The Fate of RBCs

- The number of RBCs in circulating blood is kept constant by balancing

- the rate of production (erythropiesis) to the rate of destruction (hemolysis).

- RBCs have a normal lifespan of 120 days in circulating blood.

The Fate of RBCs cont'd

- Towards the end of their normal lifespan, the permeability and flexibility of RBC membranes become altered.
- The cells then easily rupture during their transit through the capillaries, resulting into release of Hb.
- The Hb so released is degraded in order to conserve the Fe^{2+}.

The Fate of RBCs cont'd

- This aging is accompanied by change in shape of the RBCs,

- progressive loss of lipid membrane and reduction of the glycolytic enzyme activity.

- They become smaller and more dense (crowded closely together).
- Degenerate RBCs are removed from circulation by marcophages in the bone marrow, spleen and liver, then degraded.

Haemoglobin (Hb)

- Hb is the pigment of RBCs containing a complex, Fe^{2+} containing protein called **heme**, and a simple protein known as **globin.**
- Four heme groups combine with 4 globin molecules to form one Hb molecule.

Haemoglobin synthesis

- Biosynthesis of heme starts in the mitochondria with condensation of **succinly-CoA** and **glycine** to form **δ-aminolaevulinic acid (ALA)** or **pyrrole**, which is the rate limiting step.

Haemoglobin synthesis cont'd

- This step is catalyzed by the enzyme ALA synthetase which requires pyridoxine (vit. B_6) as cofactor.
- After several steps of biochemical transformations, **protopophyrin IX** is formed in the mitochondria.
- The enzyme ferrochelatase then catalyses the insertion of ferrous iron, Fe^{2+} into the protopophyrin ring to form **heme.**
- The **heme** then combines with **globin** to form **Hb.**
- Biosynthesis of Hb starts at the rubricyte stage of erythropoiesis and continues until the end of reticulocyte stage

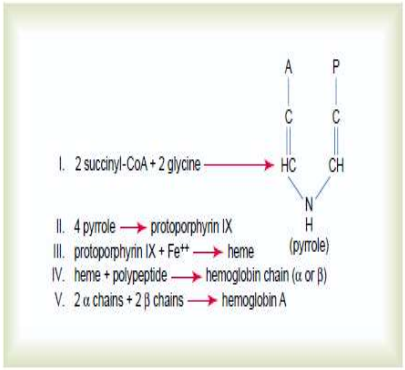
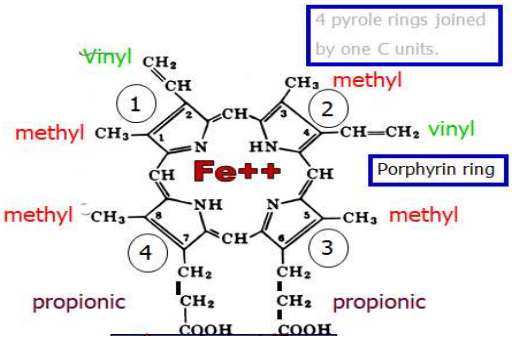


Figure 32-5

Formation of hemoglobin.



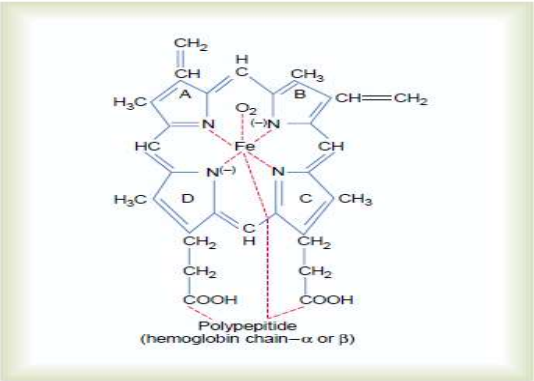
The functioning of hemoglobin

- Hb is the principal O_2-carrying pigment of blood.
- As blood transits the pulmonary capillaries, Hb combines with O_2 to form oxyhemoglobin.
- This combination is loose and reversible.
- The complex in turn loses its O_2 as blood travels through the capillary bed of body tissues to become free Hb again.

The functioning of hemoglobin cont'd

- The O_2 carrying capacity of Hb is due to the Fe^{2+} in the heme.
- The combination of the Fe^{2+} with O_2 is not a chemical oxidation,

- but rather through a co-ordination bond in which the oxygen remains in molecular form.



The functioning of hemoglobin cont'd

- Fe^{2+} forms only 0.04 – 0.05% of the blood.
- Therefore, careful conservation of the Fe^{2+} by the body is one of the key features of Hb catabolism.
- Oxy-Hb, its aqueous solution and arterial blood are all bright red in colour,

- while deoxygenated Hb, its aqueous solution and venous blood are dark or purplish red.

Fate of Hemoglobin

- The Hb molecules are broken down, with the globin protein separated from the heme.
- The heme is broken down into Fe^{2+} and protopophyrin.
- The Fe^{2+} released is transported in the blood stream by a β-globulin called transferrin to

- the erythropoietic organs for further production of RBCs and to skeletal muscles for myoglobin synthesis.

Fate of Hemoglobin cont'd

- Some of the Fe^{2+} is stored in the liver, the spleen, bone marrow and intestinal mucosa as ferritin.
- The remnant pigment, known as the protopophyrin is converted in the liver into bile pigments which are eventually excreted.

Myoglobin

- **Myoglobin (muscle Hb)** is a molecule containing 1 heme and 1 globin, but has the same functions as Hb.
- It has a higher affinity for O_2 than Hb and releases O_2 more readily when the partial pressure of O_2 falls.
- It is found in skeletal muscles, where it stores O_2 briefly during the inter-contraction intervals.
- Its O_2 is replenished during the resting stage.

Evaluation of status of RBCs

1. Erythrocyte sedimentation rate (ESR)

- Erythrocyte sedimentation rate (ESR) is defined as the speed with which

- the uppermost column of RBCs descend when anticoagulated whole blood is placed in a standard tube known as the Wintrobe tube.

- ESR is measured in mm/hr.
- The normal ESR is 3–5mm/hr in men & 4–7 mm/hr in women.

Erythrocyte sedimentation rate cont'd

- The ESR is governed by the principle of "free-fall-through-a-medium" in which the object descends

- under the influence of gravity, but the frictional forces due to viscosity of the medium oppose the fall, reducing the speed.

- The higher the effective mass of the falling object, the higher the speed of descend.
- **Increase in the viscosity of plasma, due to increasing concentration of plasma proteins, will reduce the ESR.**

Process of erythrocyte sedimentation rate

- When a sample of blood containing anticoagulant is left to stand in a vertical tube,

- different fractions of blood will separate out due to differences in relative densities or specific gravity (s.g.).

- Blood cells settle under the influence of gravity because they have higher s.g. than plasma.

Process of erythrocyte sedimentation rate cont'd

- This is called gravity sedimentation and occurs in three stages:
- a slow initial stage of rouleaux formation,
- followed by a faster phase of rapid descend,
- and finally a slower stage of packing.

- RBCs have the highest s.g., & will settle at the bottom, followed by the WBCs, leaving the straw-coloured plasma column.

Factors affecting ESR

- In usual laboratory conditions, ESR is affected by:

a) Increase in rouleaux formation (RF): RF is the adherence of RBCs to one another by their broad sides to form what resembles a stack of coins.

- Increased RF in the sample will increase ESR due to increase in effective particle size and reduction on the frictional forces.

- RF depends on the concentration of α_2 and γ-globulins, which tend to promote clumping.

Factors affecting ESR cont'd

b) Agglutination: the random attachment of RBCs to one another through interaction of antigens on the RBC membranes with anti-bodies in plasma.

c) Clot formation: particles of blood clot are effectively larger than single RBCs. Clotting therefore increases ESR.

- This can result from increased levels of fibrinogen.

d) Anaemia: reduction of the RBC counts in circulation also increases ESR, this time due to reduced total frictional forces on the RBCs.

- However, sickle cell anemia reduces ESR due to poor tendency of rouleaux formation by the RBCs.

ESR cont'd

Clinical application of ESR:

- It is used as a non-specific screening test for generalized conditions and wasting diseases. In all cases, the ESR is significantly elevated.
  - **Tuberculosis:** the ESR is elevated by increase in α_2 and γ-globulins and fibrinogen
  - **Cancers:** associated with increased production of fibrinogen, leading to increased clotting tendencies
  - **Other inflammatory conditions:** especially those that are highly destructive to tissues

Evaluation of RBCs cont'd

2. Packed cell volume (PCV)

- Packed cell volume (PCV) or hematocrit is the proportion of whole blood that consists of RBCs.
- In the Wintrobe method of determination of ESR, the column of packed RBCs as a percentage of the total length of whole blood at the end of sedimentation represents PCV.
- Man has normal PCV of 46% & woman, 40%

Packed cell volume cont'd

- PCV is used as an indicator of anaemia or polycythemia.
- When PCV has fallen to 10%, then anaemia is present.
- When PCV has been elevated to 65%, the condition of polycythemia exists.
- Anaemia is a term used to describe deficiency of RBCs in blood.
- It is merely a symptom of shortage of RBCs, either due to excessive loss or destruction or inadequate synthesis of RBCs.

Evaluation of RBC status cont'd

3. Hemoglobin status

- Normal RBCs can accumulate Hb up to 34 gm/dL in each cell, this being the metabolic limit of the Hb synthetic machinery of the cell.
- Normal cells usually have Hb concentrations near the maximum.
- When Hb synthesis in the bone marrow is deficient, the percentage of Hb in the cell falls

- and the RBC volume may decrease (microcytosis) due to shrinkage of the cell membranes.

Hemoglobin status cont'd

- When the PCV (usually 40-46%) and the quantity of Hb in each cell are normal,

- the whole blood of men will contain 16 gm Hb and women, 14 gm Hb per dL of blood.

- This is known as the Hb concentration.

Hemoglobin status cont'd

- Each gm of Hb combines with 1.39 mL of O_2, therefore in a normal man, more than 22 mL of O_2

- can be carried in combination with each dL of blood. In the woman, its is 19 mL

- Two types of Hb are found in human beings:
  - Hb A found in adults
  - Hb F found in the fetus and newborn child

26