 mg/dL** indicate "impaired glucose tolerance", and levels above **200 mg/dL** (11.1 mmol/L) at 2- hours confirms a diagnosis of diabetes.

Sample Method

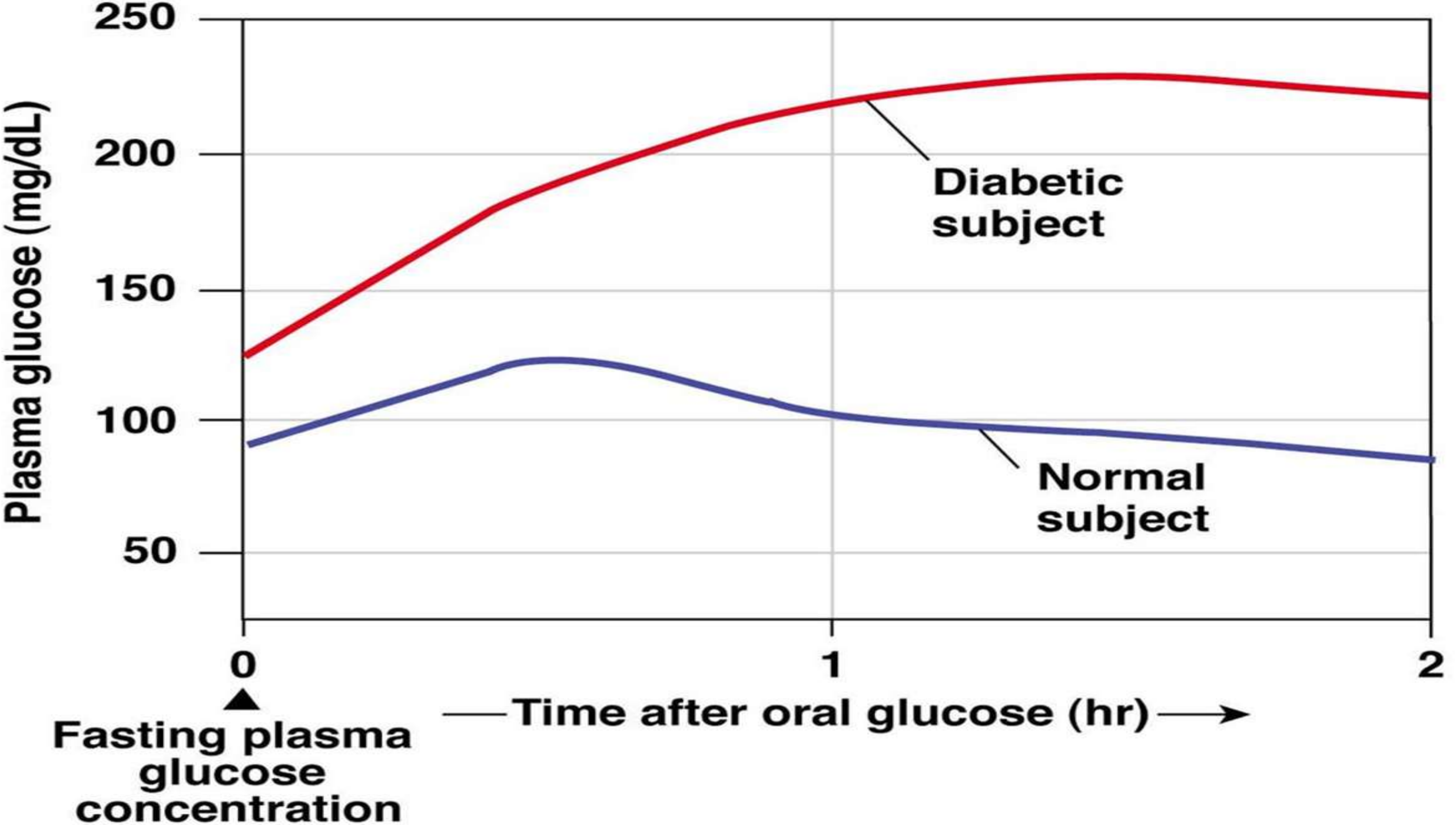
- Is for venous samples only (i.e. a blood sample taken from a vein in the arm). An increasingly popular method for measuring blood glucose is from a **capillary** or **finger-prick** sample. This is less invasive, more convenient for the patient and requires minimal training to conduct.

Casual Plasma Glucose

- Casual is defined as any time of day without regard to time since last meal
- > 200 mg/dl (11.1mm/L) plus symptoms of diabetes Polyuria, polydipsia, unexplained weight loss, confirm on subsequent visit with fasting blood glucose or oral glucose tolerance test.

Diagnostic Criteria

- DM if [glu] > 200 mg/dl at 2 hours
- IGT if [glu] >140 - 199 at 2 hours
- normal if [glu] < 140 mg/dl
- IGT and IFT recently termed "**pre-diabetic**".


250
200
150
100
50
Plasma glucose (mg/dL)
Diabetic subject
Normal subject
0
1
2
Time after oral glucose (hr)
Fasting plasma glucose concentration

Diagnostic Cut Points

Category	FPG (mg/dL)	2h 75g OGTT	A1C
Normal	< 100	< 140	5.7
Prediabetes	100 -125	140 -199	
Diabetes	> 126**	> 200 **	6.5

- or patients with classic hyperglycemic, symptoms with plasma glucose >200.

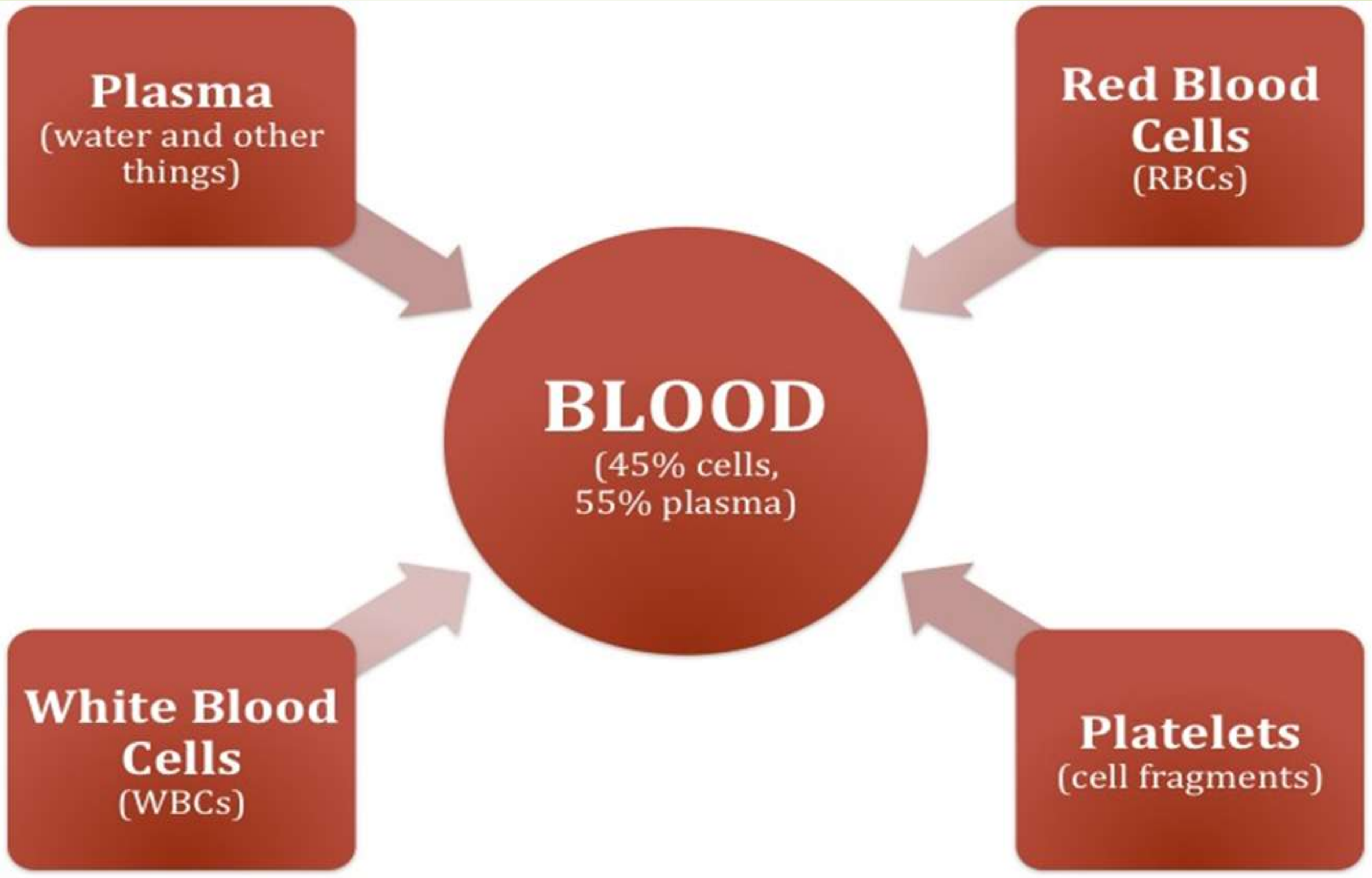
Gestational Diabetes (GDM)

- Overnight fast 75g OGTT
- Fasting >92 mg/dl
- 1 h post glucose >180 mg/dl
- 2 h post glucose >153 mg/dl
- Any one abnormal value is adequate


92
M
S


THANK YOU

The Blood

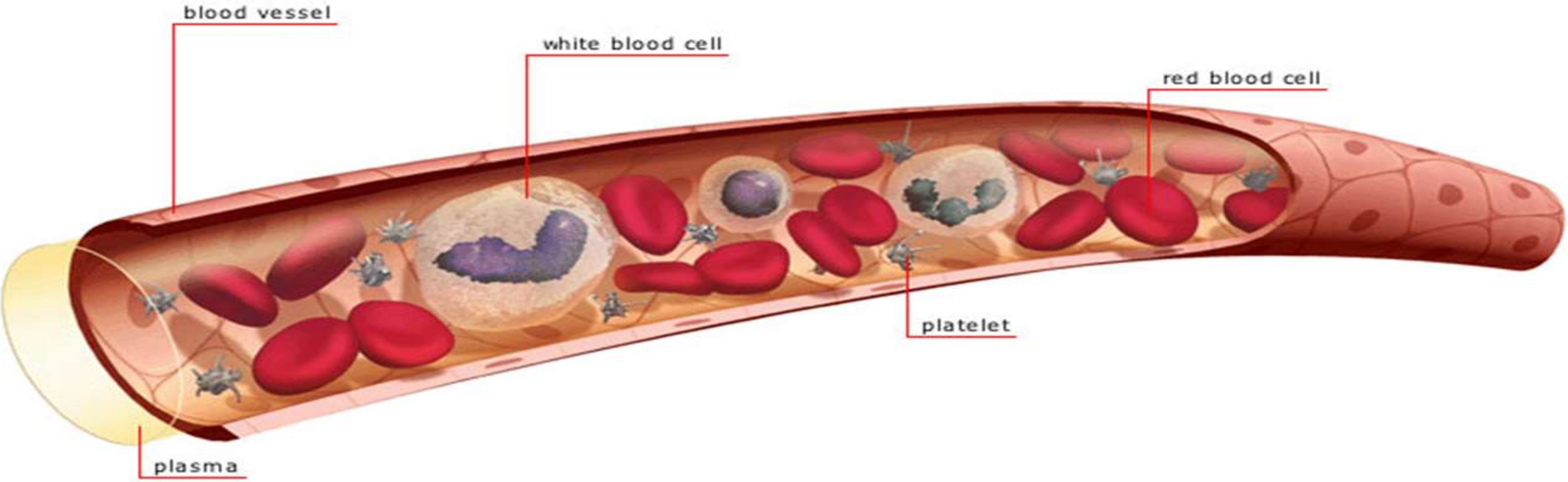

Plasma
(water and other things)
Red Blood Cells
(RBCs)
BLOOD
(45% cells, 55% plasma)
White Blood Cells
(WBCs)
Platelets
(cell fragments)

Haematopoiesis

- is the formation of blood cellular components.

- Blood is a mixture of cellular components suspended in plasma.
- The normal total circulating blood volume is about 8 % of body weight.
- The cells present in the blood are also known as formed elements.

- Blood pH: The acidity or alkalinity of blood. The pH of any fluid is the measure of the hydrogen ion (H-) concentration. A pH of 7 is neutral. The lower the pH, the more acidic the blood. A variety of factors affect blood pH including what is ingested, vomiting, diarrhea, lung function, endocrine function, kidney function, and urinary tract infection. The normal blood pH is tightly regulated between 7.35 and 7.45.
- is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly alkaline.
- Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too alkaline.


blood vessel
white blood cell
red blood cell
platelet
plasma

Functions of Blood

- Supplies oxygen and nutrients to different tissues of our body.
- Removes waste products like, urea, lactic acid and carbon dioxide from our body.
- Provides immunity to body against foreign particles.
- Helps in transportation of substances as (hormones) throughout the body.

- Aids in blood clotting which is a natural repair mechanism of cells or arrest of bleeding (hemostasis).
- Regulates and maintains normal temperature in our body.
- Maintains pH balance inside the body.
- Maintenance of a stable internal environment (homestasis).

Transports:

- Nutrients
- Electrolytes
- O_2 & CO_2
- Waste Products
- Hormones

Defense:

- Foreign organisms
- Injury/infection
- Clotting process
- Body temperature

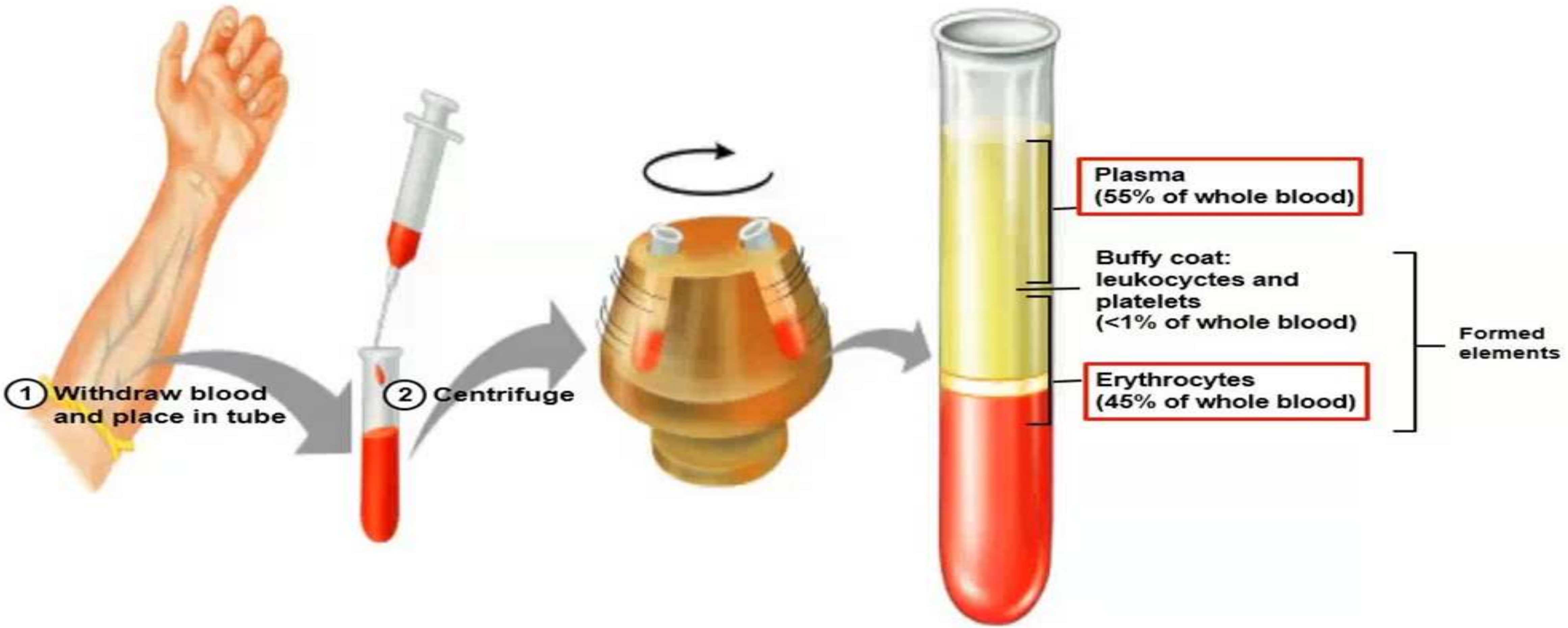
Maintains Hoemostasis

Two tubes of EDTA –anticoagulated blood.

Left tube: after standing, the RBCs have settled at the bottom of the tube.

Right tube: contains freshly drawn blood.

Composition of the blood

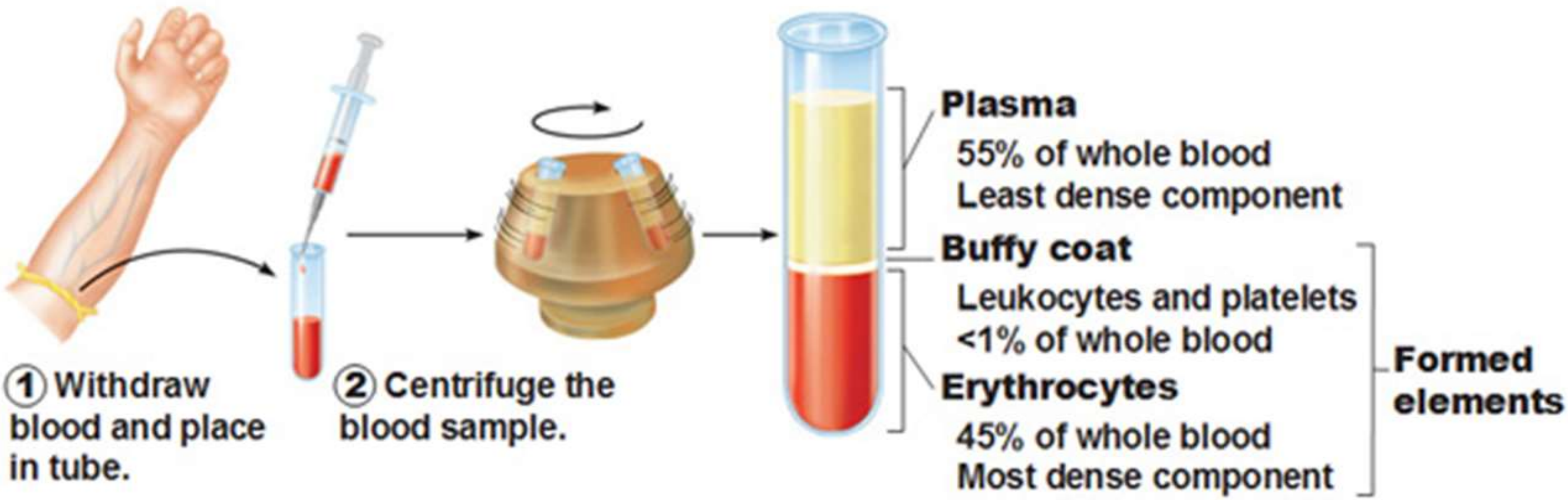


Major Components of Whole Blood

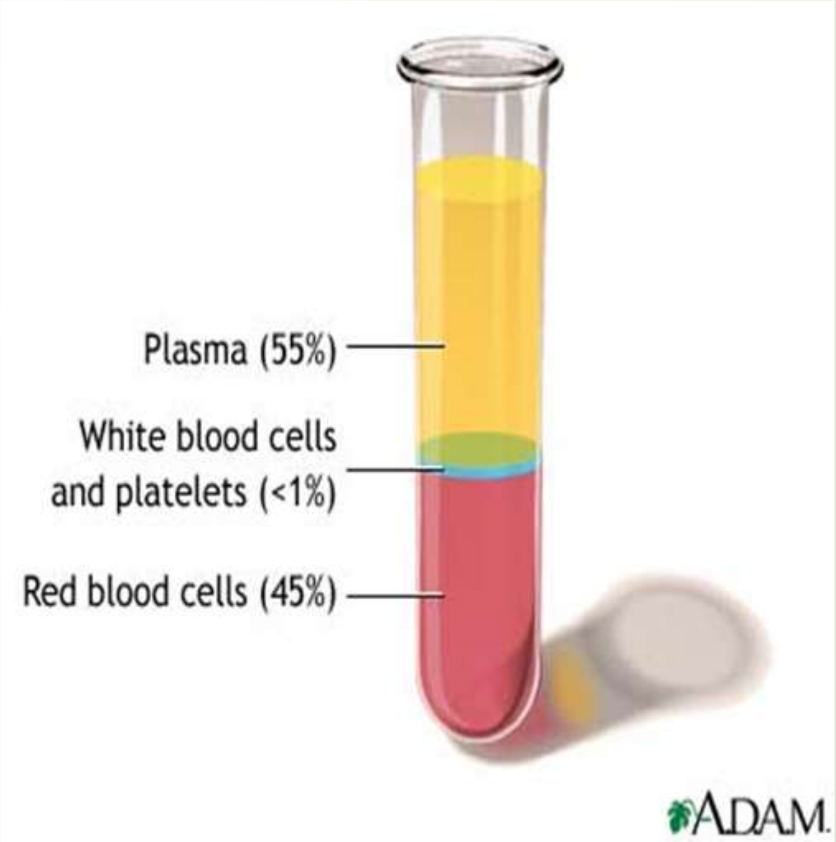
Hematocrit = %-age of blood volume that is RBCs

Males = 47% +/- 5%

Females = 42% +/- 5%



- **Blood plasma Consists of:**
  - Water 90%
  - Plasma Proteins 8 %
  - Electrolytes (Na^+ & Cl^-) 1%
- **Other components:**
  - Nutrients (e.g. Glucose and amino acids)
  - Hormones
  - metabolic end products
  - Blood gases (e.g. CO_2, O_2)



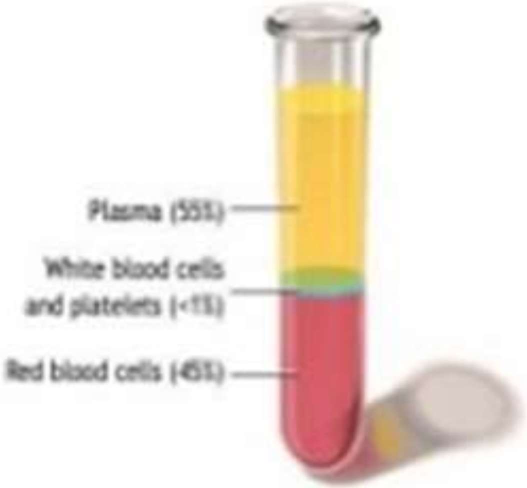
- Blood plasma is prepared by spinning a tube of fresh blood containing an anti- coagulant in a centrifuge until the blood cells fall to the bottom of the tube. The blood plasma is then poured or drawn off.
- Blood serum is blood plasma without fibrinogen or the other clotting factors (i.e., whole blood minus both the cells and the clotting factors).

- **Plasma** is the liquid, cell-free part of blood, that has been **treated with anti-coagulants**.

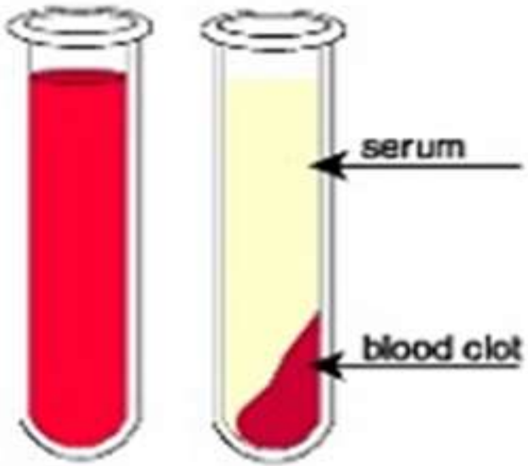
Serum is the liquid part of blood **AFTER coagulation**, therfore devoid of clotting factors as fibrinogen.

Anticoagulated

Clotted



- serum= plasma - fibrinogen



1. **Water:**

* Transport medium; carries heat

2. **Electrolytes:**

* Membrane excitability

* Osmotic distribution of fluid b/t ECF & ICF

* Buffering of pH changes

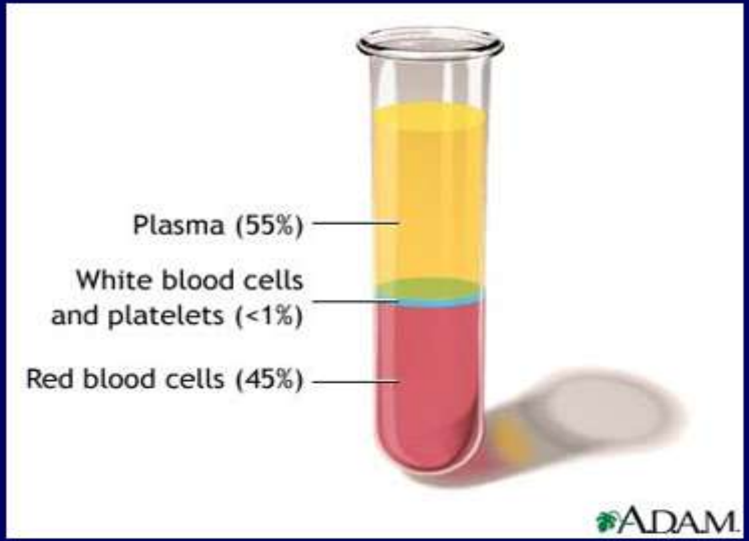
3. **Nutrients, wastes, gases, hormones:**

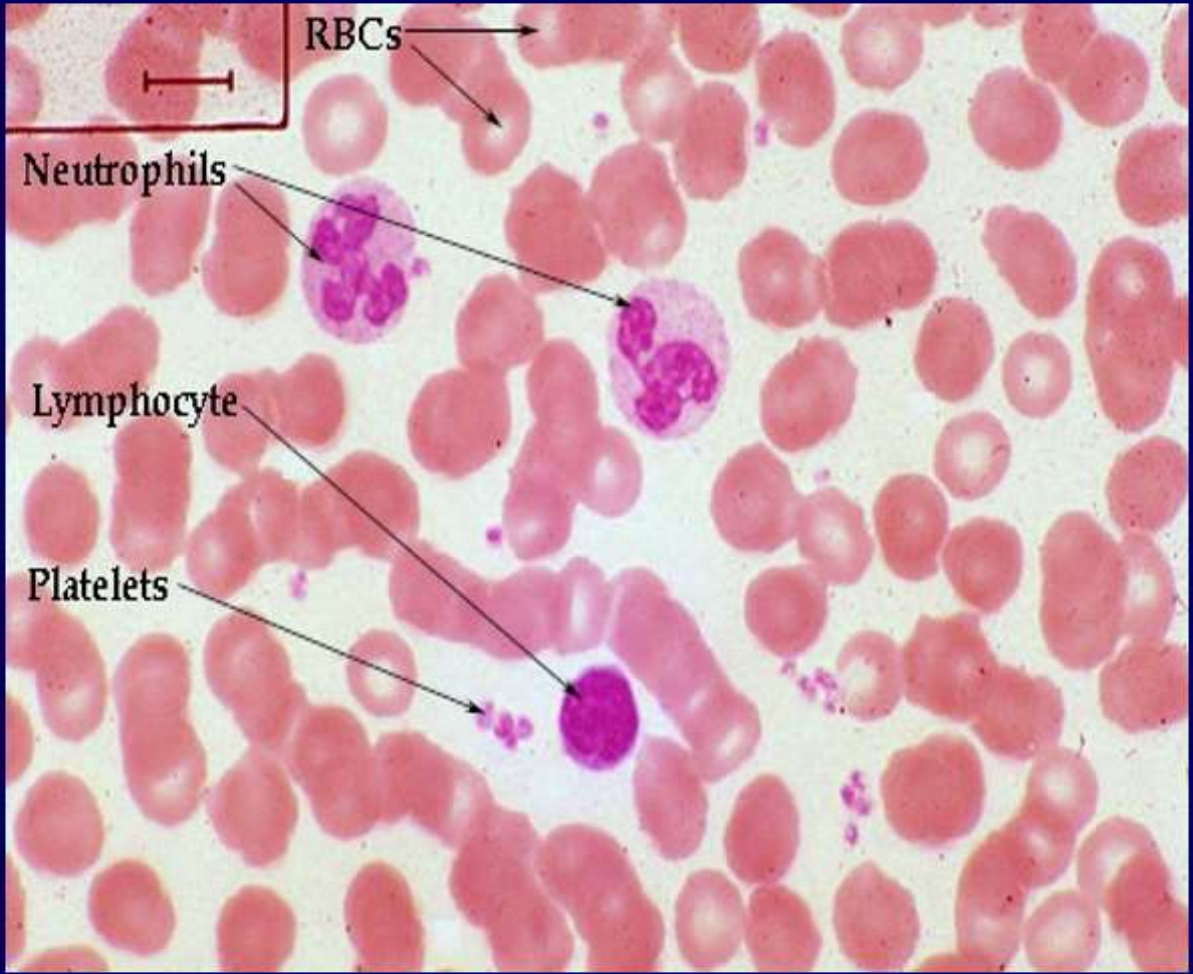
No function – just being transported

4. **Plasma Proteins**

- **Plasma Proteins: (albumins, globulins, fibrinogen)**

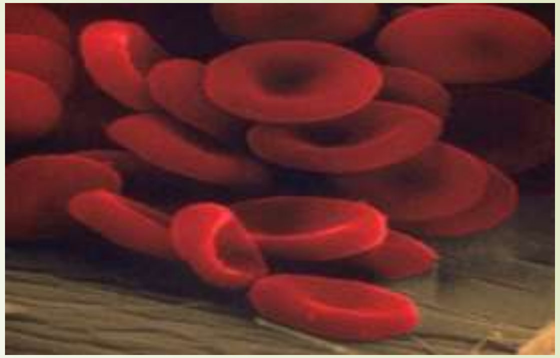
1. Maintaining colloid osmotic balance (albumins)
2. Buffering pH changes
3. Transport of materials through blood (such as water insoluble hormones)
4. Antibodies (e.g. gamma globulins, immunoglobulins)
5. Clotting factors (e.g. fibrinogen)





1. **Red Blood Cells**
2. **White Blood Cells**
3. **Platelets**

1. RBC'S (Erythrocytes)



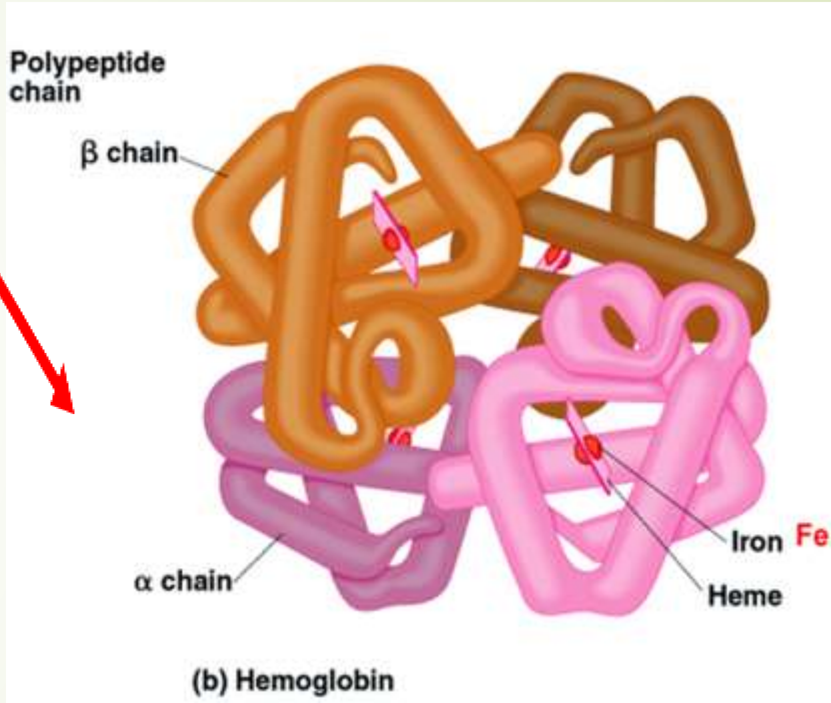
- Shape – a biconcave disc with large surface area
- Can change shape
- No Nucleus / organelles
- Contains hemoglobin
- Primary Function = Transport oxygen from the lungs to the cells of the body & assist with CO_2 removal

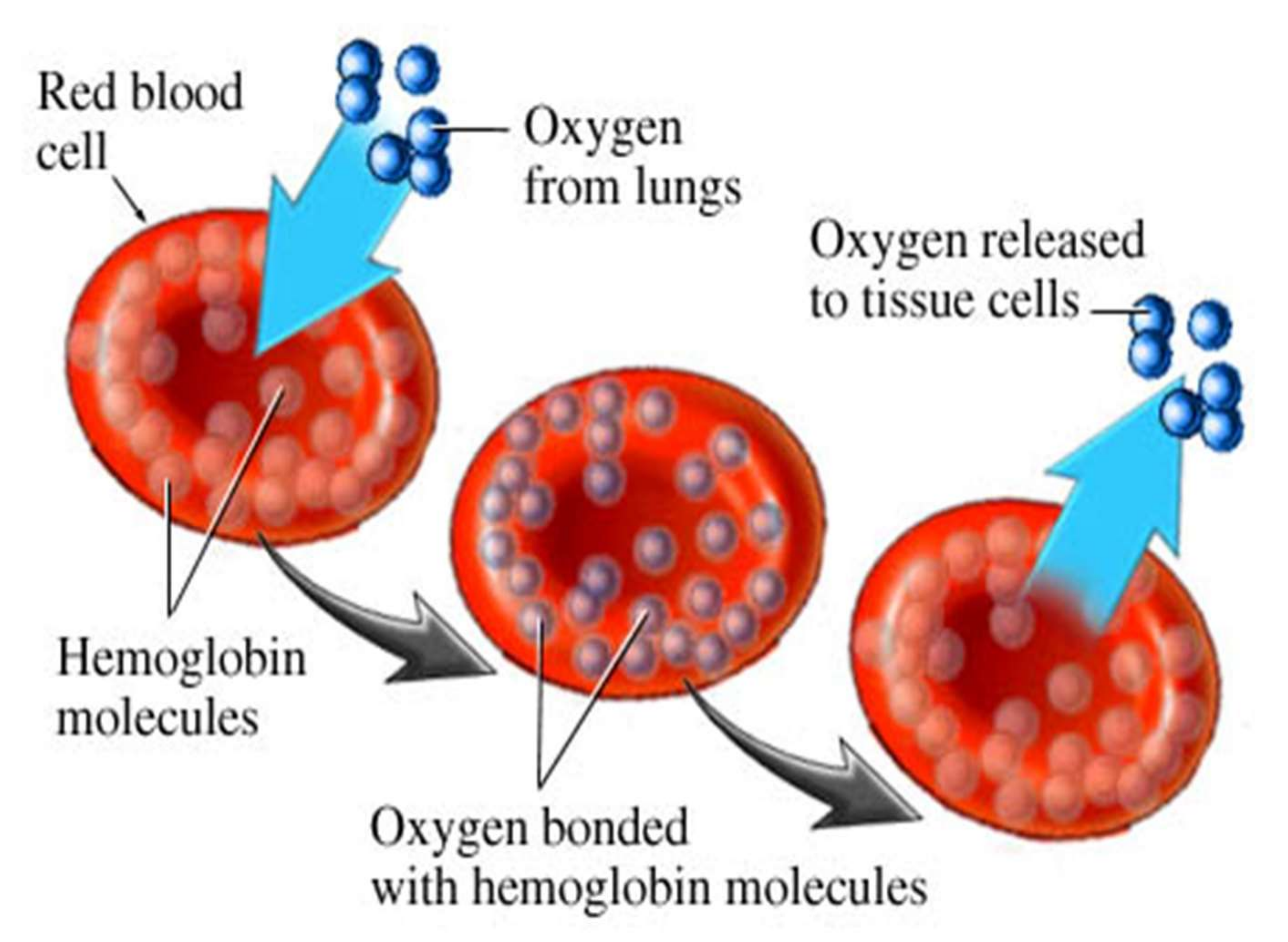
Mechanism of Transport

Hemoglobin

4 Heme Molecules =4 Oxygen Molecules

Oxygenated Hemoglobin
  Bright Red (systemic)
*Deoxygenated Hemoglobin
  Blue (venous circulation)

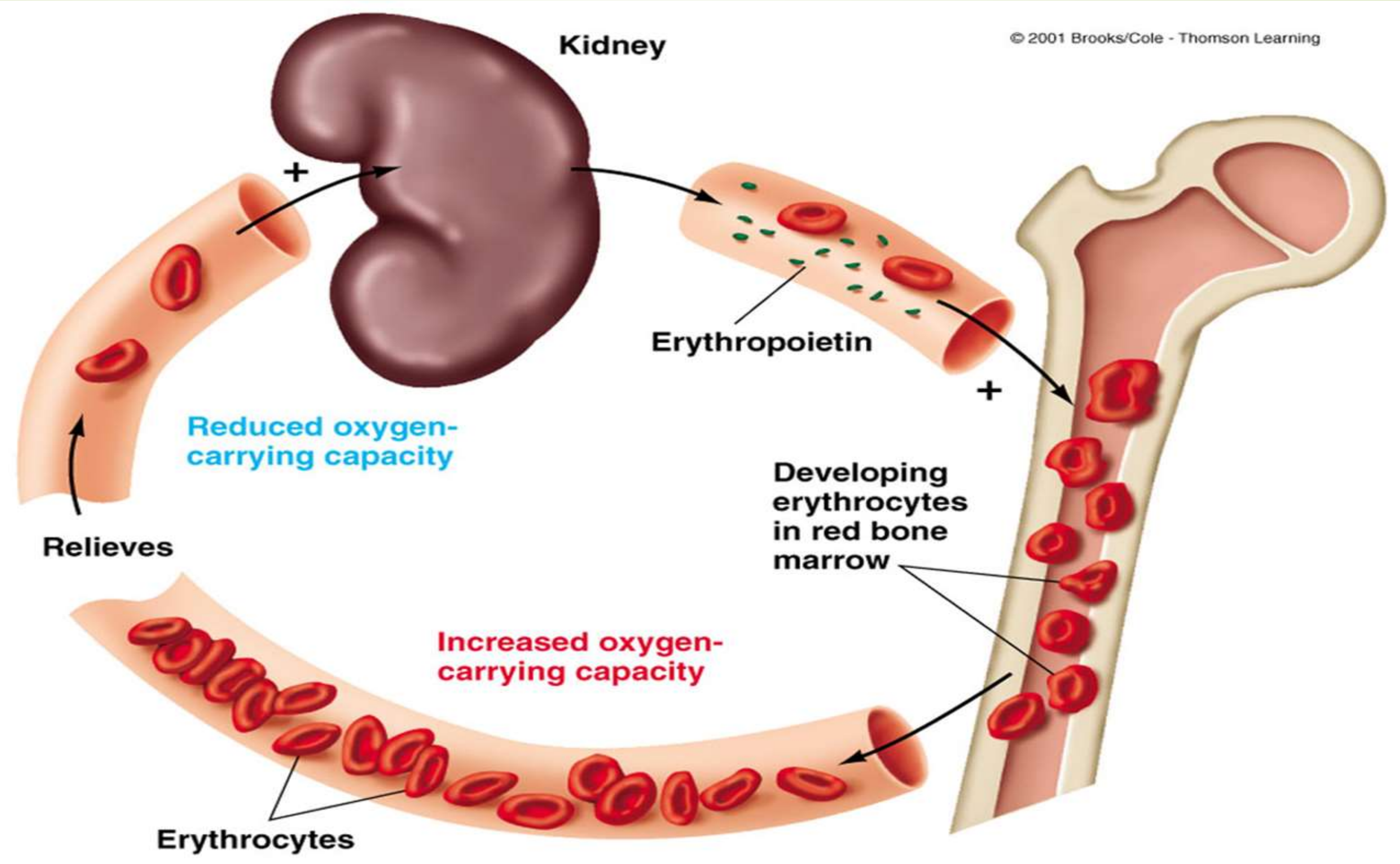



Red blood cell
Oxygen from lungs
Oxygen released to tissue cells
Hemoglobin molecules
Oxygen bonded with hemoglobin molecules

RBC'S (Erythrocytes) count…

- Short Life Span (~120 days)
- Aged RBC , Fragile – prone to rupture

- Erythrocytes are produced in the bone marrow and destroyed in the spleen and liver.
- Iron, folic acid and vitamin B_{12} are essential for erythrocyte formation.
- The erythropoietin which is produced by the kidney in response to low oxygen supply stimulates RBCs production by the bone marrow.


Kidney
© 2001 Brooks/Cole - Thomson Learning
+
Erythropoietin
+
Reduced oxygen-carrying capacity
Relieves
Developing erythrocytes in red bone marrow
Increased oxygen-carrying capacity
Erythrocytes

Formation of New RBC's

- Ruptured cells must be replaced by new cells by a process called......... ..Erythropoiesis.
- Secretion of the hormone erythropoietin
- New RBC's (and platelets & leukocytes) are produced in the bone marrow.

2. White Blood Cells (Leukocytes)

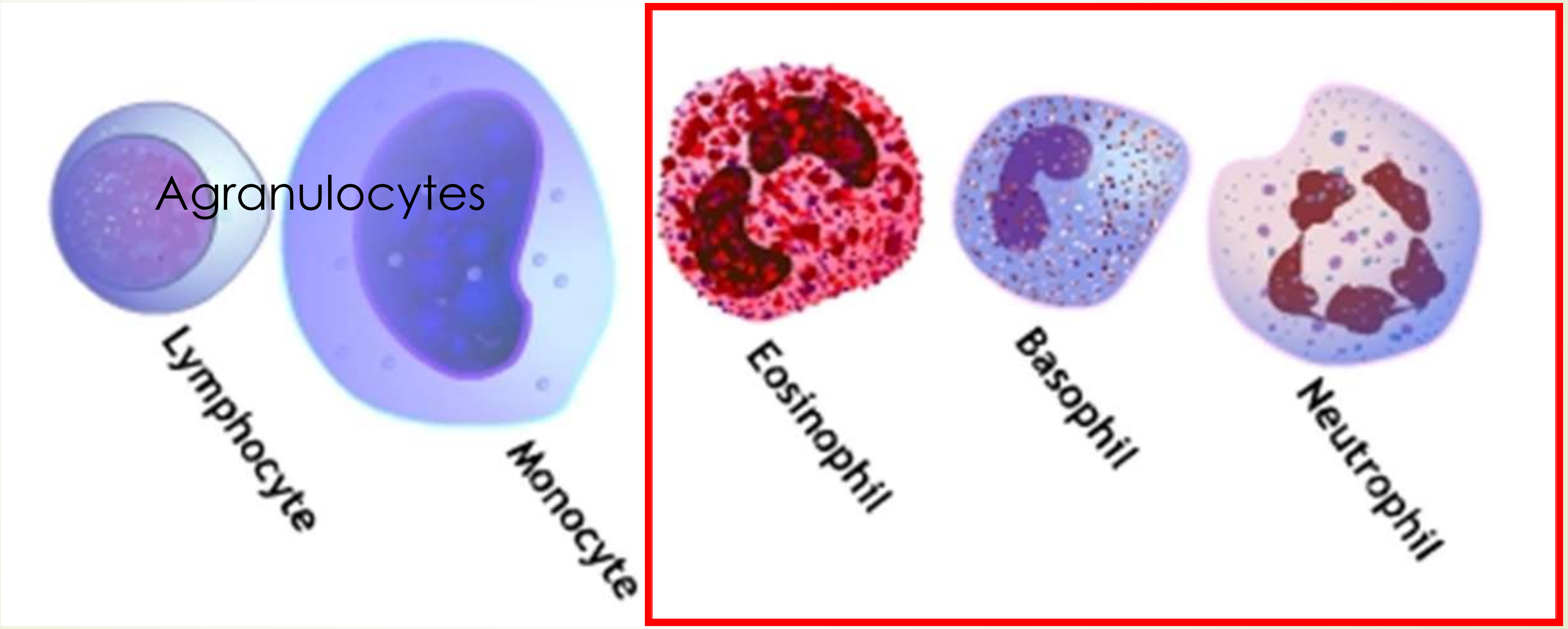
1. Mobile units of body's defense system:

2. "Seek and Destroy" Functions:

- . Destroy invading microorganisms
- Destroy abnormal cells (i.e.: cancer)
- Clean up cellular debris (phagocytosis)

3. Assist in injury repair

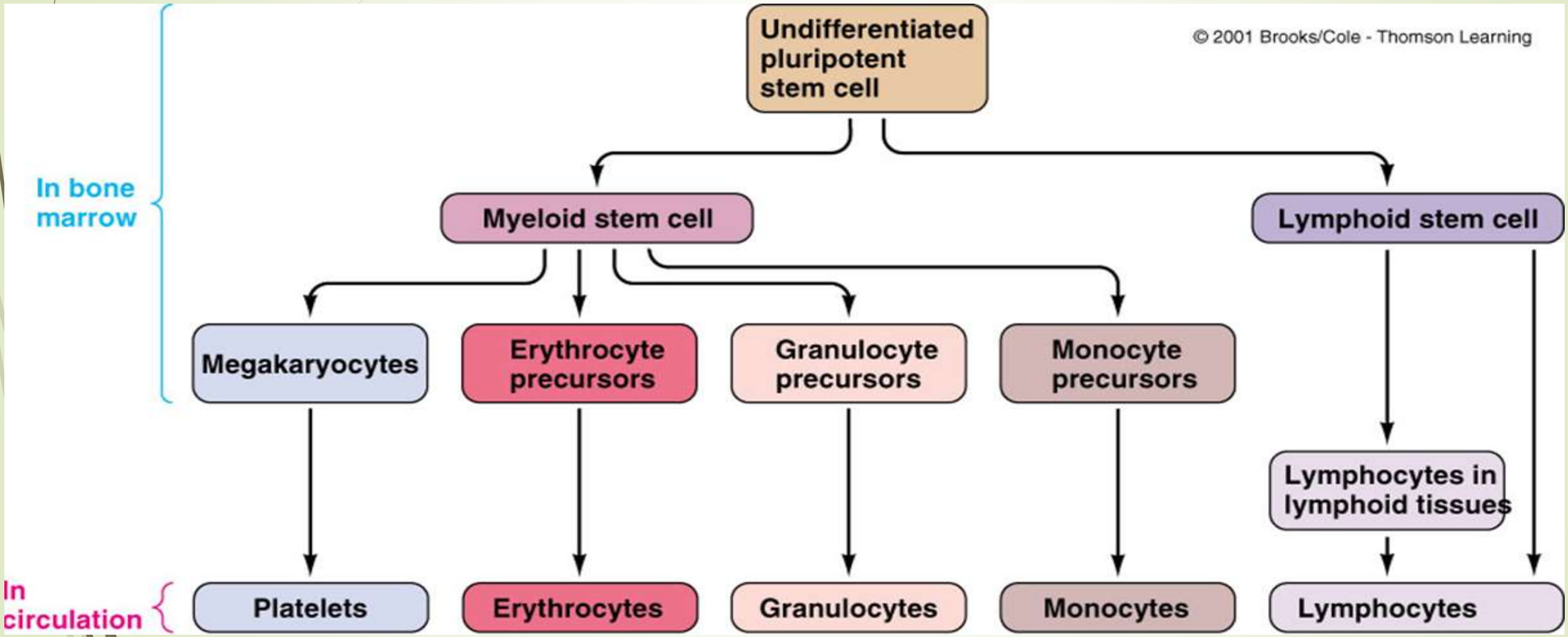
Types of WBC's
Each WBC has a specific function



Types of WBC’s

- **Polymorphonuclear Granulocytes**
- Neutrophils
- Eosinophils
- Basophils

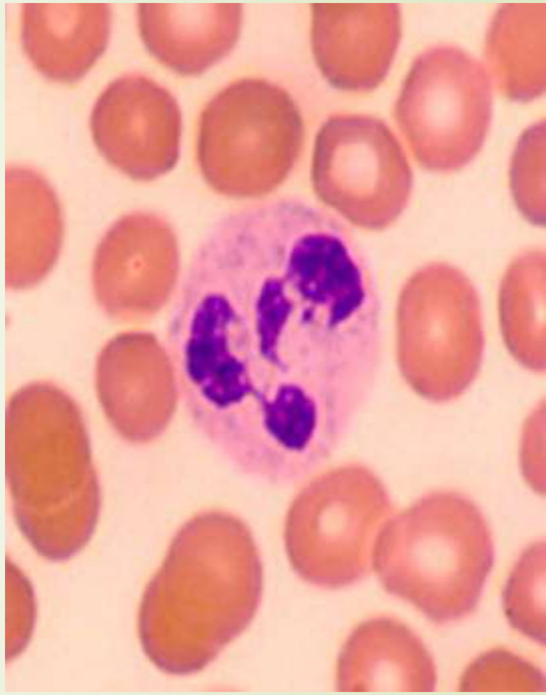
Blood cell origin and Production



- All leukocytes ultimately originate from the same undifferentiated multipotent stem cells in the red bone marrow that also give rise to erythrocytes and platelets
- All new WBCs except for lymphocytes are produced in the bone marrow. Most new lymphocytes are produced by colonies of cells in lymphoid tissues, such as lymph nodes and tonsils.

1. Neutrophils

- 50-70% of all leukocytes (most abundant of WBC's).
- Important in inflammatory responses.
- Phagocytes that engulf bacteria and debris.

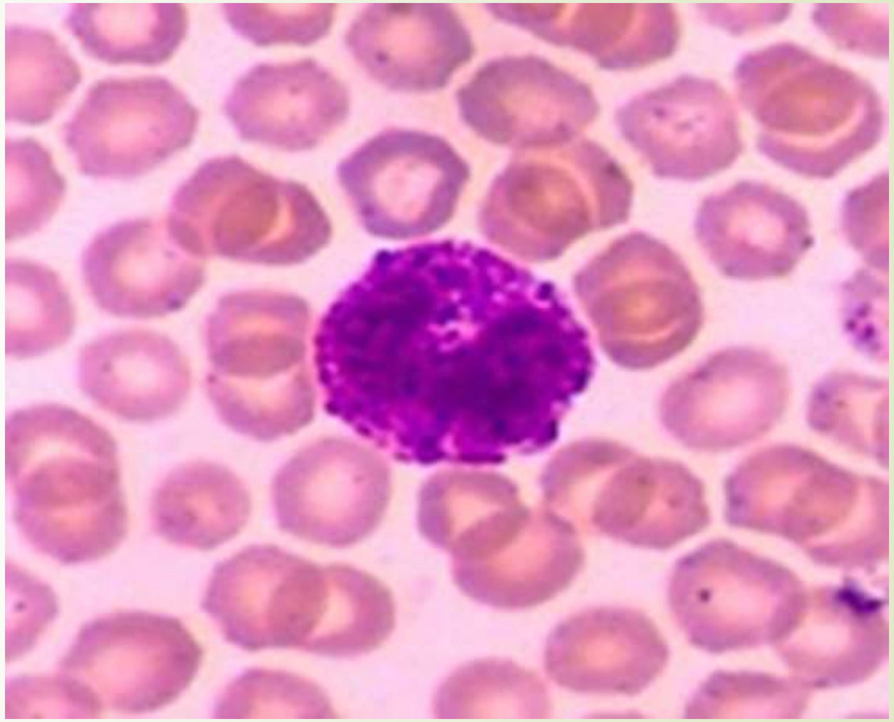


2. Eosinophils.

- 1-4% of the WBC's.
- Attack parasitic worms.
- Important in allergic reactions.

3. Basophils

- 0.5% of the WBC's.
- Release histamine and heparin.
- Important in allergic reactions.

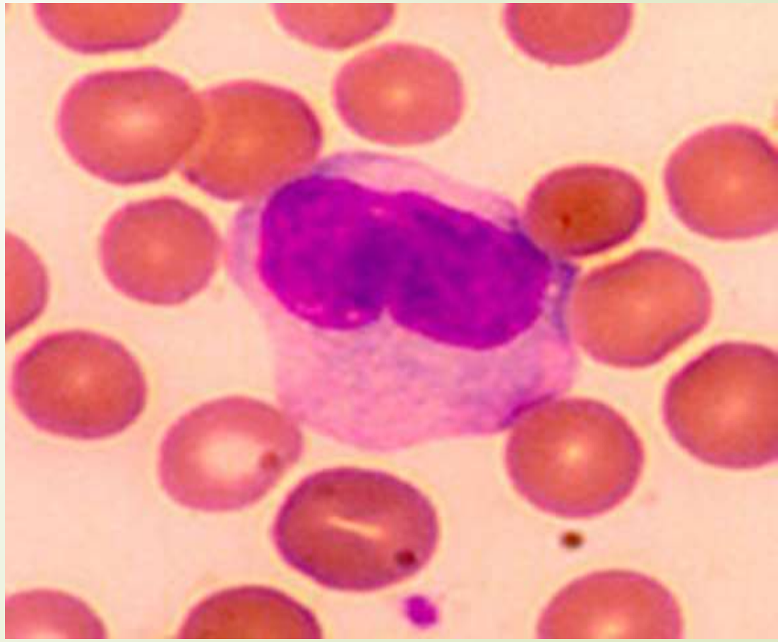


Mononuclear a granulocytes of WBCs

- 4. Monocytes
- 5. Lymphocytes (B and T cells)

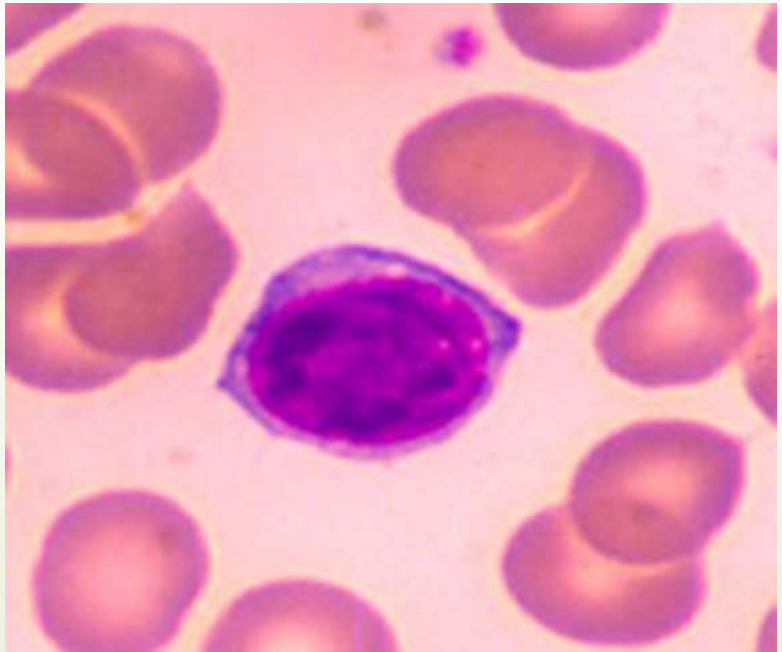
4. Monocytes

- 2-6 % of the WBC's.
- Exit blood to become macrophages inside the tissues.
- Phagocytic = defend against viruses and bacteria.



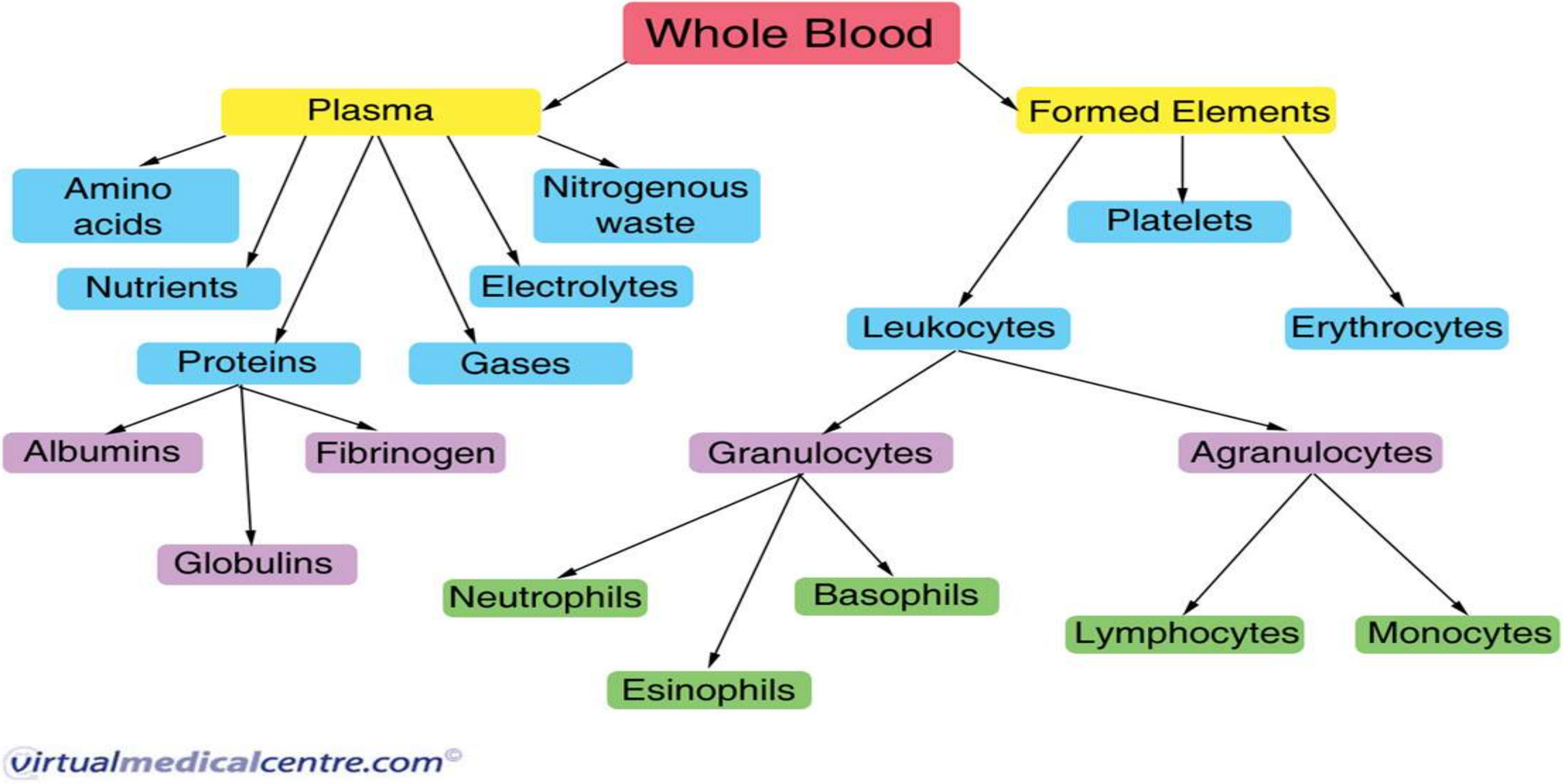
5. Lymphocytes

- 25-33 % of the WBC's
- B-lymphocytes: Produce Antibodies.
- T-lymphocytes: Directly destroy virus- invaded cells and cancer cells.

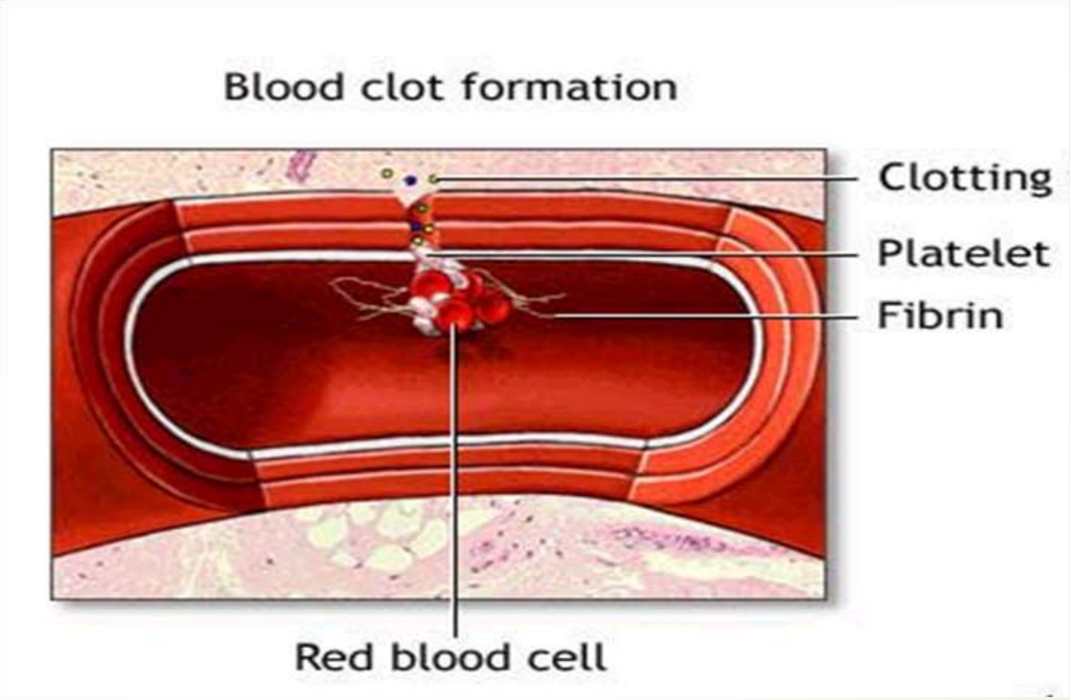


3-Platelets:

- Platelets are cell fragments essential for blood clotting.

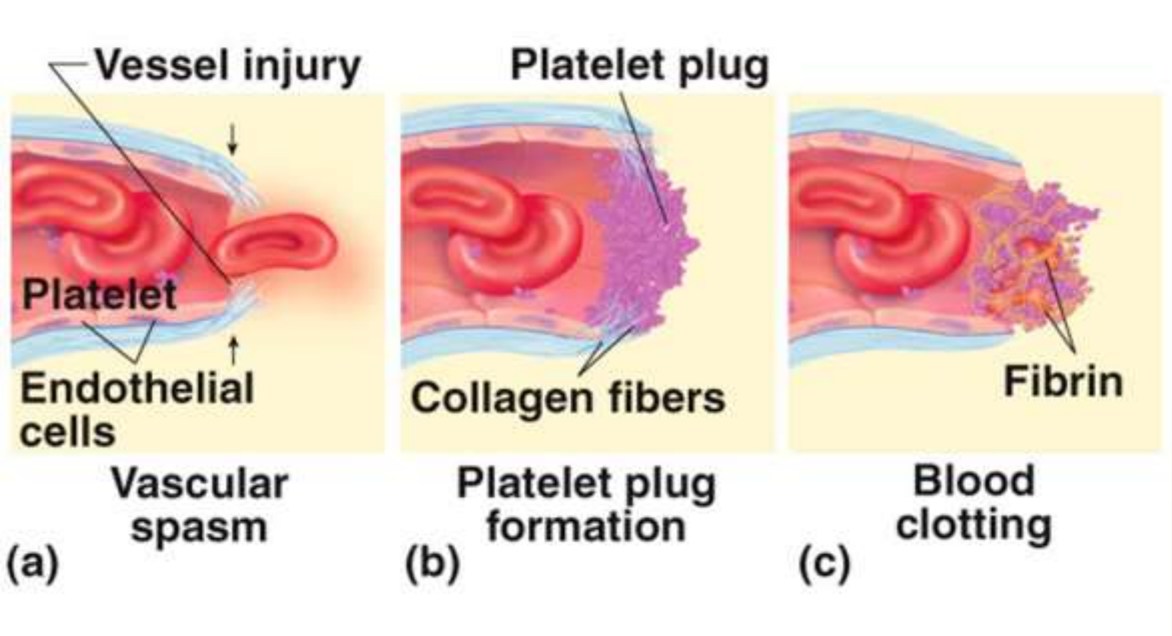

Whole Blood
Plasma
Formed Elements
Amino acids
Nutrients
Proteins
Gases
Electrolytes
Nitrogenous waste
Albumins
Globulins
Fibrinogen
Platelets
Leukocytes
Erythrocytes
Granulocytes
Agranulocytes
Neutrophils
Esinophils
Basophils
Lymphocytes
Monocytes
virtualmedicalcentre.com©

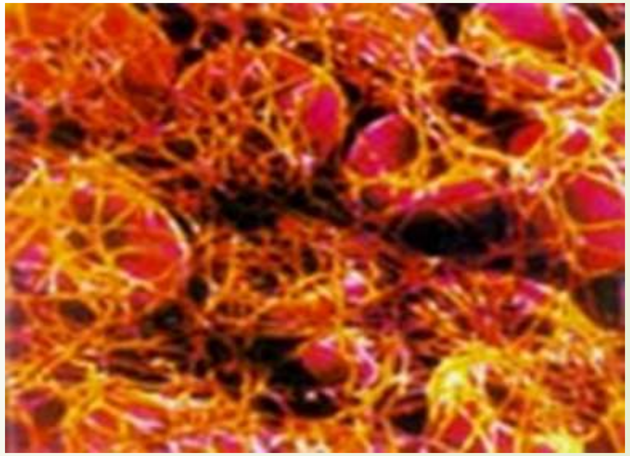

Thank you



Bleeding and clotting time

- **Hemostasis** („hemo"=blood; sta=„remain") (the cessation of blood loss from a damaged vessel).
- Following an injury to blood vessels several actions may help prevent blood loss, including:





Formation of a clot

Definition

- the natural process of stopping blood flow or loss of blood during an injury.

- Hemostasis can be organized into three major separate but interrelated events:

1- vascular spasm or vasoconstriction (Narrowing of blood vessels)

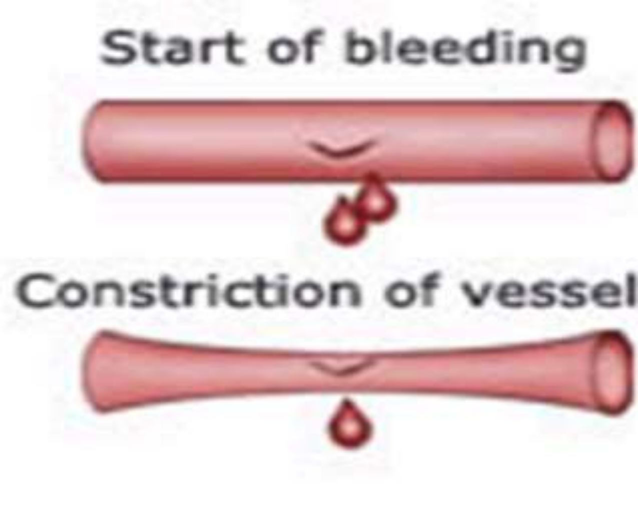
2- primary hemostasis(the formation of a temporary loose platelet plug).

3- secondary hemostasis or coagulation (formation of the more stable fibrin clot retraction and dissolution clot).

Local vasoconstriction

- is the **first step in hemostasis**, and is a brief reflex
- Vasoconstriction is due to local spasm of the smooth muscle (symp. reflex)
- can be maintained by platelet vasoconstrictors
- platelets are responsible for much of the vasoconstriction by releasing thromboxane A2.
- (TXA2), contributes to the vasoconstriction
- A spasm constricts the vessel and reduces blood flow.
- It is a **transient event that usually lasts less than**

1 minute.

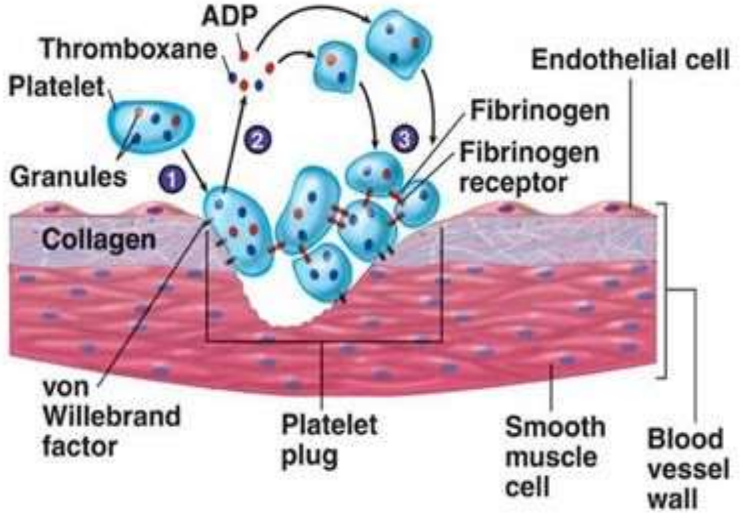


Formation of platelet aggregate

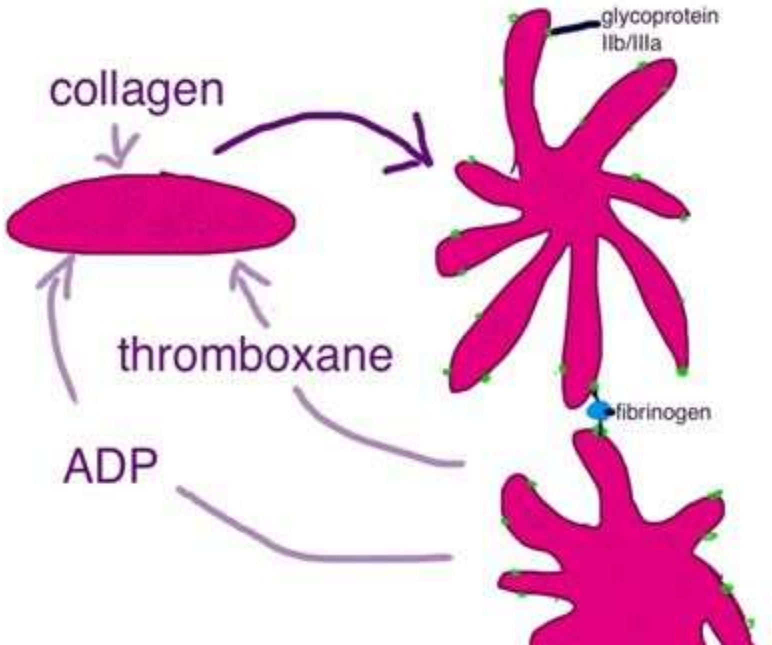
- is the second essential step in hemostasis. It involves the adhesion, activation, and aggregation, of platelets into a plug that serves as a barrier against blood flow.
- Injured blood vessel releases ADP, which attracts platelets (PLT)
- PLT coming in contact with exposed collagen release: serotonin, ADP, TXA2, which accelerate vasoconstriction and causes PLT to swell and become more sticky
- .

Mechanism of the Platelet Plug

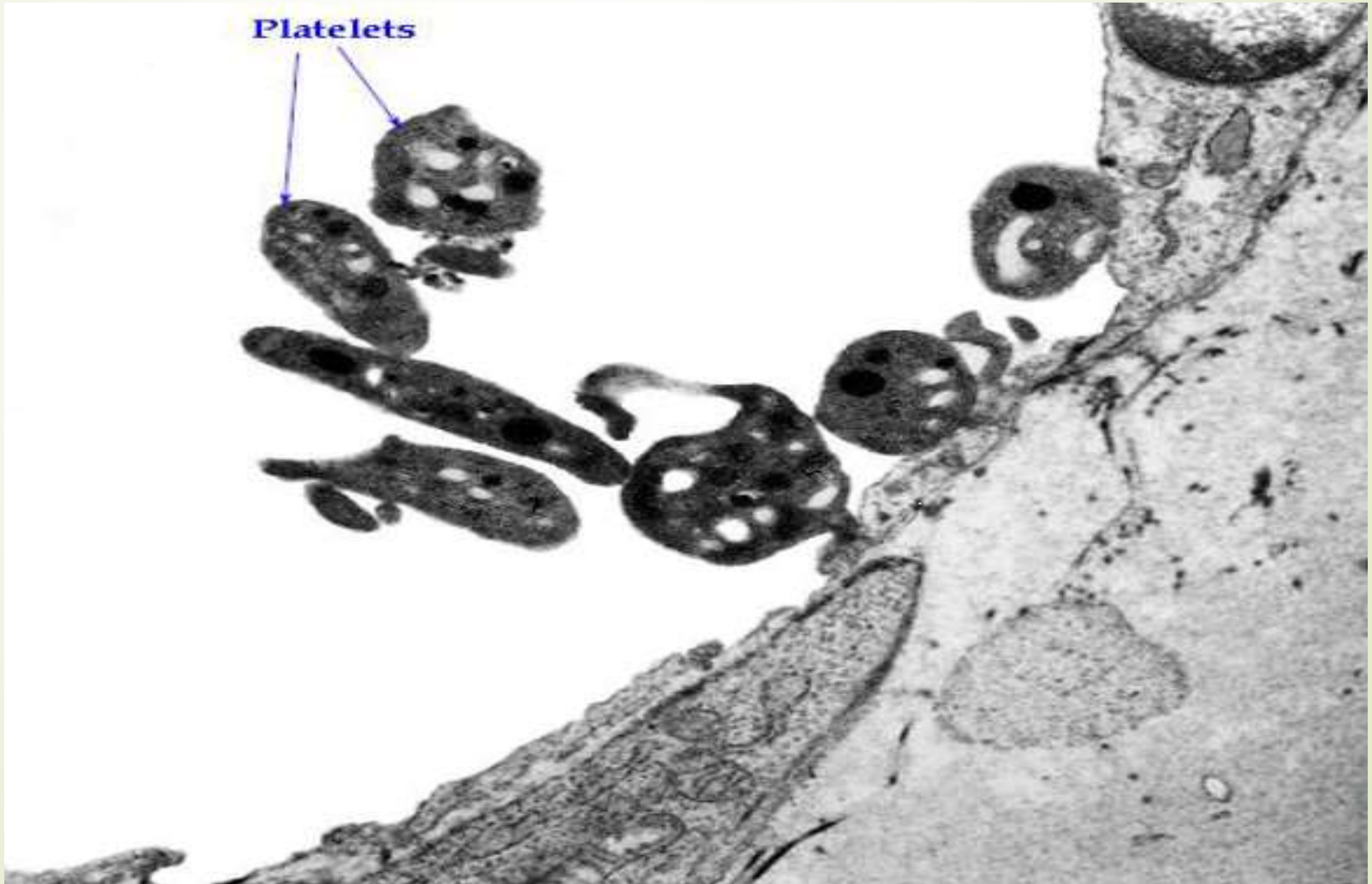
1. The vessel wall is injured, this leads to collagen exposure
2. Platelets contact with injured wall especially with collagen
3. Platelets will change their characteristics dramatically:
   - they swell
   - become irregular in shape
   - produce many protrudes on their surfaces
   - they contract and release their active products
   - they become sticky
4. Platelets adhere to collage and a protein called von willbrand factor(vWF) *(Congenital absence of vWF causes bleeding disorder) vWF is secreted from endothelial cells and platelets*
5. They secrete ADP and thromboxane A2
6. ADP and thromboxane A2 in turn will activate more platelet, increasing the stickiness of the platelets further, so more platelets adhere to the site of injury forming the plate plug



Platelet activation



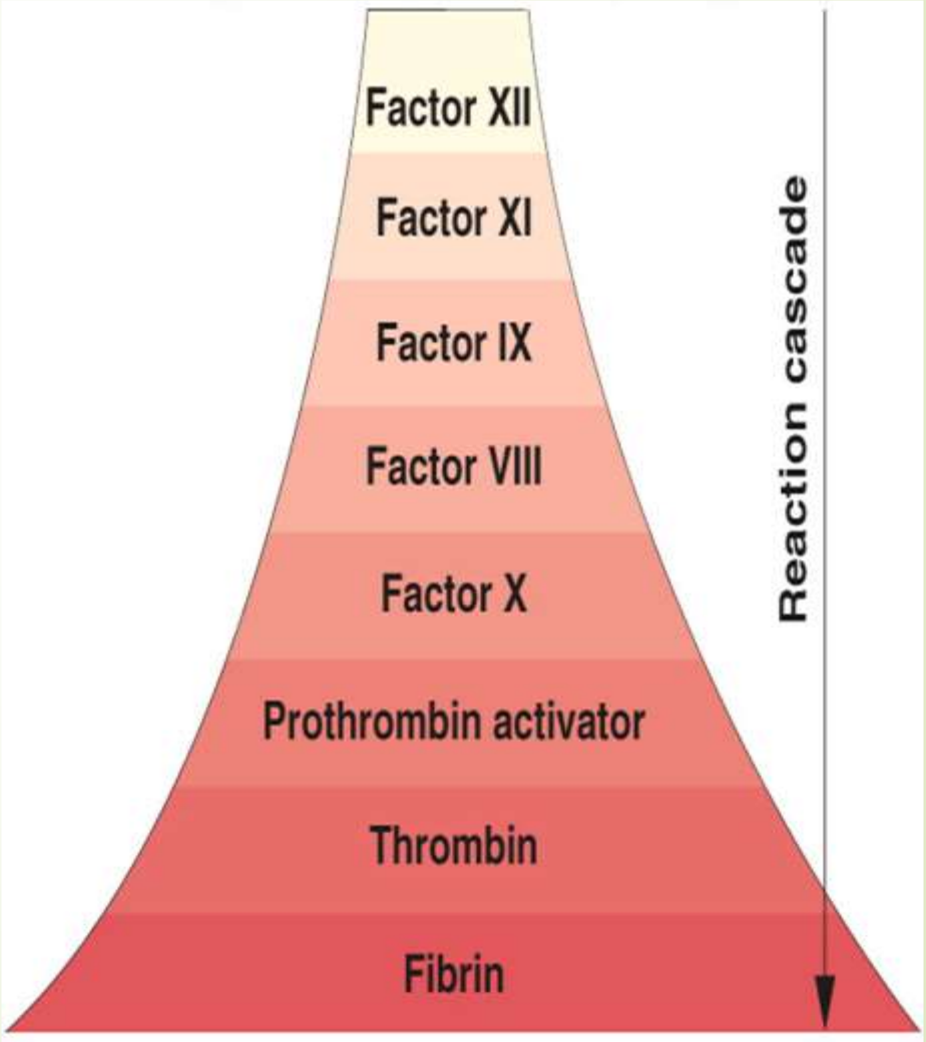
The micrograph shows activated platelets adhering to some damaged cells



Formation of blood clot(coagulation phase)

- In the formation of the clot, an enzyme called thrombin converts fibrinogen(soluble) into insoluble protein, fibrin

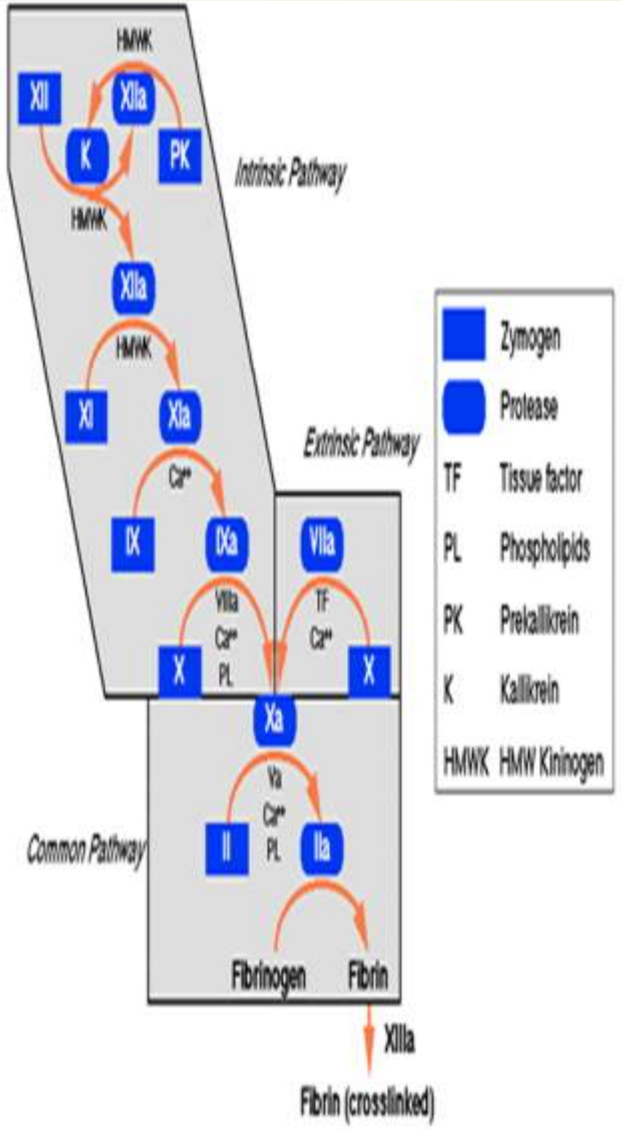
- Fibrin aggregates to form a meshlike network at the site of vascular damage



Detailed Events of Coagulation (Phases of blood clot formation)

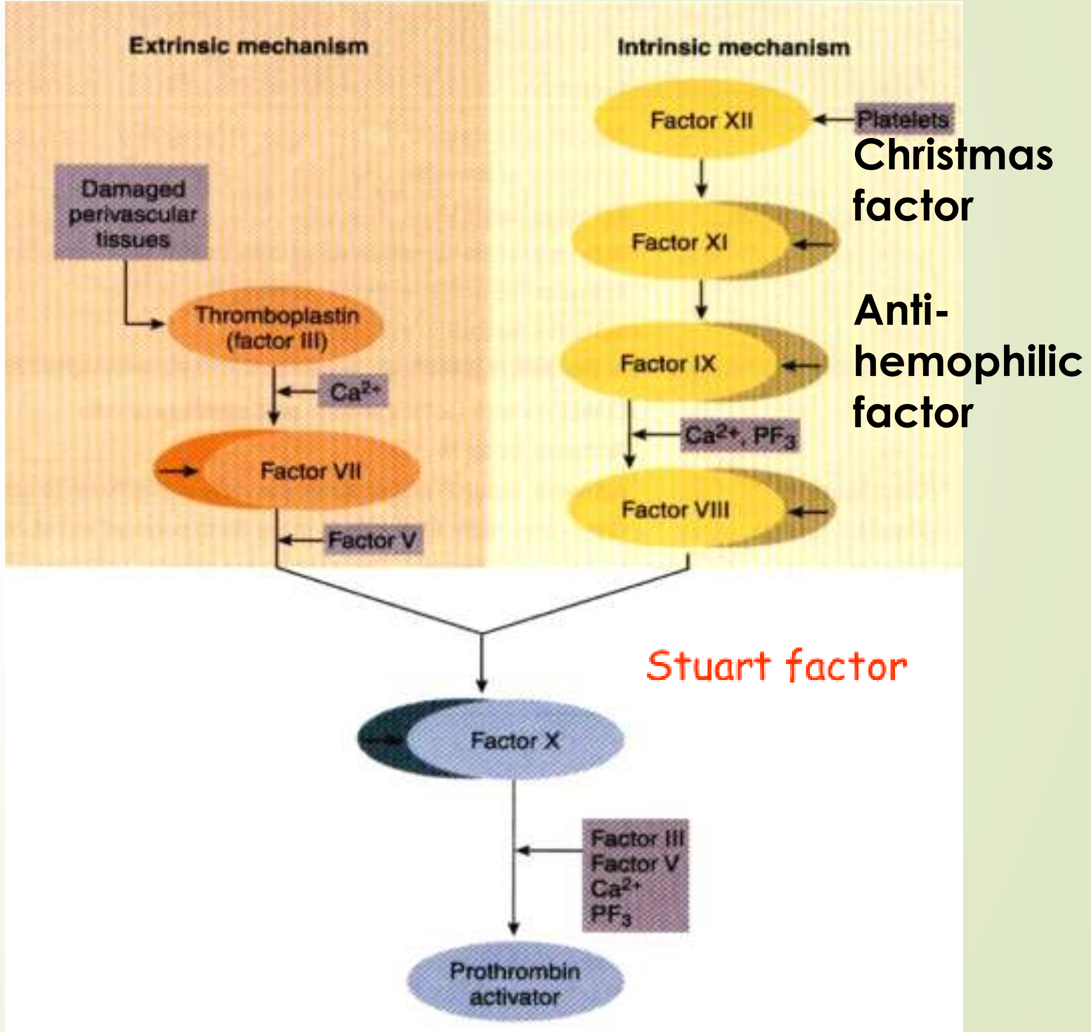
- The complex sequence of events

that produce fibrin are divided into three stages:



Coagulation Phase 1: Two Pathways to Prothrombin Activator

- Initiated by either the intrinsic or extrinsic pathway
- Each pathway cascades toward factor X(Common pathway)
- The intrinsic system is more complex and present only in „higher" life forms (e.g. birds and reptiles possess only extrinsic system).

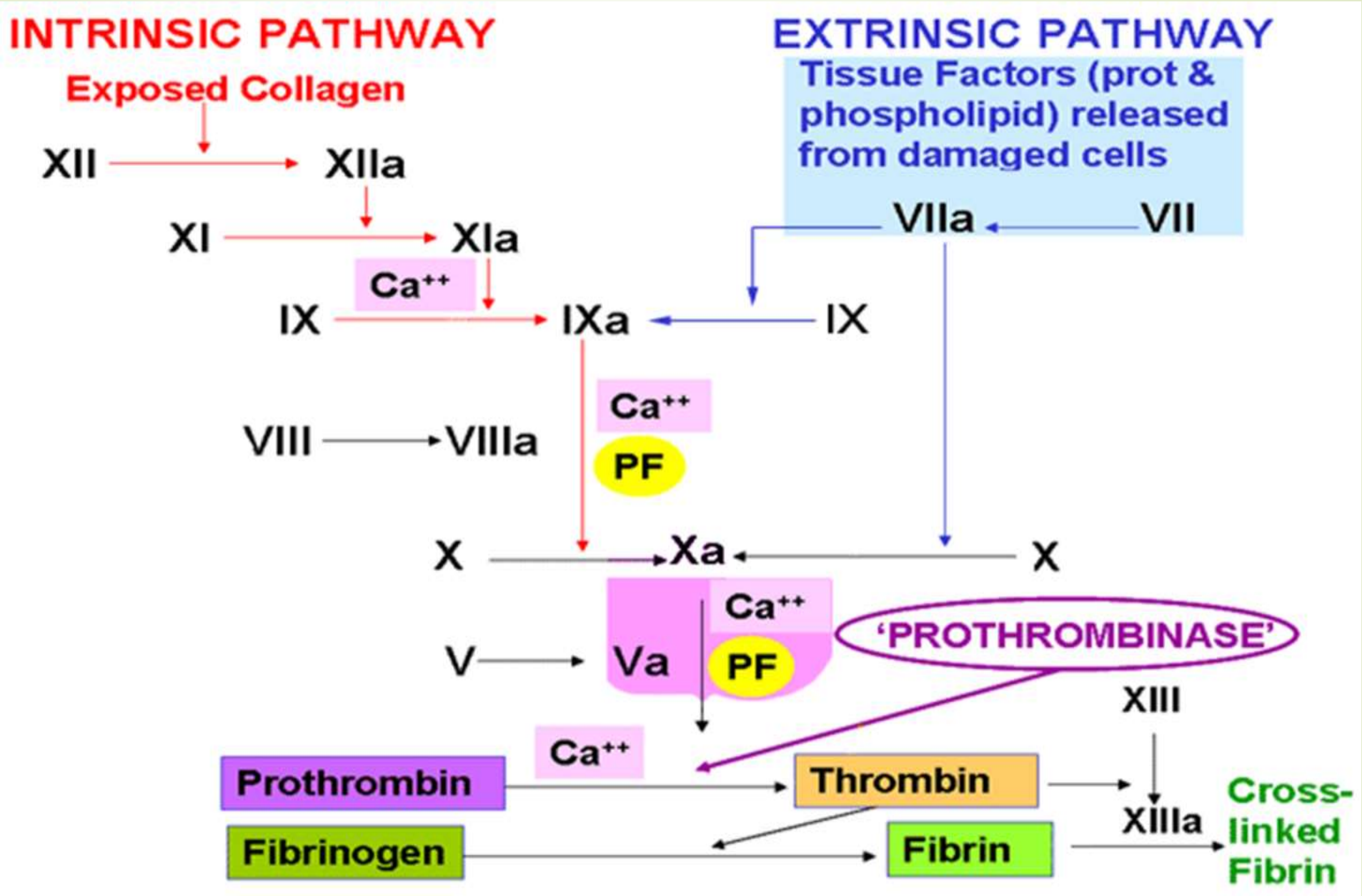


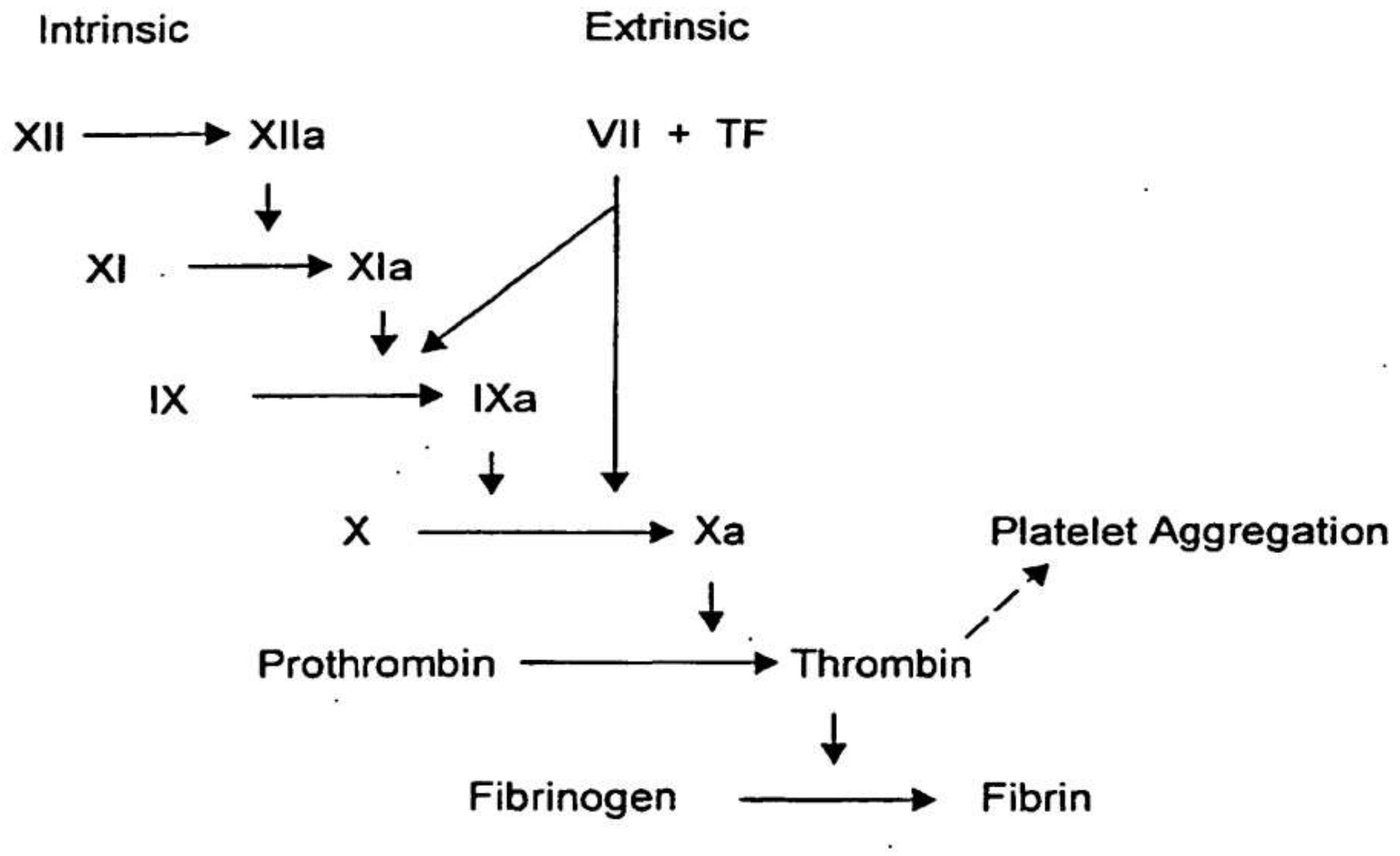
1.Extrinsic pathway for coagulation:

- begins with trauma to vascular wall and surrounding tissues
- 1. When blood comes in contact with injured tissue, tissue cells release – tissue factor(TF) activates F VII to VIIa
- 2. Tissue factor (TF) interacts with (VIIa) and Ca^{2+} to form (TF/VIIa complex)
- 3. TF/VIIa complex will activate Stuart factor (F X)

Intrinsic pathway for coagulation :

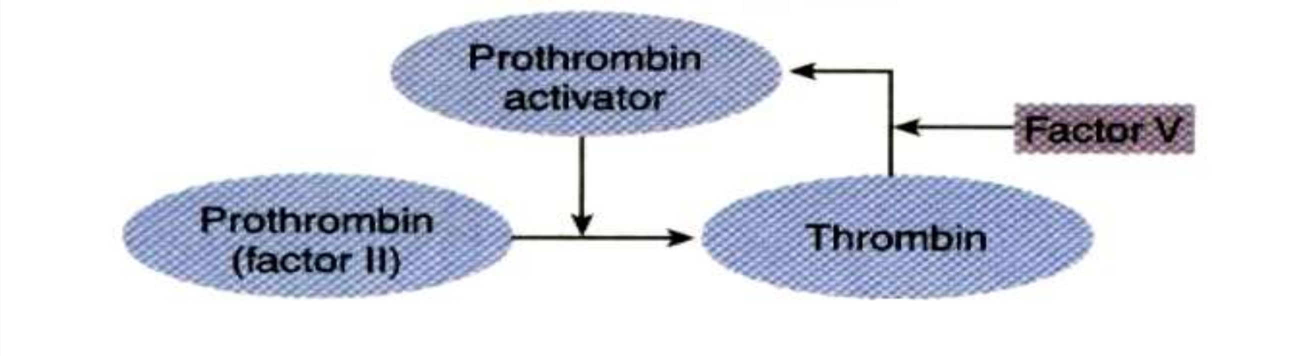
- Exposed collagen activates (F XII)to XIIa ,which ultimately lead to activate Stuart factor (F X) through a complex(called IXa/ VIIIa complex)


INTRINSIC PATHWAY
Exposed Collagen
XII
XIIa
XI
XIa
Ca++
IX
IXa
VIII
VIIIa
Ca++
PF
EXTRINSIC PATHWAY
Tissue Factors (prot & phospholipid) released from damaged cells
VIIa
VII
IX
X
Xa
X
Ca++
V
Va
PF
'PROTHROMBINASE'
XIII
Ca++
Prothrombin
Thrombin
XIIIa
Fibrinogen
Fibrin
Cross-linked Fibrin



Scheme 1: Blood coagulation cascade

Coagulation Phase 2: Pathway to Thrombin

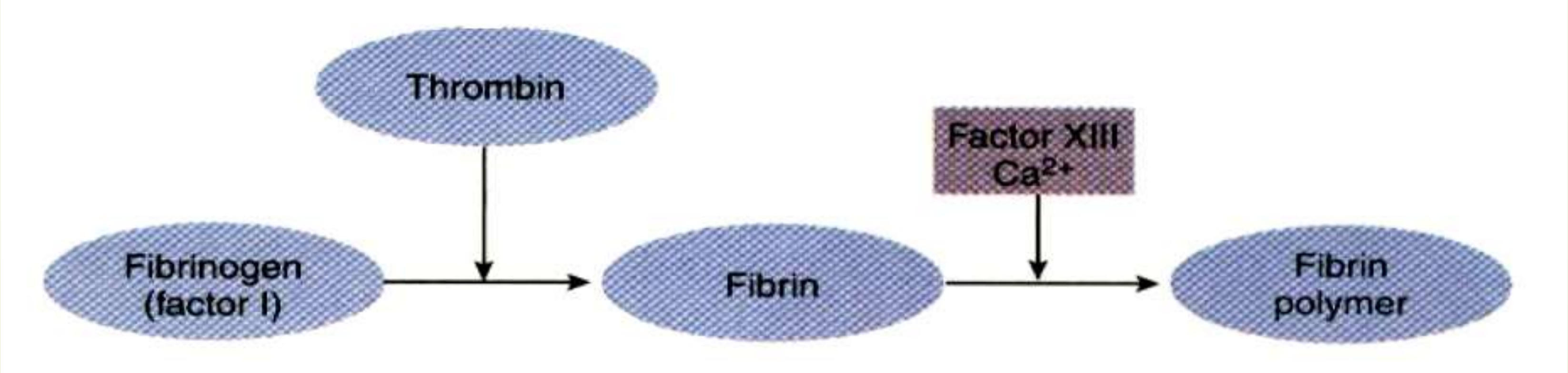


- Prothrombin activator catalyzes the transformation of prothrombin (II) to the active enzyme thrombin in presence of Ca^{+2} and F Va **which form complex with F Xa.**

Prothrombin – inactive precursor of enzyme thrombin
In the presence of prothrombin activator and Ca2+ prothrombin is converted to thrombin
Thrombin itself increases its own rate of formation (positive feedback mechanism)

Phase 3: Common Pathways of conversion of fibrinogen to the Fibrin Mesh.

- Fibrinogen – plasma protein produced by the liver, Thrombin converts fibrinogen to fibrin.
- Thrombin also activates fibrin-stabilizing factor (F XIII), which in the presence of Ca2+, stabilizes the fibrin polymer through covalent bonding of fibrin monomers



Calcium ions Ca^{2+}

- Are required for promotion and acceleration of almost all blood clotting reactions
- Except: activation of XII and XI (intrinsic mechanism)

Clot Dissolution

1. Plasmin is formed from plasminogen - enzyme called activator (e.g. enzymes from urine, tears, saliva or bacterial enzyme streptokinase)

2. Plasmin as an enzyme is involved in breaking down fibrin into soluble fragments (fibrinolysis)

Plasminogen —Activator (e.g. t-PA)→ Plasmin

Fibrin → soluble fragments (Plasmin)

Plasminogen may be produced by eosinophils

Bleeding time

- This is a test that measures the **speed** in which small blood vessels close off (the condition of the blood vessels and platelet function)
- This test is useful for detecting bleeding tendencies.
- Using the ear lobe method, a normal bleeding time is between 1 and 4 minutes.

Purpose

- The test helps identify people who have defects in their platelet function. This is the ability of blood to clot following a wound or trauma. **Normally**, platelets interact with the walls of blood vessels to cause a blood clot. There are many factors in the clotting mechanism, and they are initiated by platelets. The **bleeding time** test is usually used on patients who have a history of prolonged bleeding after cuts, or who have a family history of bleeding disorders. Also, the bleeding time test is sometimes performed **as a preoperative** test to determine a patient's likely bleeding response during and after surgery. However, in patients with no history of bleeding problems, or who are not taking anti-inflammatory drugs, the bleeding time test is not usually necessary.

- **Bleeding time: it is a test for :**

a-capillary response to injury.

b-platelet function:

1-stick to each other and form plug (aggregate).

2-breake and release thromboplastine.

Bleeding time procedure

- Clean the earlobe with an alcohol.
- Prick the earlobe with a lancet
- Note the time when blood first appears on the skin
- After half a minute (30sec) place the edge of the filter paper on the top of the drop of blood.
- Perform the operation at half minute (30 sec) interval
- The end point or bleeding time when no blood is seen on the filter paper.

Duke Method

- With the Duke method, the patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip, after having been swabbed with alcohol.
- The prick is about 3–4 mm deep. The patient then wipes the blood every 30 seconds with a filter paper.
- The test ceases when bleeding ceases.
- The usual time is about 2–5 minutes.

Sources of Error

- If the patient has taken aspirin or aspirin-containing compounds 7 to 10 days prior to the procedure, the bleeding time may be prolonged. ???

- Results may be affected by an improperly performed puncture. A puncture that is too shallow, too deep, or in an inappropriate location will adversely affect test results.
- The alcohol must be completely dried before making the puncture. If residual alcohol is on a puncture site, the bleeding time will be erroneously prolonged.

- If the technician does not initiate timing of the procedure at the same time with the puncture, the results will be adversely affected.
- If the technician allows the filter paper to touch the wound, the platelet clot may be dislodged, causing falsely elevated results.

- If the stopwatch has not been appropriately calibrated, it may keep incorrect time. Stopwatches should be calibrated on a regular basis as a part of the quality assurance program.
- The direction of the incision should be consistent. A horizontal incision gives a longer bleeding time than a vertical incision.

- Bleeding time is affected by ?????????????/

The blood clotting process requires:

- Platelets
- Von well brand factor (helps blood platelets clump together and stick to the blood vessel wall, which is necessary for normal blood clotting).
- Clotting factors synthesized in the liver using vitamin k.

Abnormal Bleeding Time

- **Prolonged bleeding time may indicate:**
- A vascular (blood vessel) defect
- A platelet function defect
- platelets count defect (low platelets)
- Drugs that may increase times include dextran, indomethacin, and salicylates (including aspirin).

Clotting time

- is the time required for a sample of blood to coagulate in vitro under standard conditions .
- There are various methods for determining the clotting time, the most common being the capillary tube method.
- It is affected by calcium ion levels and many diseases.
- Normal value of clotting time is 5 to 8 minutes.

- **Clotting time** : It is the time interval in between onset of bleeding and appearance of jelly like semisolid mass i.e. blood clot.
- **Bleeding time:** is lesser than clotting time, since bleeding is stopped by vascular spasm and platelet plug formation.
- While clotting involves a series of enzymatic reaction taking more time.

Whole blood clotting time procedure:

- Clean the tip of the finger with an alcohol
- Prick the finger tip with a lancet
- Note the time when blood first appears on the skin
- Touch the tube to the drop of blood
- Break gently 1cm of the tube at the end of 2 min, and every 30 sec these after
- When fibrin is formed between the two broken pieces of tube the coagulation or clotting time is noted

Overview

- Blood clotting normally occurs when there is damage to a blood vessel. Platelets immediately begin to adhere to the cut edges of the vessel and release chemicals to attract even more platelets. A platelet plug is formed, and the external bleeding stops.
- Next, small molecules, called clotting factors, cause strands of blood-borne materials, called fibrin, to stick together and seal the inside of the wound. Eventually, the cut blood vessel heals and the blood clot dissolves after a few days.

Thank you