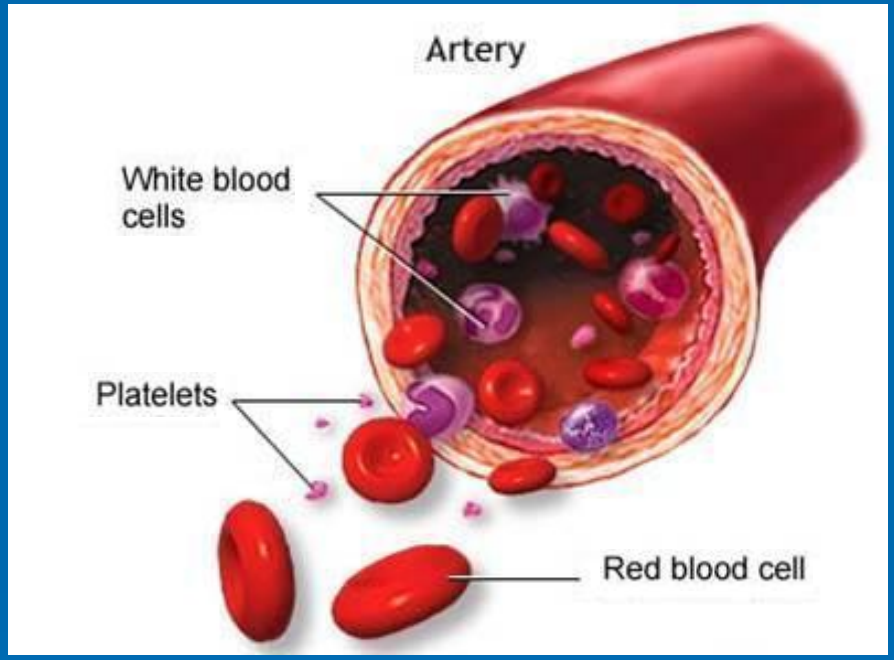
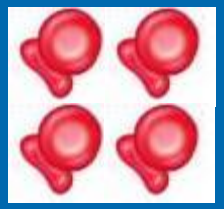
Physiology of erythrocytes Blood groups

Blood Cells

- RBCs, Red blood cells or erythrocytes
- WBCs, white blood cells or Leukocytes
- Platelets (thromobocytes)



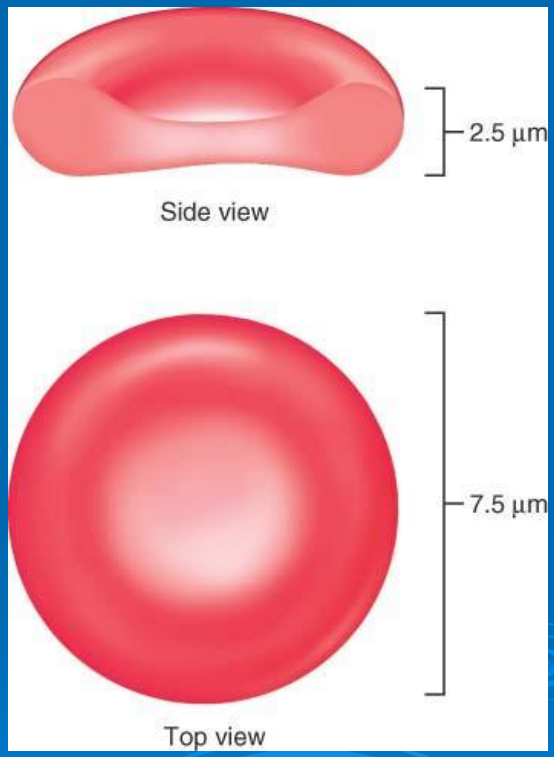
Erythrocytes



- Cell Type
  - **Erythrocytes** (Red blood cells, RBCs)
- Description
  - Bicancavae, anucleate disc, salmon-colored, sacs of hemoglobin,most organelles ejected, diameter 7-8 μm
- Cells/mm^3 (μl) of blood
  - 4-6 millions
- Duration of development (D) & Life Span (LS)
  - D: 5-7 days
  - LS: 100-120 days
- Function
  - Transport oxygen bound to hemoglobin and also small amount of CO_2

Erythrocytes (RBCs)

- Red, oxygen carrying, hemoglobin containing, non-nucleated cells, present in the blood
- Shape → Bi-concave Discs
- Size:
  - Diam → 7.5 - 7.8 µm
  - Thickness:
    - Thickest → 2.5 µm
    - Thinnest → 1 µm or <1 µm
  - Thin centers appear lighter in colour than edges
- Volume: 90-95 μm^3
- Life Span:
  - Adults: 100-120 Days
  - Neonates: 70-90 Days
- Count:
  - Males: 5.2 million $\pm$ 3,00,000 cells/mm^3
  - Females: 4.7 million $\pm$ 3,00,000 cells/mm^3
  - Newborn: 6 – 6.5 million cells/mm^3
  - Fetus: 7.8 million cells/mm^3
    - Why count is different? [1]



Leuckocytes

- Cell Type
  - **Leukocytes** *(lecuko- white)* (White blood cells, WBCs)
- Description
  - Spherical, nucleated cells
- Cells/mm^3 (µl) of blood
  - 4800-10,800
- Types
  - Granulocytes
    - Neutrophils
    - Eosinophils
    - Basophils
  - Agranulocytes
    - Lymohocytes
    - Monocytes

Platelets (Thrombocytes)

- Not cells
- Cytoplasmic fragments of extraordinary large cells (60μm) → *Megakaryocytes*
- Cytoplasm stain *blue*, granules Stain *Purple*
- Essential for the clotting process when blood vessels are ruptured or their lining is injured.
- ***Components of Granules***
  - *Serotonin*
  - *Ca 2+*
  - *Different Enzymes*
  - *ADP*
  - *Platelets derived Growth Factors (PDGF)*
- When not involved in clotting mechanism, they are kept inactive by molecules (NO, PG I_2) secreted by endothelial cells lining blood vessels.

Hematopoiesis

- Hematopoiesis or hemopoiesis (Hemato, hemo = blood, Poiesis = to make)
- Process occurs in Red bone marrow
- Red bone marrow composition
  - It is composed of a soft network of reticular connective tissue bordering on wide blood capillaries called *blood sinusoids*. With in this network are immature red blood cells, fat cells, reticular cells (secrete the fibers).
  - On average, the marrow produces 1 ounce of new blood every day
  - Cells produced are about 100 billion
- All cells arise from the same type of stem cells the *PHSC* or *hemocytobalsts (Cyte = cell , blast = bud)* that reside in red bone marrow.
- But the maturation pathway is different form each other, once a cell is *committed* to a specific blood cell pathway, it can not change
- This commitment is signaled by *appearance of membrane surface receptors* that respond to specific *hormones or growth factors*, which in turn push the cell towards further specialization.

Erythrocytes (RBCs)

Composition of RBCs:

The composition of RBCs is same as that of a normal cell except that mature RBCs contain *Hb* and don't contain *nucleus, mitochondria, and other important organelles*.

- Water = 65 %
- Solid and semisolids = 35 %
  - Hb (33 %)
  - Organic and inorganic substances (2%)
    (Amino Acids, Cholesterol, Creatinine, Proteins, Phospholipids, Urea)

How RBCs Change and Maintain Shape:

- Main protein – Hb - 97 %
- Other Proteins
  - Anti-Oxidant Enzymes (Get rid body of harmful O_2 radicals)
  - Maintenance proteins

Bi-concave shape of RBCs is maintained by network of proteins, especially one called **spectrin**, it is attached to the cytoplasmic side of the plasma membrane, as **spectrin** net is deformable, it gives erythrocytes the flexibility to change their shape as necessary- to twist, turn and become cup shaped when pass through small capillaries – and then resume their normal shape.

Erythrocytes (RBCs)

- Energy Production:
- For energy RBCs depend on plasma glucose, metabolic break down takes place through
  - Embden Meyerhof Glcolytic pathway
  - Pentose phosphate Pathway (PPP) or (Hexose Monophosphate shunt)
- Structural Characterstics VS Function
  - Small size and Biconcave shape provides huge surface area (about 30 % more area than comparable spherical cells).
  - Excluding water content RBC is 97 % Hb that transports resp. gases.
  - Don't use oxygen themselves as produce energy by anaerobic mechanisms.
- Functions or RBCs:
  - O_2 Transport:
    - Contains Hb, that carries oxygen bound to 'Heme' portion
  - CO_2 Transport:
    - CO_2 Transport takes place in combination with 'globin' protion. (20%)
  - Acid-Base balance
    - By buffering action of Hb
  - Blood Viscosity
  - Ionic balance

Factor needed of Erythropoiesis

1. Erythropoietin (Released in response to Hypoxia)
2. Vitamin B 6 (Pyridoxine)
3. Vitamin B 9 (Folic Acid)
4. Vitamin B 12 (Cobolamin)
    - Essential for DNA synthesis and RBC maturation
5. Vitamin C → Helps in iron absorption (Fe+++ → Fe++)
6. Proteins → Amino Acids for globin synthesis
7. Iron & copper → Heme synthesis
8. Intrinsic factor → Absorption of Vit B 12
9. Hormones

Physiological Variations in RBC count

1. Diurnal Variation (During 24 hours)
    - 5 %
    - Lowest - Sleep and early morning hours
    - Highest - Evening
2. Temperature
3. High Altitude
4. Hypoxia
5. Radiations

    X-rays

Fate and destruction of RBCs [1]

- Anucleate → certain limitations.
  - No synthesis of new proteins, No growth, No division.
- However they do have Cytoplasmic enzymes (hexokinase, Glu-6-phosphate dehydrogenase) that are capable of metabolizing glucose and forming small amounts of ATP. These enzymes also perform following actions
  - maintain pliability of the cell membrane,
  - maintain membrane transport of ions,
  - keep the iron of the cells' hemoglobin in the ferrous form rather than ferric
  - Prevent oxidation of the proteins in the red cells.
- Erythrocytes become "old" as they lose their flexibility and become *pikilocytes (spherical),* increasingly rigid and fragile. Once the cell become fragile, they easily destruct during passage through tight circulation spots, especially in spleen, where the intra-capillary space is about 3 micron as compared to 8 micron of cell size

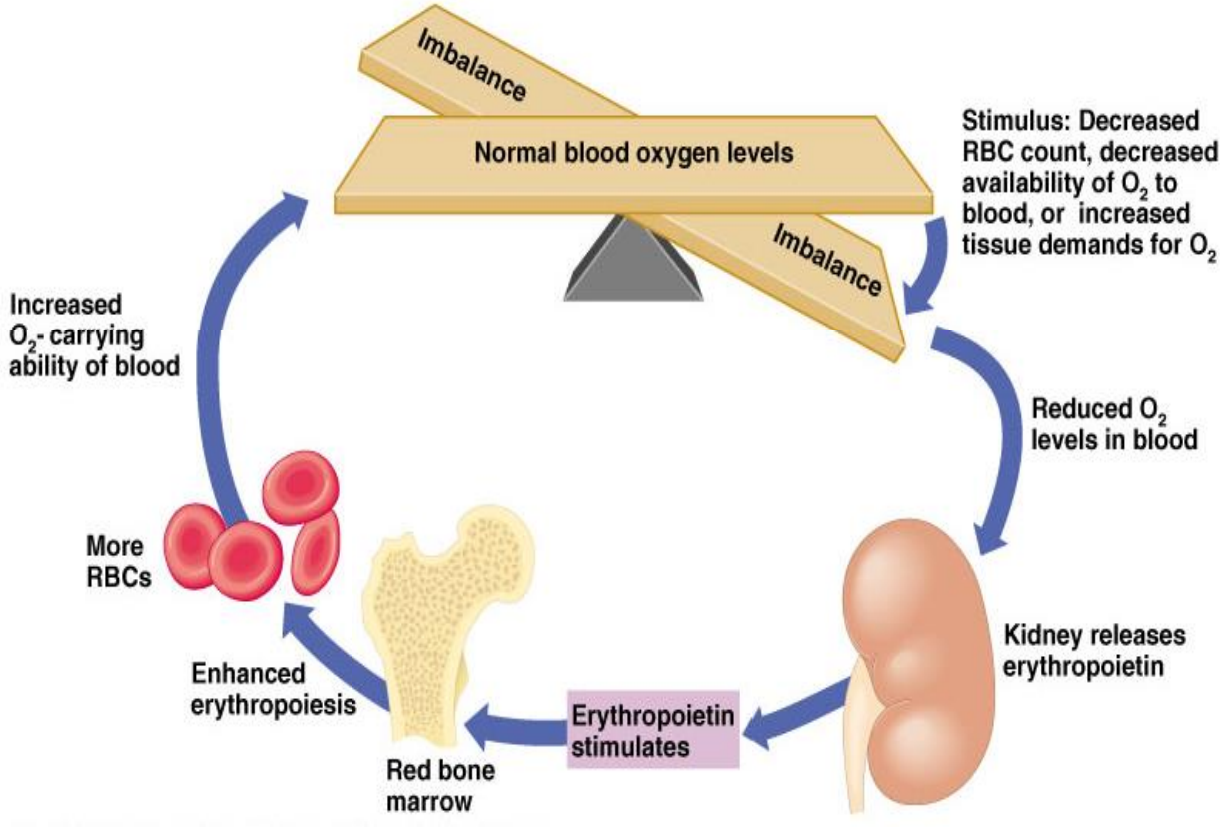
  RBCs useful life span is 100 to 120 days,After which they become trapped and fragment in smaller circulatory channels, particularly in those of the spleen. For this reason, the spleen is sometimes called the "red blood cell graveyard."
- Dying erythrocytes are engulfed and destroyed by macrophages.

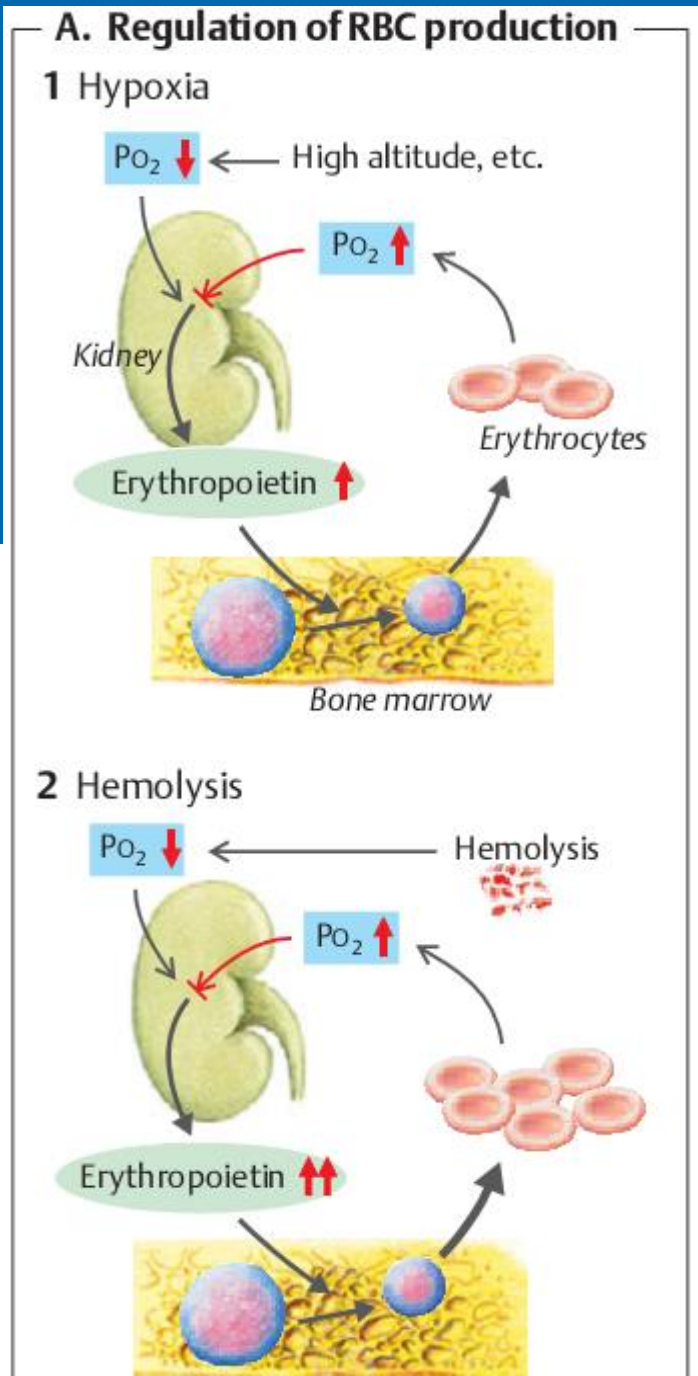
Regulation of RBCs production

- Control of rate of erythropoiesis is based on ability of RBCs to transport sufficient oxygen to tissues as per demand, not the number
- Tissue Oxygenation
  - Drop in normal blood oxygen levels may result due to
    - Reduced number of RBCs
      - Hemorrhage
      - Excess RBC Destruction
    - Reduced Availability of Oxygen
      - High Altitude
      - Lung Diseases
    - Increase Tissue demands of Oxygen
      - Aerobic Exercises
- Erythropoietin (Formation & role)[1]
  - Glycoprotein, Mol wt= 34,000.
  - Erythropoietin, a hormone, produced mainly by the kidneys(90%) and also by liver(10%), stimulates erythropoiesis by acting on committed stem cells to induce proliferation and differentiation of erythrocytes in bone marrow.
  - Site of Action: BONE Marrow

Regulation of RBC production

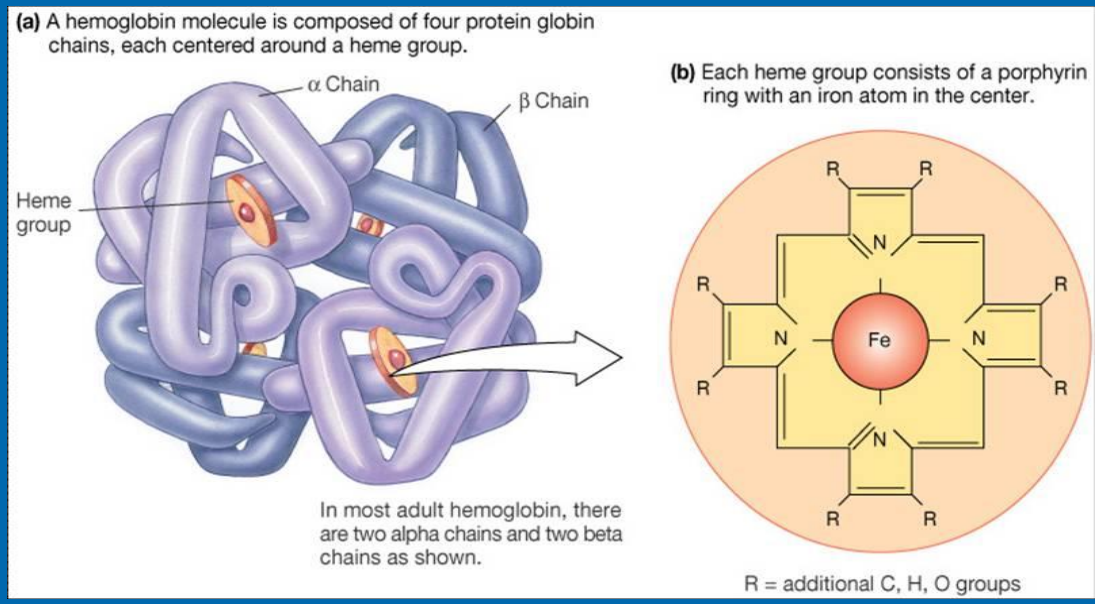
A negative Feed back mechanism



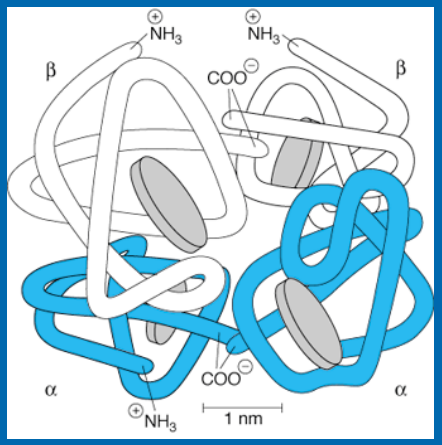


Hemoglobin (Hb)

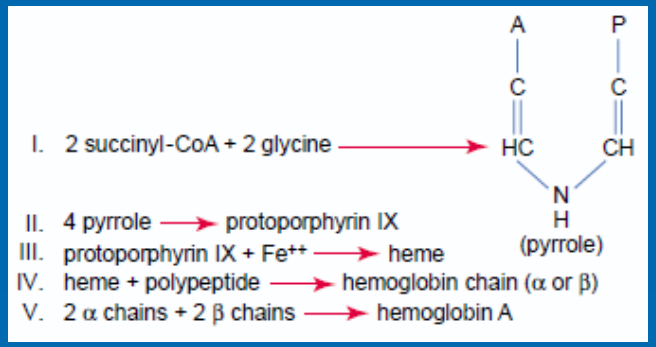
- Red, oxygen carrying pigment present in RBCs.
  - Heme (4%)
  - Globin (96%)
- Quantity
  - 700-900g in body
  - 29-32 peco gram/RBC
- RBCs
  - Male= 36g/100ml
  - Female = 34g/100ml
- Whole Blood
  - Newborn = 14-20g/100ml
  - Male= 14-16g/100ml
  - Female = 12-14g/100ml
- Molecular Weight
  - 64,450
- Types
  - 4 types of poly peptide chains based on amino acid composition and sequence.
  - alpha, beta, gamma, delta
  - Adult Hb
    - Hb A = 2 alpha (141 AA)+ 2 beta (146 AA) chains ($\alpha_2\beta_2$)
    - Hb A_2 = 2 alpha (141 AA)+ 2 delta (146 AA) chains (2.5%) [1] ($\alpha_2\delta_2$) (10 AA differ)
  - Fetal Hb
    - Hb F = 2 alpha (141 AA)+ 2 gamma (146 AA) chains [2] ($\alpha_2\gamma_2$) (37 AA differ)
    - 99% replaced with adult Hb with in a year of birth.



Hemoglobin (Hb)



- 250 million Hb molecules / RBC
- So carry 1 billion oxygen molecules / RBC
- Synthesis of Hb
  - Starts at proerythroblastic stage
- Synthesis steps:
  - Heme is made from *acetic acid* and glycine in mitochondria
  - Acetic Acid → α-ketoglutaric Acid → Succinyl Co A (Krebs Cycle)
  - Globin (polypeptide chain) is synthesized by Ribosomes



- Reactions of Hb:
  - Oxyhemoglobin (oxygen + Hb) Ruby Red (in lungs) (Co-ordination bonds)
  - Deoxyhemoglobin (Reduced Hb) Dark Red (in tissues)
  - Carbaminohemoglobin (Co_2 + Hb) (Globin's amino acids) (20 %)
  - Caroboxyhemoglobin (Co + Hb)
  - Methemoglobin (Fe+++ instead of Fe++)

Reactions of Hb:

- Hemoglobin binds O_2 to form oxyhemoglobin, O_2 attaching to the Fe^{2+} in the heme. The affinity of hemoglobin for O_2 is affected by
  - pH,
  - Temperature,
  - The concentration of 2,3-diphosphoglycerate (2,3-DPG) in the red cells.
- 2,3-DPG and H+ compete with O_2 for binding to deoxygenated hemoglobin, decreasing the affinity of hemoglobin for O_2 by shifting the positions of the four peptide chains (quaternary structure).
- Each of the four iron atoms can bind reversibly to one O_2 molecule. The iron stays in the ferrous state, so that the reaction is an oxygenation, not an oxidation. It has been customary to write the reaction of hemoglobin with O_2 as

$$Hb + O_2 \leftrightarrow HbO_2$$

- Since it contains four Hb units, the hemoglobin molecule can also be represented as Hb_4, and it actually reacts with four molecules of O_2 to form Hb_4O_8 as following.

$$Hb_4 + O_2 \rightleftharpoons Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftharpoons Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftharpoons Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftharpoons Hb_4O_8$$

- The reaction is rapid, requiring less than 0.01 s.
- The deoxygenation (reduction) of Hb_4O_8 is also very rapid.

Hb Abnormalities

- Globin Genes[1] determine the AA sequence in Hb.
- Two types of Abnormalities:
  - Hemoglobinopathy
    - Abnormal polypeptide chains are produced
      - Sickle cell disease due to Hb-S
  - Thalassemia
    - In which the chains are normal in structure but produced in decreased amounts or absent because of defects in the regulatory portion of the globin genes.
      - The α and β thalassemias are defined by decreased or absent α and β polypeptides, respectively.
- 1000 Abnormal Hbs due to mutant genes in humans. usually identified by letter—Hb-C, E, I, J, S, etc.
- Mostly, the abnormal Hbs differ from normal Hb-A in the structure of the polypeptide chains.
- For example, In hemoglobin S,
  - α chains normal
  - β chains abnormal, among the 146 AA residues in each β polypeptide chain, one *glutamic acid* residue has been replaced by a *valine* residue.

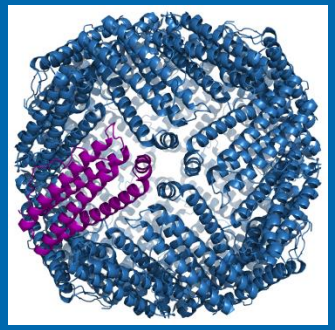
Hb Abnormalities

- Heterozygous →Half the circulating hemoglobin is abnormal and half is normal.
  - Have sickle cell trait
- Homozygous → all of the hemoglobin is abnormal.
  - Develop the full blown disease
- Results of abnormality
  - Many of the abnormal hemoglobins are harmless.
  - Abnormal O2 equilibriums.
  - Anemia.
    - Hb-S polymerizes at low O_2 tensions, and this causes the red cells to become sickle-shaped, hemolyze, and form aggregates that block blood vessels.
    - The result is the severe hemolytic anemia known as sickle cell anemia.
- The sickle cell gene is an example of a gene that has persisted and spread in the population.
- It originated in the black population in Africa, and it confers resistance to one type of malaria.
- Africa = 40% of the black population have the sickle cell trait.
- In United States 10 %
- Treatment:
  - Bone marrow Transplatation
  - Hb-F production by hydroxyurea.

Hemoglobin Metabolism

- The heme of the hemoglobin is split off from globin.
  - Its core of iron is saved, bound to protein (as ferritin or hemosiderin), and stored for reuse.
  - The heme is converted to biliverdin. In humans, most of the biliverdin is converted to bilirubin, a yellow pigment that is released to the blood and binds to albumin for transport.
  - Bilirubin is picked up by liver cells, which in turn secrete it (in bile) into the intestine, where it is metabolized to urobilinogen.
  - Most of this degraded pigment leaves the body in feces, as a brown pigment called stercobilin.
  - Exposure of the skin to white light converts bilirubin to lumirubin, which has a shorter half-life than bilirubin.
  - Phototherapy (exposure to light) is of value in treating infants with jaundice due to hemolysis.
- The protein (globin) part of hemoglobin is metabolized or broken down to amino acids, which are released to the circulation.

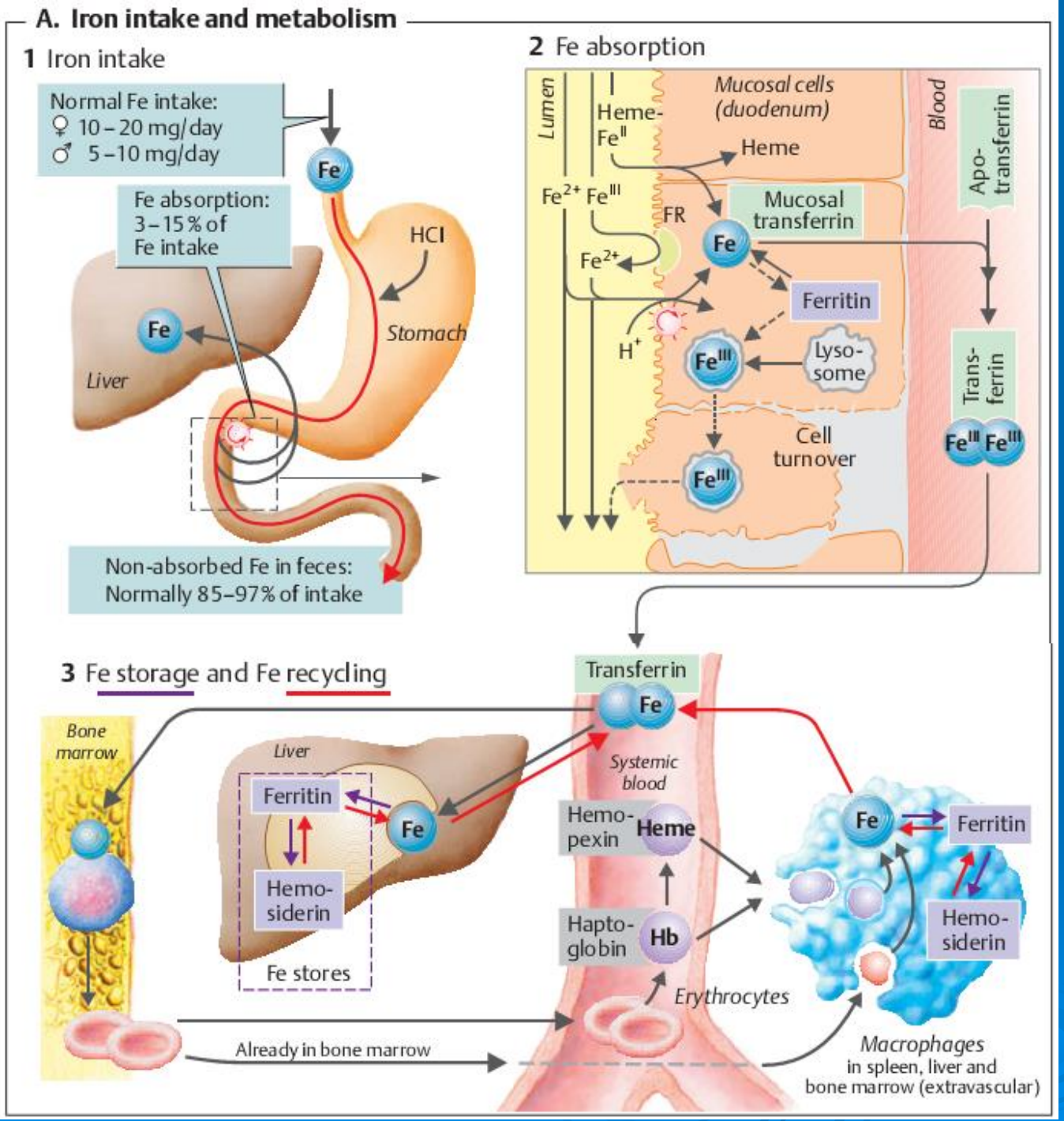
Iron metabolism



Ferritin

- Iron = 4-5g Per person
- Hb→ 65 % of total iron
- Reticuloendothelial system + liver = 15-30 %
- Myoglobin = 4%
- Intracellular oxidating heme compounds = 1%
- Transferrin = 0.1 %
- Absorption of Iron:
  - Mianly from Duodenum.
  - Heme-Fe^{+2} from Meat (Myoglobin, hemoglobin)
  - Fe^{+2} from small intestine (Fe^{+3} reduced by Vit C & *ferrireductase*(FR) to Fe^{+2} for absorption)
- Transport of Iron:
  - Iron + Apotransferrin [protein from liver] → Transferrin (Bound) → is taken up by endocytosis into erythroblasts and cells of the liver, placenta, etc. with the aid of *transferrin receptors.*
- Storage & Recycling:
  - *Ferritin* → one of the chief forms in which iron is stored in the body, storage occurs mainly in the intestinal mucosa, liver, bone marrow, red blood cells, and plasma. (4500 Fe^{+3} ions i.e. 600mg as readily available store).
  - *Hemosidrin* → In marcophages of liver and bone marrow (250mg) slow release.

    97 % recycled by phagocytes of liver, spleen and bone marrow



FR=*ferrireductase*

Daily Iron Loss
Male: 1mg/day
Females: 2mg/day

Daily Iron Requirement
Male: 1mg/day
Females: 2mg/day

Blood Transfusion

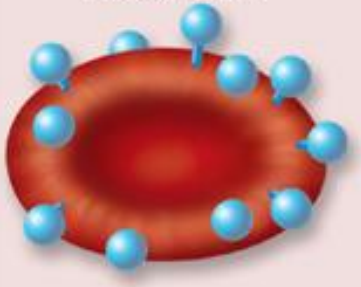
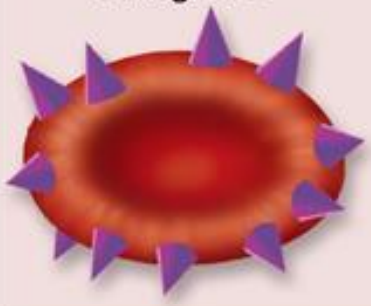
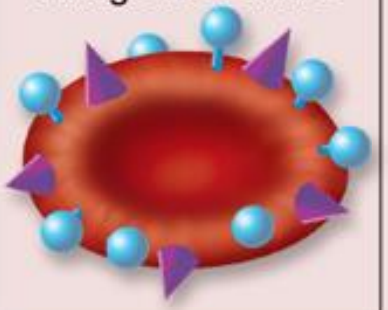
- Whole blood transfusions are routine when blood loss is rapid and substantial.
- In all other cases, infusions of packed red cells (whole blood from which most of the plasma has been removed) are preferred for restoring oxygen-carrying capacity.
- The usual blood bank procedure involves collecting blood from a donor and then mixing it with an anticoagulant, such as certain citrate or oxalate salts, which prevents clotting by binding with calcium ions.
- The shelf life of the collected blood at 4°C is about 35 days.
- Because blood is such a valuable commodity, it is most often separated into its component parts so that each component can be used when and where it is needed.

ABO Blood Group

BLOOD TYPES

- The membranes of human red cells contain a variety of blood group *antigens*, which are also called *agglutinogens*.
- *Antibodies* against red cell *antigens* are called *agglutinins*.
  - When the plasma of a type A individual (containing Anti-B antibodies) is mixed with type B red cells, the anti-B antibodies cause the type B red cells to clump (agglutinate).
- The most important and best known of these are the A and B antigens, but there are many more. eg
  - MNSs, Lutheran, Kell, Kidd,

ABO Blood Types

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

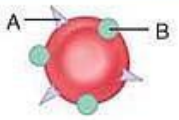
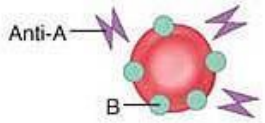
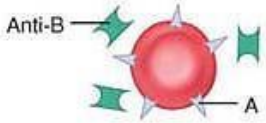
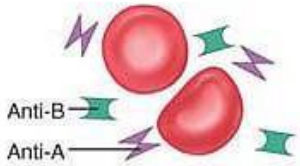
ABO Blood Group

- The individuals are divided into four major blood types on this basis of presence of these antigens.
  - Type A individuals have the A antigen,
  - Type B have the B,
  - Type AB have both, and
  - Type O have neither.
    - These antigens are found in many tissues in addition to blood:
    - E.g.. salivary glands, saliva, pancreas, kidney, liver, lungs, testes, semen, and amniotic fluid.
- <u>Chemsitry of Anitgens:</u>
  - The A and B antigens are complex oligosaccharides that differ in their terminal sugar.
  - On red cells they are mostly glycosphingolipids,
  - whereas in other tissues they are glycoproteins.
  - An *H* gene codes for a fucose transferase that puts a fucose[1] (hexose dexoy sugar) on the end of these glycolipids or glycoproteins, forming the H antigen
  - H-antigen is usually present in individuals of all blood types.

ABO Blood Group

- Individuals who are type A have a gene which codes for a transferase that catalyzes placement of a terminal *N*-acetylgalactosamine on the H antigen,
- Individuals who are type B have a gene which codes for a transferase that places a terminal galactose.
- Individuals who are type AB have both transferases.
- Individuals who are type O have neither, so the H antigen persists.

TABLE 17.4 ABO Blood Groups

RBC ANTIGENS (AGGLUTINOGENS)	ILLUSTRATION	PLASMA ANTIBODIES (AGGLUTININS)	BLOOD THAT CAN BE RECEIVED
A B		None	A, B, AB, O (Universal recipient)
B		Anti-A (a)	B, O
A		Anti-B (b)	A, O
None		Anti-A (a) Anti-B (b)	O (Universal donor)

ABO Blood Group

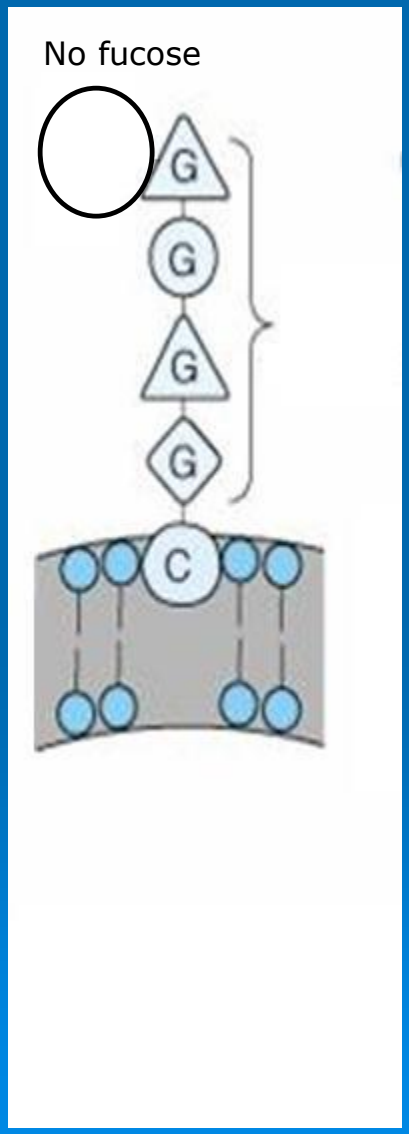
- Subgroups of blood types A and B
  - Most important being A1 and A2.
    - A1 cell has about 1,000,000 copies of the A antigen on its surface,
    - A2 cell has about 250,000 copies of the A antigen on its surface
- Antibody Development:
    - Antigens very similar to A and B are common in intestinal bacteria and possibly in foods to which newborn individuals are exposed.
    - Therefore, infants rapidly develop antibodies against the antigens not present in their own cells.

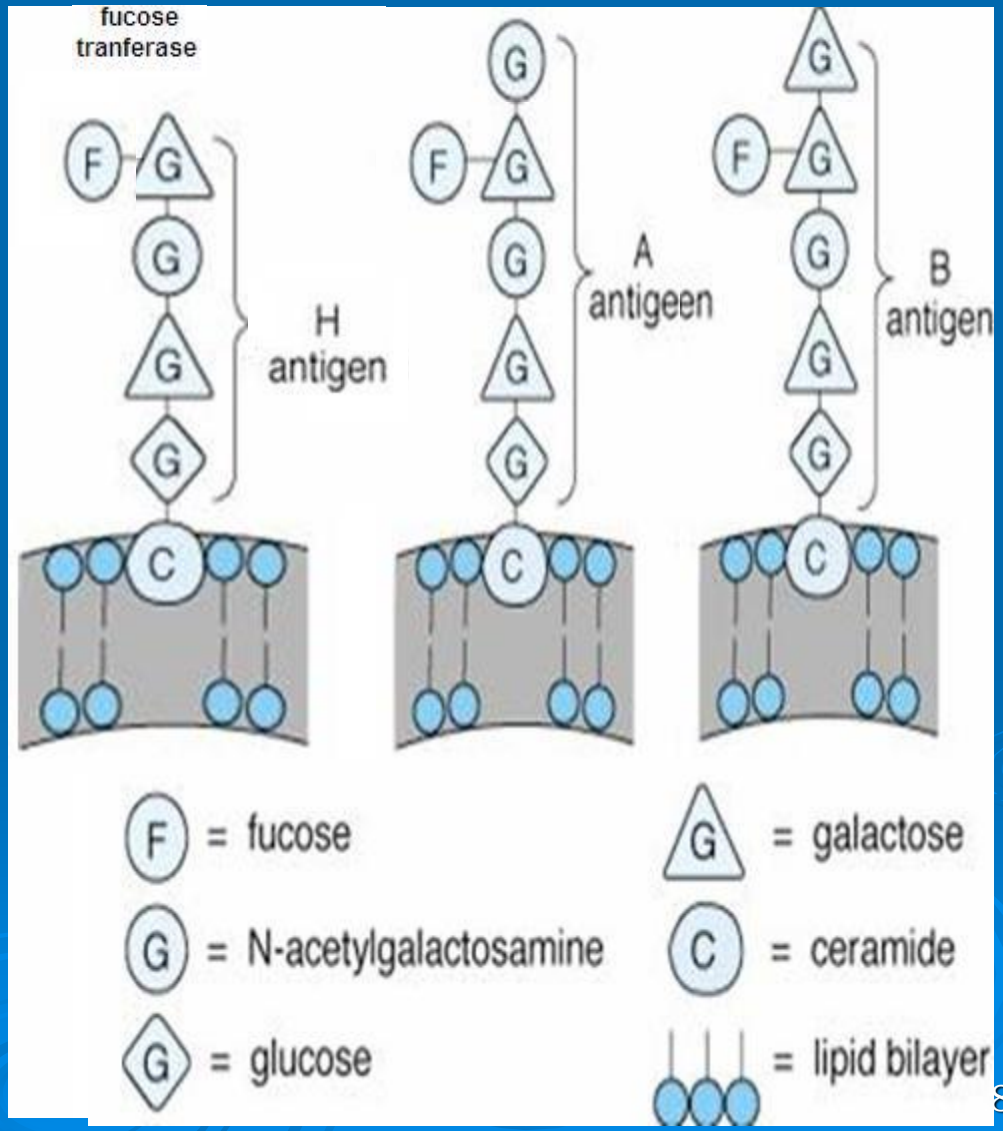
  Thus,
    - type A individuals develop anti-B antibodies,
    - type B individuals develop anti-A antibodies,
    - type O individuals develop both,
    - and type AB individuals develop neither.
- Blood Typing Test:

  Blood typing is performed by mixing an individual's red blood cells with antisera containing the various agglutinins on a slide and seeing whether agglutination occurs.

Bombay phenotype

Missing H-gene so no fucose tranferase so no fucose and no H-antigen that Forms the base for A and B Antigen.





Bombay Phenotype

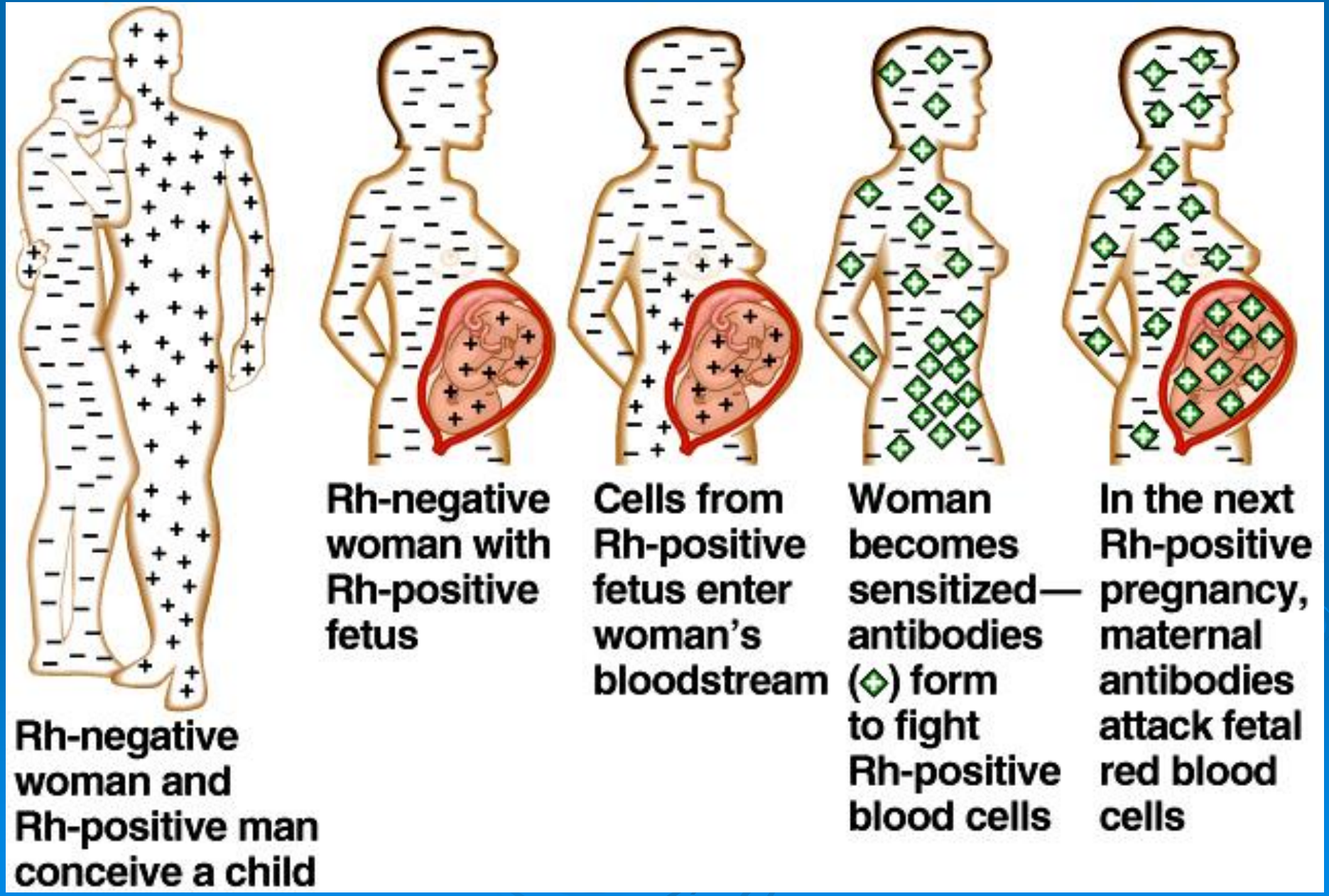
- This blood phenotype was first discovered in Bombay, now known as Mumbai, in, by Dr. Y.M. Bhende.
- **hh** is a rare blood group also called **Bombay Blood group**. Individuals with the rare **Bombay phenotype** (*hh*) do not express H antigen (the antigen which is present in blood group O).
- So whatever alleles they may have of the A and B blood-group genes, they cannot make *A-anitgen* or *B-antigen* on their red blood cells,because A antigen and B antigen are made from H antigen.
- As a result, people who have Bombay phenotype can donate to any member of the ABO blood group system (unless some other gene, such as Rhesus, is checked for compatibility), but they cannot receive any member of the ABO blood group system's blood (which always contains one or more of A and B and H antigens), but only from other people who have Bombay phenotype.
- The usual tests for ABO blood group system would show them as group O, unless the hospital worker involved has the means and the thought to test for Bombay group.

Rh Blood Groups

- 45 different types of Rh agglutinogens, each called an Rh factor.
- Three, the C, D, and E antigens, are fairly common.
- Rh antigen → first identified in rhesus monkeys.
- As a rule, ABO and Rh blood groups reported together eg, O+, A–, and so on.
- If an Rh– person receives Rh+ blood, the immune system becomes sensitized and begins producing anti-Rh antibodies against the foreign antigen soon after the transfusion.
- Hemolysis does not occur after the first such transfusion because it takes time for the body to react and start making antibodies.
- But the second time, and every time thereafter, a typical transfusion reaction occurs in which the recipient's antibodies attack and rupture the donor RBCs. eg Erythorblastosis fetalis[1]
- <u>Prevention:</u>

Anit-Rh antibodies given after every Rh+ birth. [RhoGAM]

Rh Factor


Rh-negative woman and Rh-positive man conceive a child
Rh-negative woman with Rh-positive fetus
Cells from Rh-positive fetus enter woman's bloodstream
Woman becomes sensitized— antibodies (◇) form to fight Rh-positive blood cells
In the next Rh-positive pregnancy, maternal antibodies attack fetal red blood cells

Blood Transfusion Reactions

- When mismatched blood is infused, a **transfusion reaction** occurs
- Donor's red blood cells → attacked by the recipient's plasma agglutinins.
- Donor's plasma antibodies may also agglutinate the host's RBCs, but they are so diluted that this does not usually present a serious problem.
- Initially, agglutination clogs small blood vessels throughout the body.
- During the next few hours, the clumped red blood cells begin to rupture or are destroyed by phagocytes, and their hemoglobin is released into the bloodstream.
- These events lead to two easily recognized problems:
  - The oxygen-carrying capability of the transfused blood cells is disrupted
  - The clumping of red blood cells in small vessels hinders blood flow to tissues beyond those points.
- Less apparent, but more devastating, is the consequence of hemoglobin escaping into the bloodstream.
- Circulating hemoglobin passes freely into the kidney tubules, causing cell death and renal shutdown. If shutdown is complete (acute renal failure), the person may die.

Blood Transfusion Reactions

- Transfusion reactions can also cause
  - fever,
  - chills,
  - low blood pressure,
  - rapid heartbeat,
  - nausea,
  - vomiting, and general toxicity;

  but in the absence of renal shutdown, these reactions are rarely lethal.
- Treatment of transfusion reactions is directed toward preventing kidney damage by administering fluid and diuretics to increase urine output, diluting and washing out the hemoglobin.
- Some laboratories are developing methods to enzymatically convert other blood types to type O by clipping off the extra (A- or B-specific) sugar residue.
- Autologous (auto = self) transfusions.
- The patient predonates his or her own blood, and it is stored and immediately available if needed during or after the operation. .
- Iron supplements are given, and as long as the patient's preoperative hematocrit is at least 30%, one unit (400–500 ml) of blood can be collected every 4 days, with the last unit taken 72 hours prior to surgery.