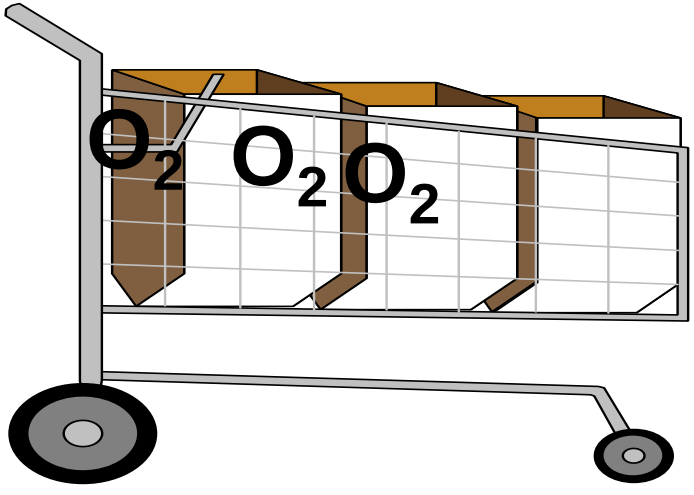


Haemoglobin

Oxygen O_2

- 98% travels in oxyhaemoglobin (in red blood cells)

- 2% is dissolved in plasma (compared to carbon dioxide, oxygen is relatively insoluble in plasma)

O_2 is not very soluble – thus needs a carrier !

Oxygen-Binding Proteins

- Myoglobin, Haemoglobin, Cytochromes bind O_2

- Oxygen is transported from lungs to various tissues via blood in association with **haemoglobin**

- In muscle, haemoglobin gives up O_2 to **myoglobin** which has a higher affinity for O_2 than heamoglobin.

- **Cytochromes** participate in redox reactions and are components of the electron transport chain

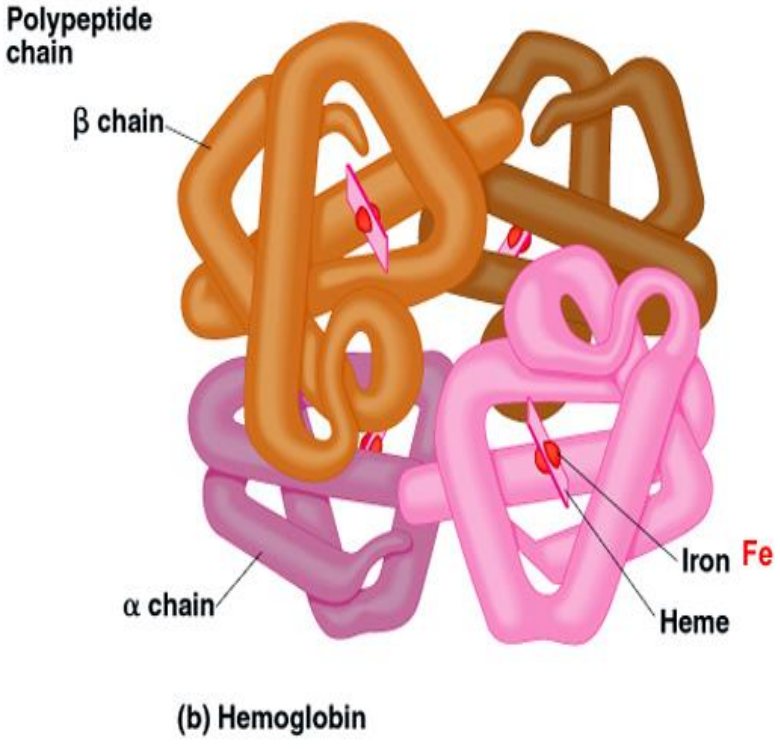
Haemoglobin

- Haemoglobin (Hb or Hgb) is the primary constituent of RBCs

- This molecule gives the characteristic red colour to erythrocytes and to the blood

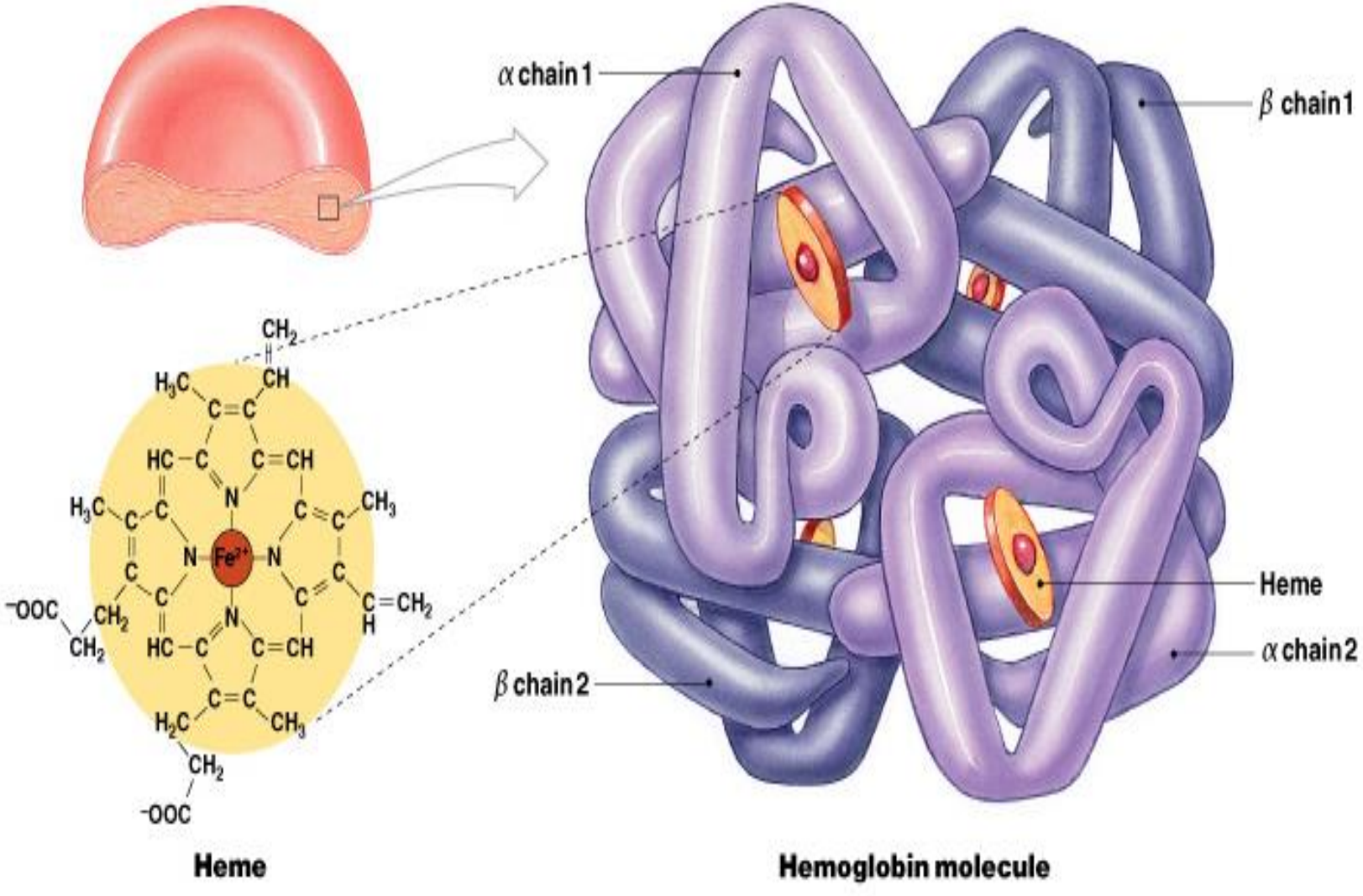
- The primary function of haemoglobin is to transport oxygen (O_2) from the lungs to the tissue cells of the body and to carry carbon dioxide (CO_2)

Structure of Hemoglobin

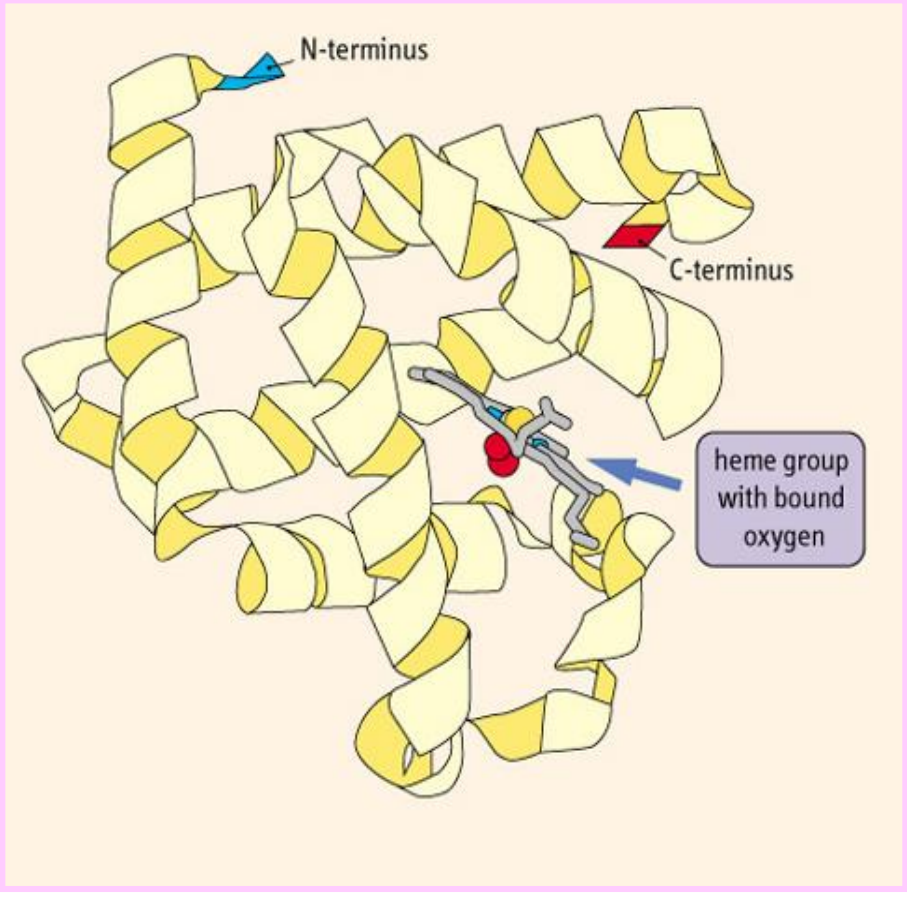


(b) Hemoglobin

- Hemoglobin (tetramer) is composed of the protein globin, made up of two alpha chains (141 a.a) and two beta chains (146 a.a), each bound to a heme group
- Alpha and beta are similar but not identical in a.a. sequence
- Each heme group bears an atom of iron, which can bind to one oxygen molecule
- Each hemoglobin molecule can transport 4 molecules of oxygen


α chain 1
β chain 1
Heme
α chain 2
β chain 2
Heme
Hemoglobin molecule

Myoglobin



- Abundant in skeletal muscles
- Consists of one heme and globin consists of single polypeptide chain (monomeric: 153 aa; 17,200 MW)

Normal haemoglobin types

1. Hb A:

- Makes up about 95%-98% of Hb found in adults;
- Contains two alpha (α) protein chains and two beta (β) protein chains

2. Hb A2:

- Makes up about 2%-3% of Hb;
- Has two alpha (α) and two delta (δ) protein chains

Normal haemoglobin types (cont.)

3. Hb F:

- Makes up to 2% of Hb found in adults;
- Has two alpha (α) and two gamma (γ) protein chains;
- The primary haemoglobin produced by the **fetus** during pregnancy, its production usually falls to a low level shortly after birth
- Foetal Hb has a higher affinity for oxygen than adult haemoglobin.
- This means that the fetus can receive oxygen from the mother across the placenta.

The Prosthetic Heme Group

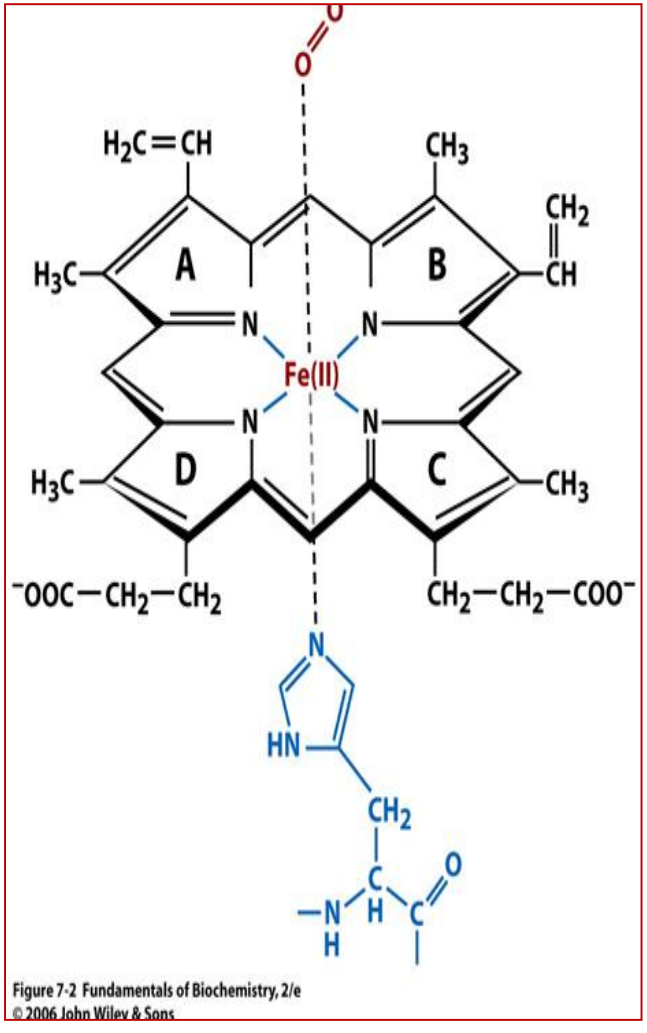


Figure 7-2 Fundamentals of Biochemistry, 2/e

- Responsible for the O_2 binding capacity of Hb.
- Consists of an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin
- The protoporphyrin made up of four pyrrole rings linked by methane bridges

The Prosthetic Heme Group (cont.)

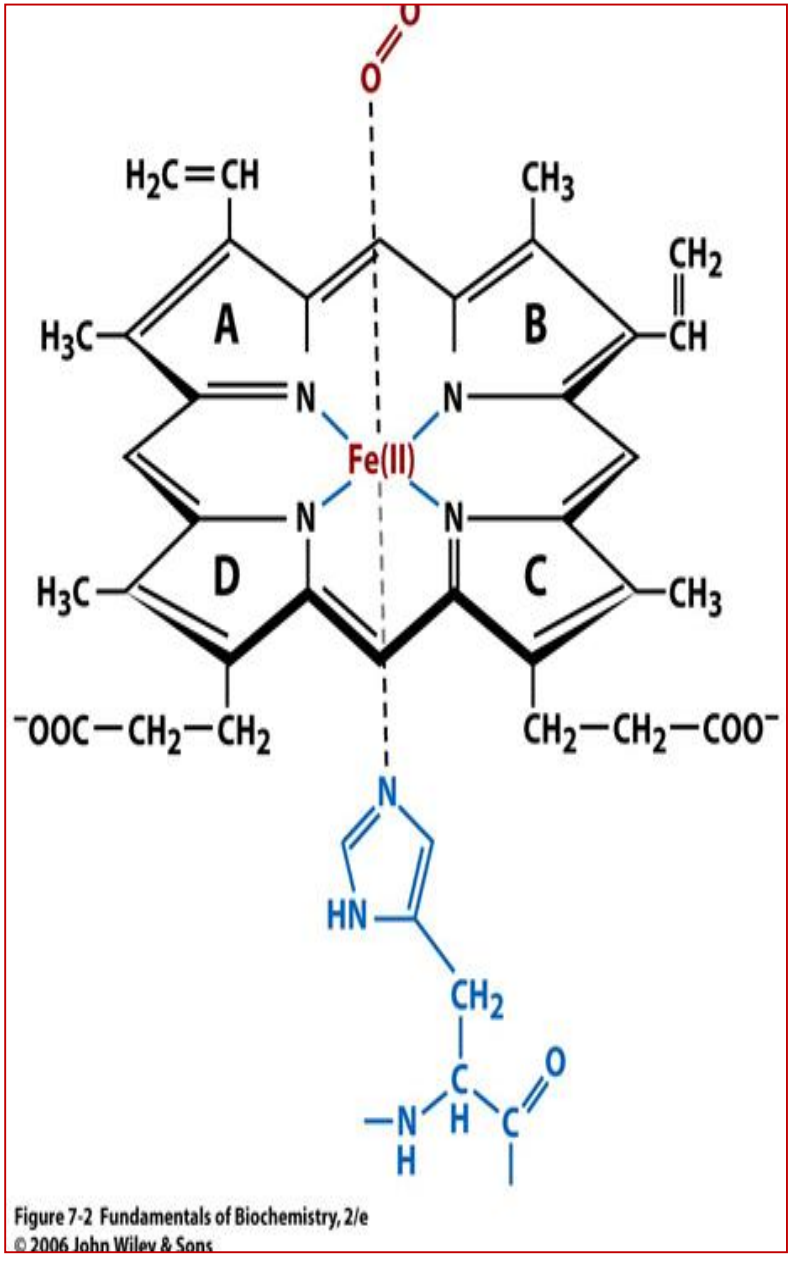


Figure 7-2 Fundamentals of Biochemistry, 2/e
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- A Fe atom in its ferrous state (Fe^{+2}) is at the center of protoporphyrin.
- Fe^{+2} has 6 coordination bonds
  - 4 bonded to the 4 pyrrole N atoms (The nucleophilic N prevent oxidation of Fe^{+2})

The Prosthetic Heme Group (cont.)

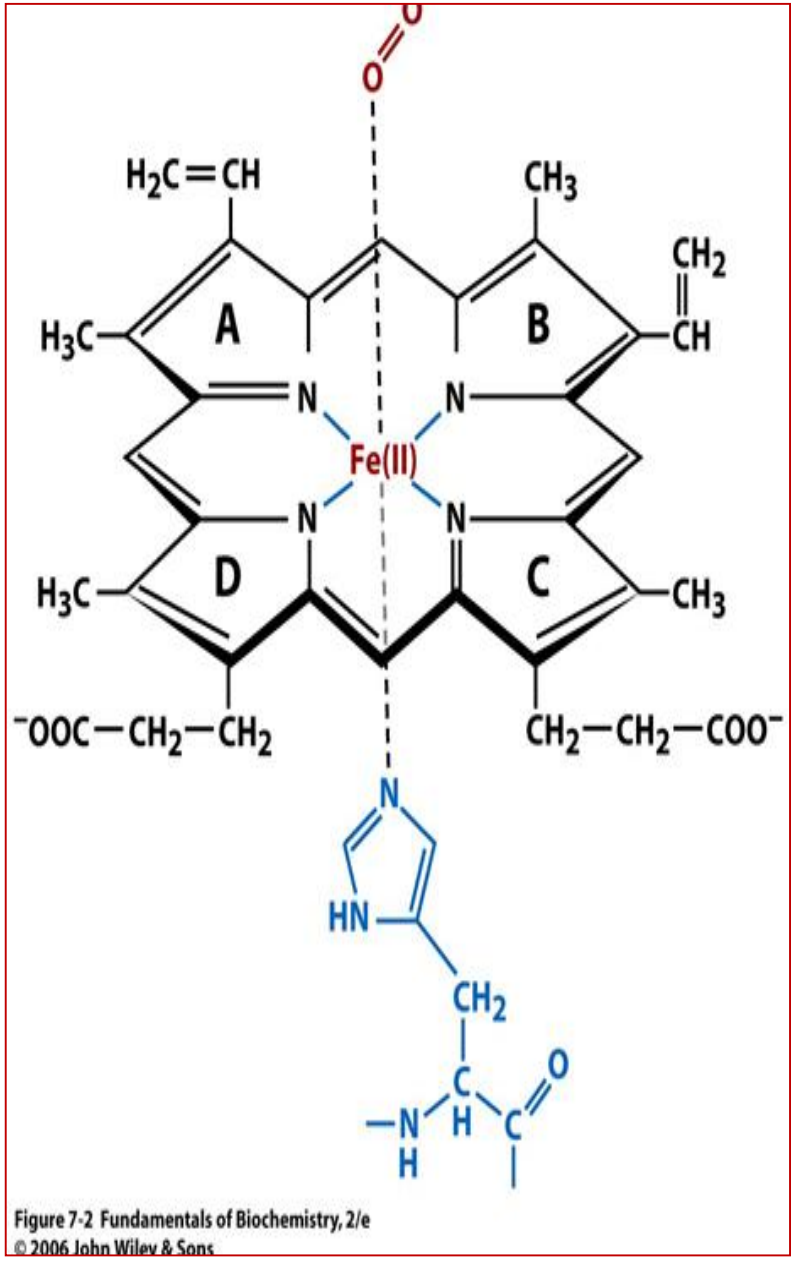


Figure 7-2 Fundamentals of Biochemistry, 2/e

- The 2 additional binding sites are one on either side of the heme plane:
  - ✓One of these is occupied by the imidazole group of His
  - ✓The second site can be reversibly occupied by O_2

Different forms of Hemoglobin

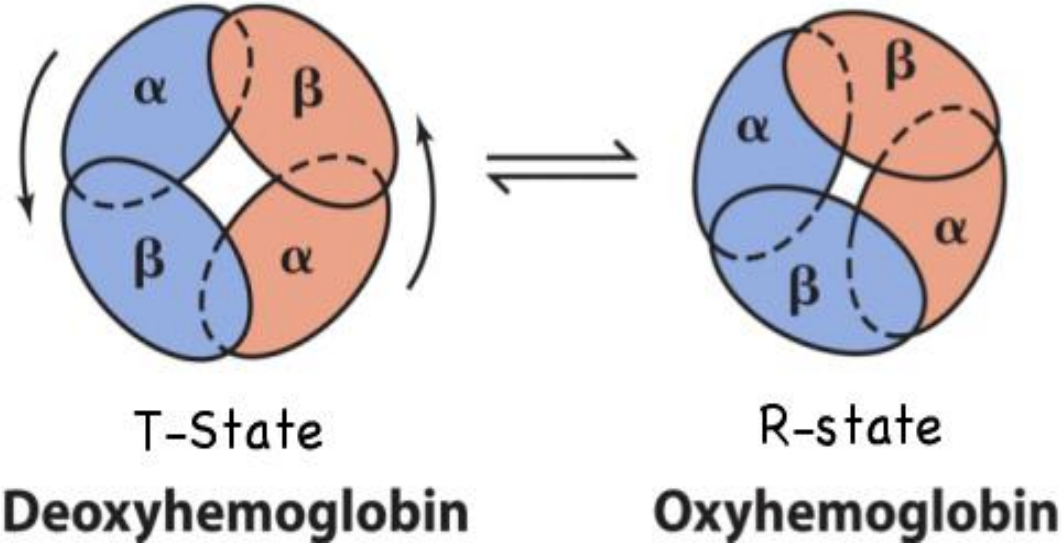
- When Hb is bound to O_2, it is called **oxyHb**. This is the **relaxed (R) state**

- The form with a vacant O_2 binding site is called **deoxyHb** and corresponds to **the tense (T) state**

- If iron is in the oxidized state as Fe^{+3}, it is unable to bind O_2

Different forms of Hemoglobin (cont.)

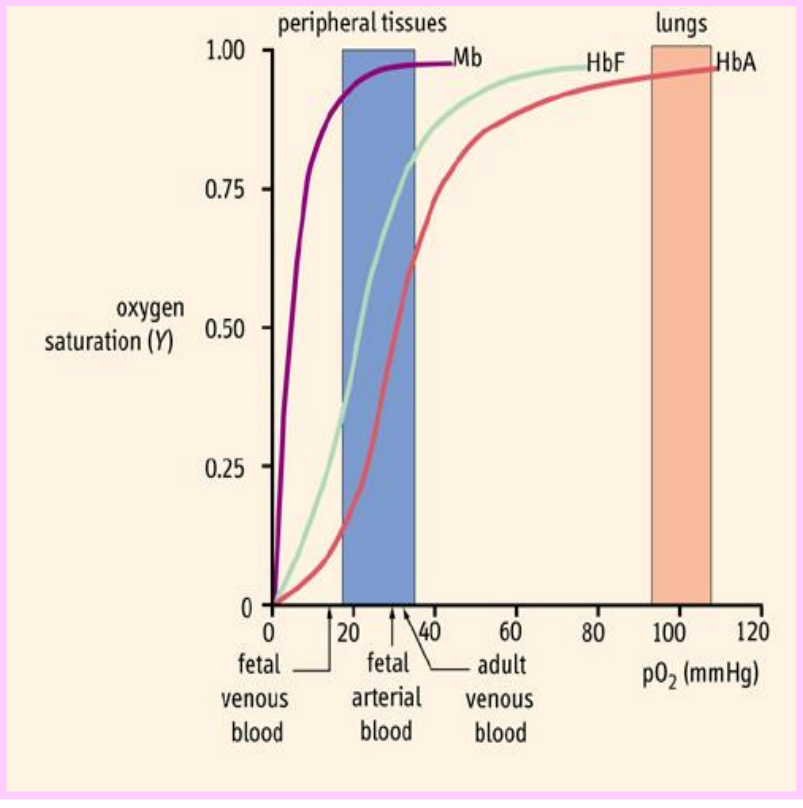
- **R state** has a higher affinity for O_2
- **T state** is more stable in the absence of O_2



Conformational change

The subunits slide and rotate making the central cavity smaller

O_2-binding kinetics

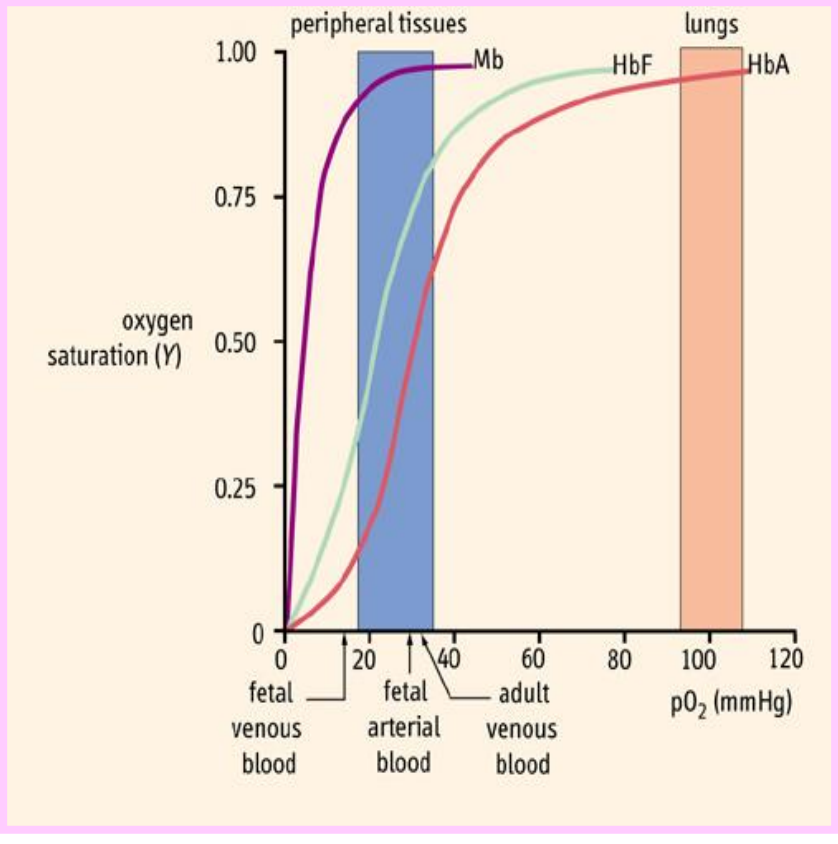


- O_2-binding curves show Hb saturation as a function of the partial pressure for O_2

- 4 subunits, so $4O_2$-binding sites: If one heme group has a bound O_2, it increases the ability of the other heme groups to bind O_2 (last O_2 affinity is 300 times greater than its affinity for 1^{st} O_2)

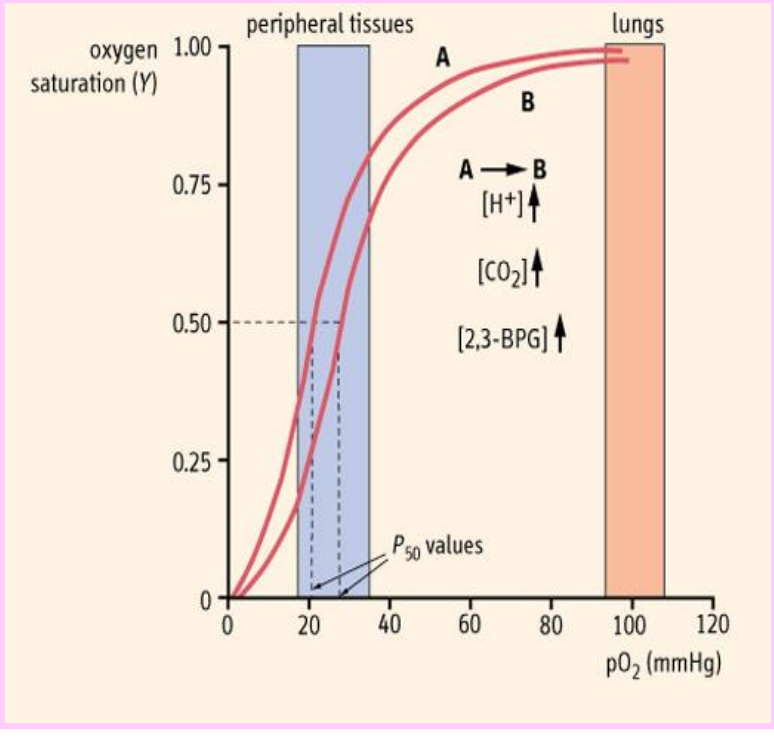
- Cooperative binding
- Segmoidal curve

O_2-binding kinetics (cont.)



- Myoglobin has a higher O_2 affinity than Hb
- Myoglobin O_2 dissociation curve is hyperbolic.

A number of factors reduce the affinity of Hb for O_2 so that more O_2 is released to tissues(****)



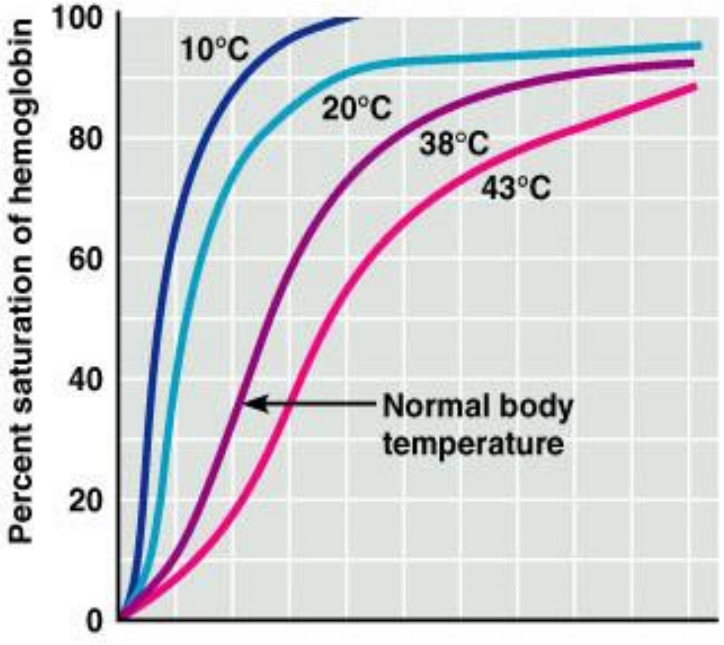
- As the curve shifts from A to B (to right) the affinity for O_2 decreases.
- H^+, PCO_2, and BPG modify the structure of Hb and alter its affinity for oxygen:

  Increases of these factors:

  - Decrease hemoglobin's affinity for oxygen
  - Enhance oxygen unloading from the blood

  Decreases act in the opposite manner.

A number of factors reduce the affinity of Hb for O_2 so that more O_2 is released to tissues (cont.)



- Increasing temperature also shift the curve to the right

Transport of CO_2

- CO_2 in blood present in 3 forms:

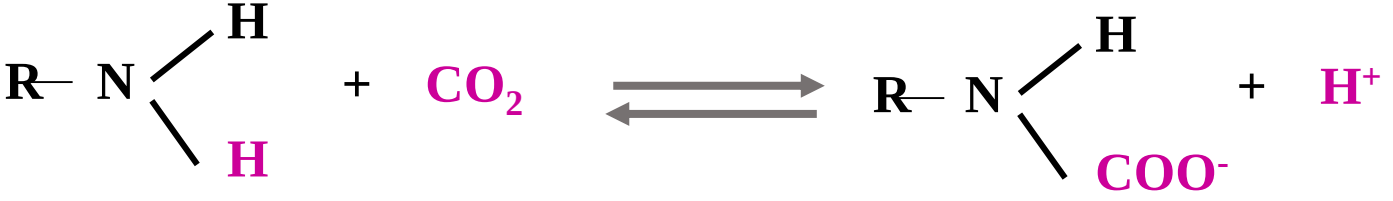
In red blood cells

1. 7% dissolved in plasma
2. 70% travels as HCO_3^- ions (hydrogencarbonate ions)
3. 23% travels as carboamino compounds

CO_2 = waste product of cellular metabolism (the end-product)

CO_2 bound as carbamate

- CO_2 reacts directly with Hb to form the carboaminoHb; reversible reaction
- Small quantity of CO_2 reacts with plasma proteins - less significant (quantity of proteins 1/4th that of Hb)



Protein **Carboamino compound**

CO_2 bound as HCO_3

- Dissolved CO_2 in blood reacts with water to form Carbonic Acid.

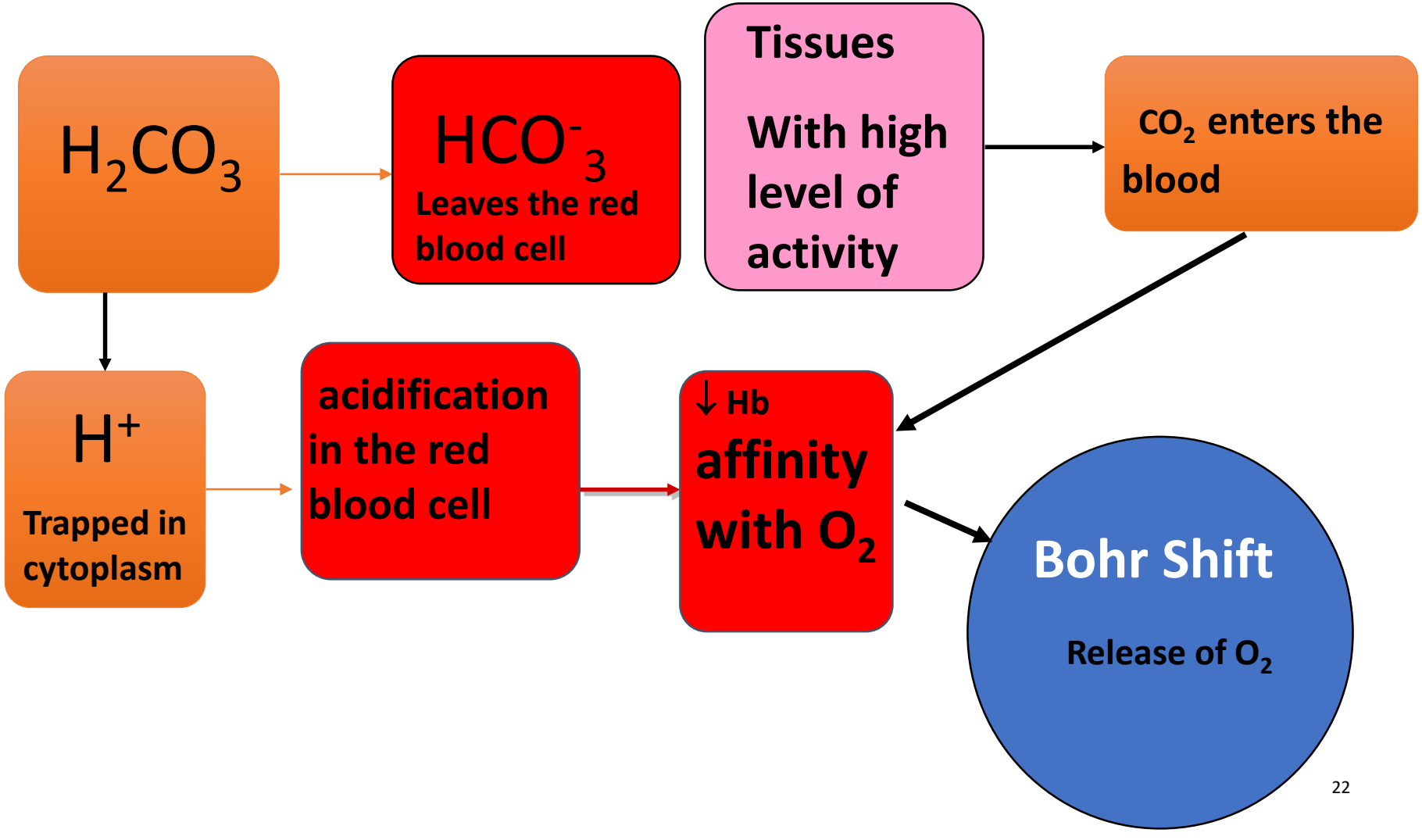
$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

- Carbonic Anhydrase present inside RBCs (but not plasma) catalyzes this reaction

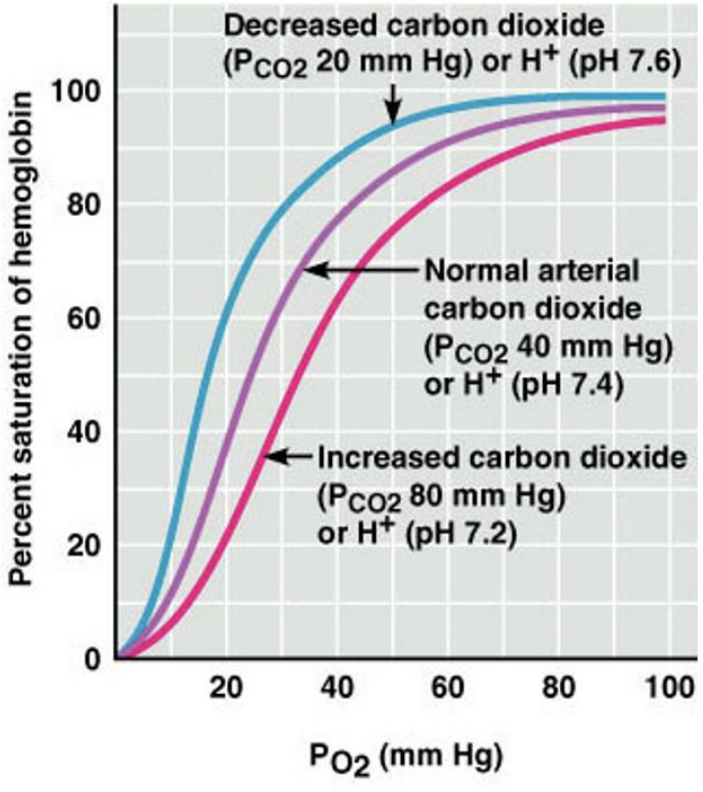
- Carbonic acid rapidly dissociates into ion H^+ and bicarbonate ion.

Bohr Shift

The relationship between the binding of O_2, H^+, CO_2 to hemoglobin (allosteric site), is knowing as Bohr effect



Bohr Effect or Bohr Shift



- The dissociation curve moves to the right at higher concentration of carbon dioxide. This shows that carbon dioxide lowers the affinity of Hb for oxygen.

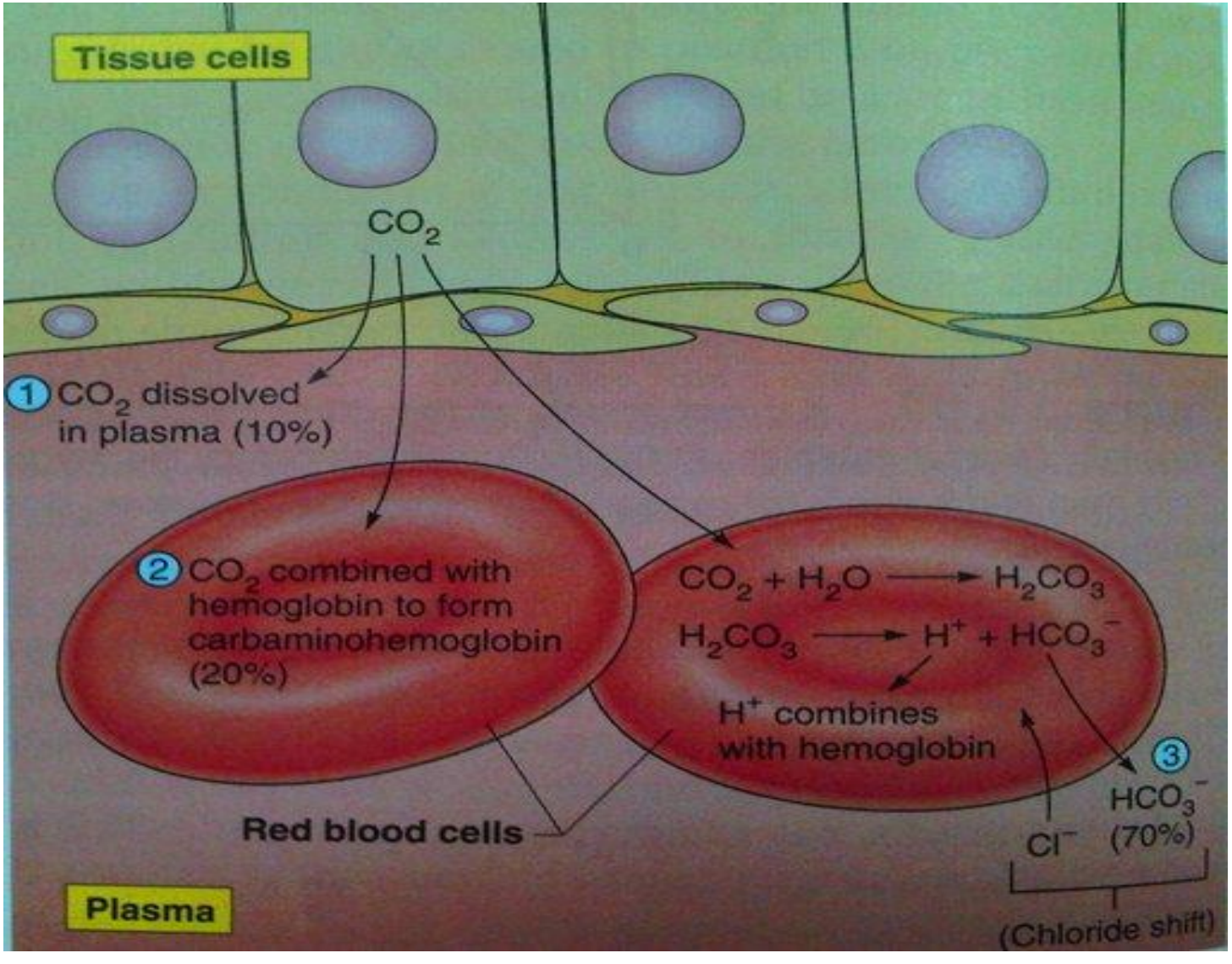
- Hb tends to give up O_2 in area of high CO_2 such as the respiring tissues that need it most.

Chloride shift

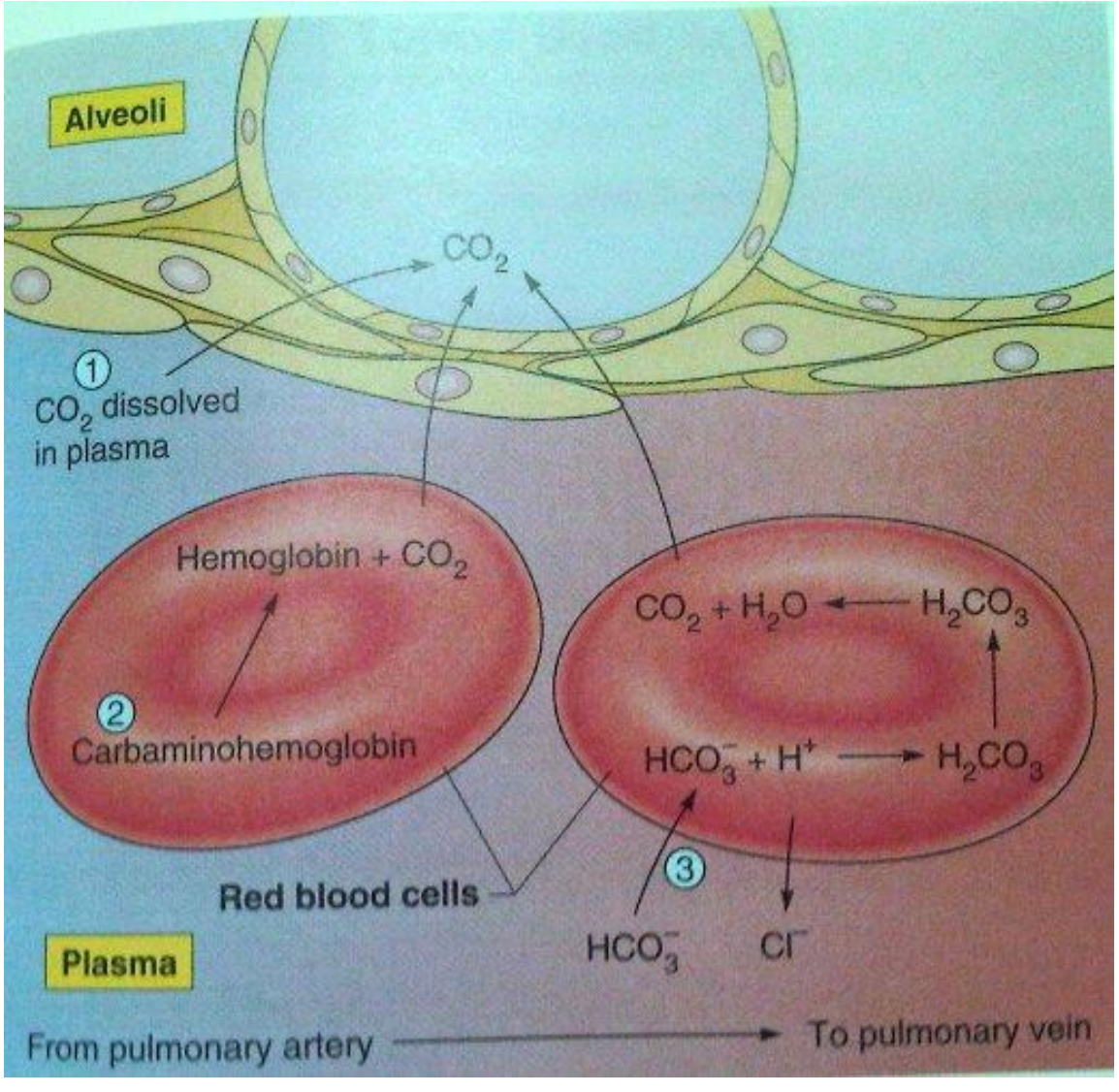
- The build up of hydrogen carbonate ions causes them to diffuse out of the RBC leaving the inside of the RBC positively charged

- In order to balance this electric charge chloride ions diffuse into the RBCs from the plasma → this is known as the **chloride shift**

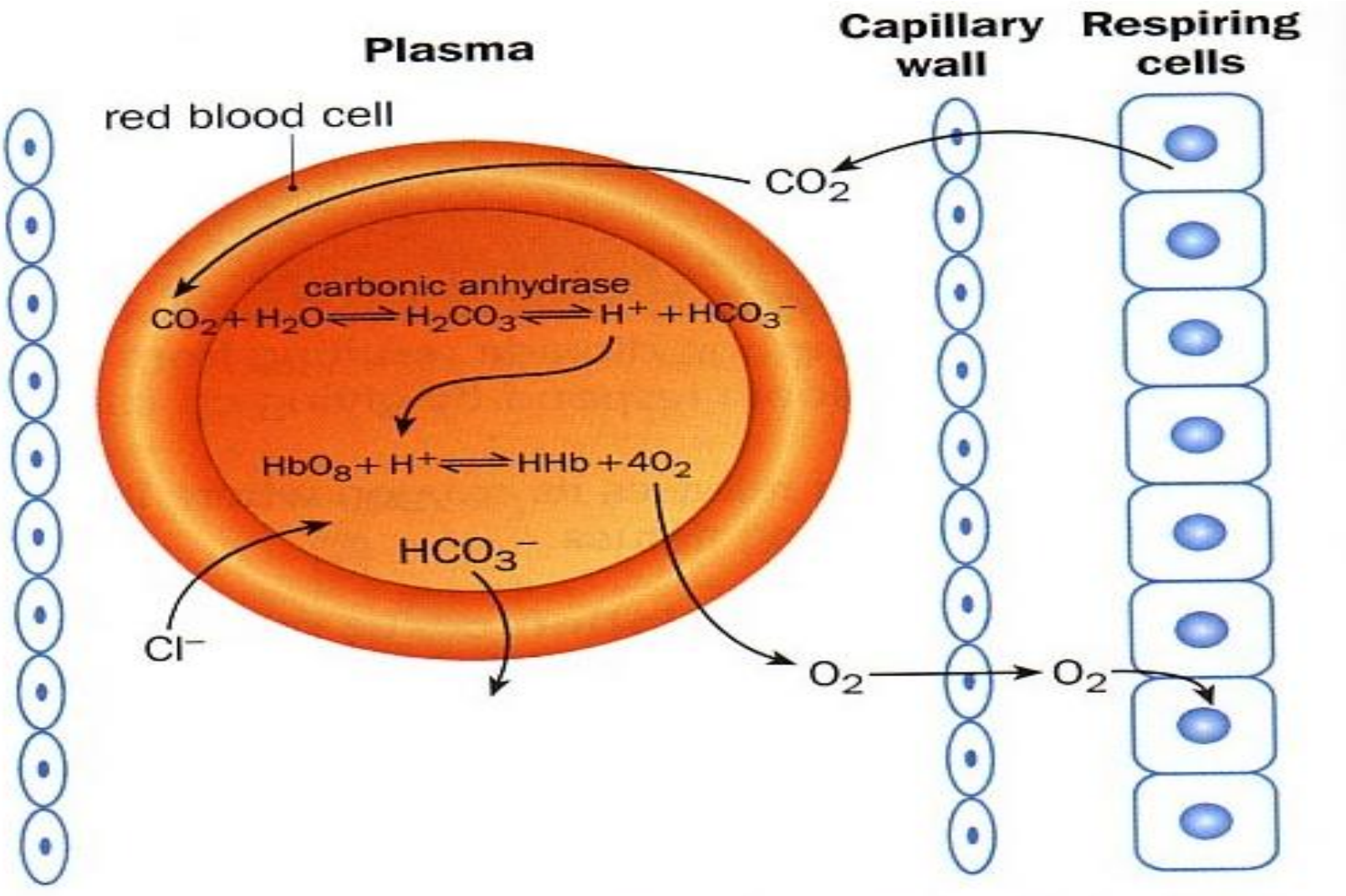
Carbon dioxide transport and the chloride shift



The reverse chloride shift in the lungs



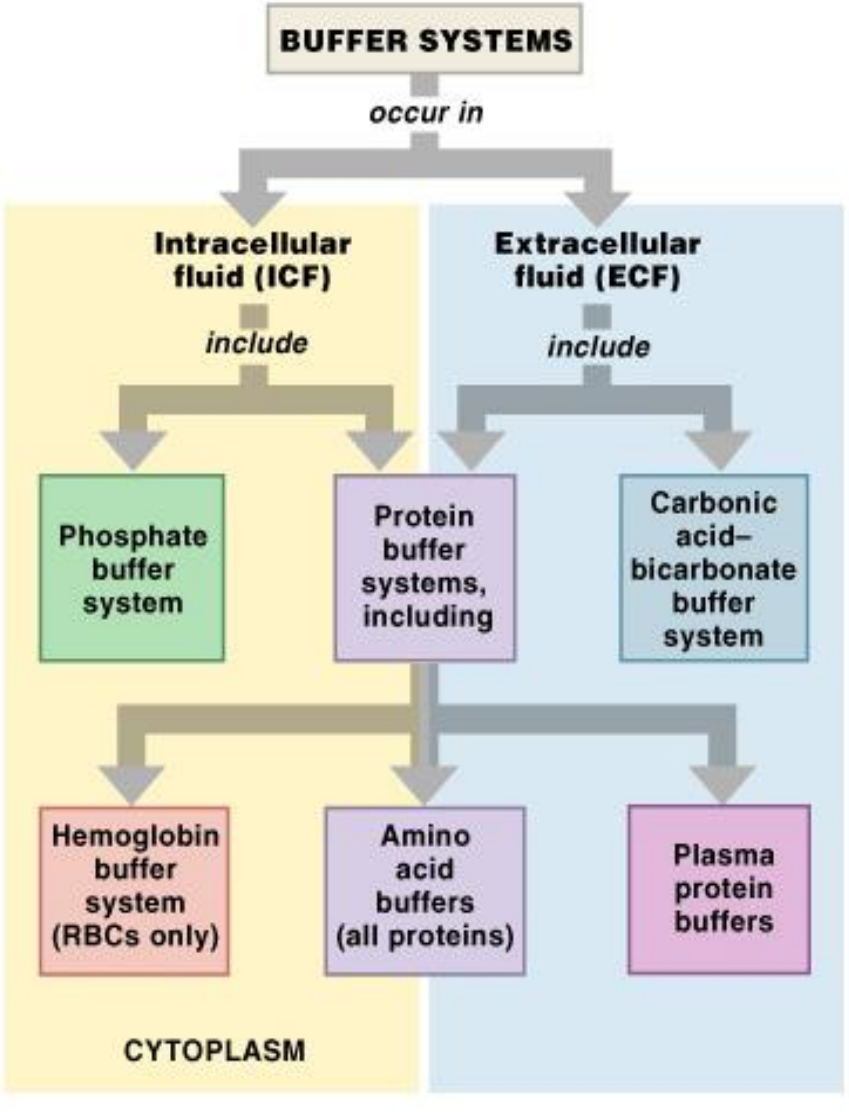
Chloride shift



Carbon dioxide transport in the plasma and red blood cell

- When blood gets to the lungs, all the reactions are reversed
- The hydrogen carbonate and hydrogen ions recombine releasing CO_2
- The chloride shift is reversed
- Carbamino-haemoglobin breaks down to release CO_2

3 Major Buffer Systems in the body



Hemoglobin as a buffer

Hemoglobin acts as a buffer in blood by picking up CO_2 or H^+

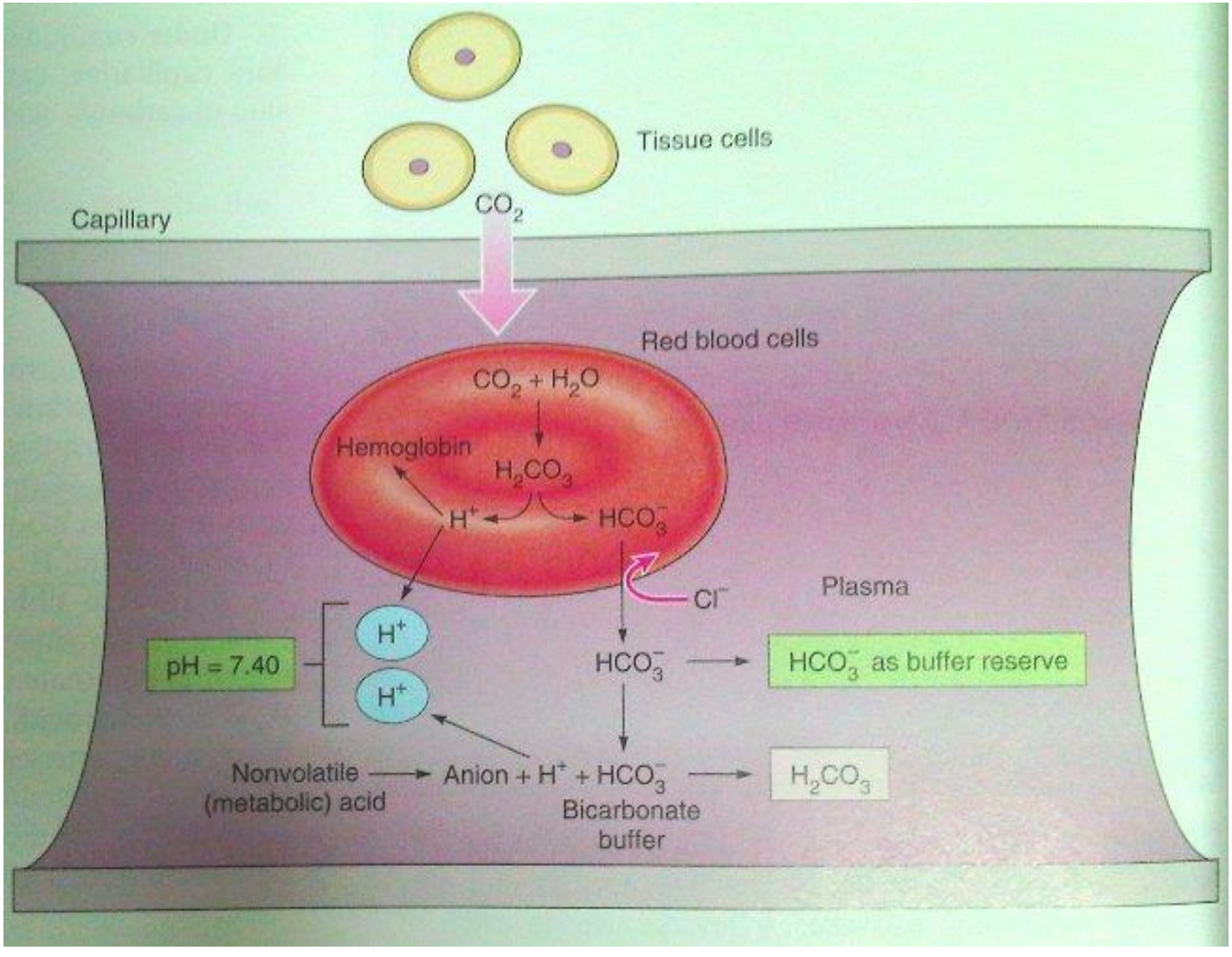
- **In tissues:**

  Hemoglobin becomes more basic when it is deoxygenated, i.e. it binds H^+ more tightly

- **In the lung:**

Hemoglobin is oxygenated, becomes more acidic, (i.e. it is a more powerful H^+ donor), and releases its H^+

The effect of bicarbonate on blood pH



Erythrocyte hemolysis
(Normal red cell destruction)

- cells are removed extravascularly by the *macrophages* of the reticuloendothelial (RE) system.
- *removed* in marrow also in the liver and spleen.

Blood-Cleansing Function of the Spleen: *Removal of Old Cells*

- Blood cells passing through the splenic pulp undergo *thorough squeezing*.
- Therefore, fragile RBCs will not withstand the trauma, and will be destroyed in the body.
- After the cells rupture, the released Hb is digested by the *phagocytic* reticuloendothelial cells of the spleen *(similar Kupffer cells in liver).*
- Digested products are reused by the body as nutrients, or for making new blood cells.

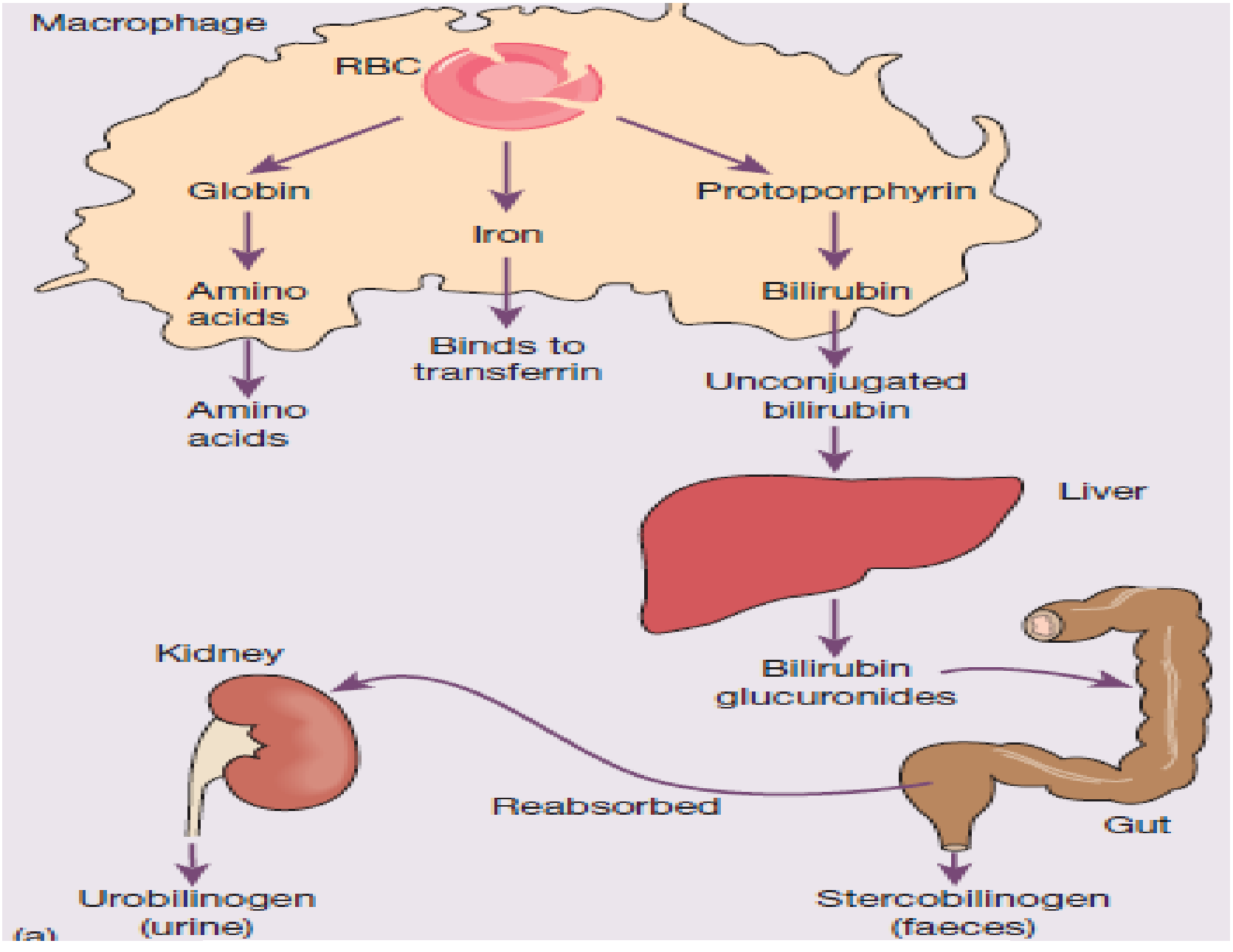
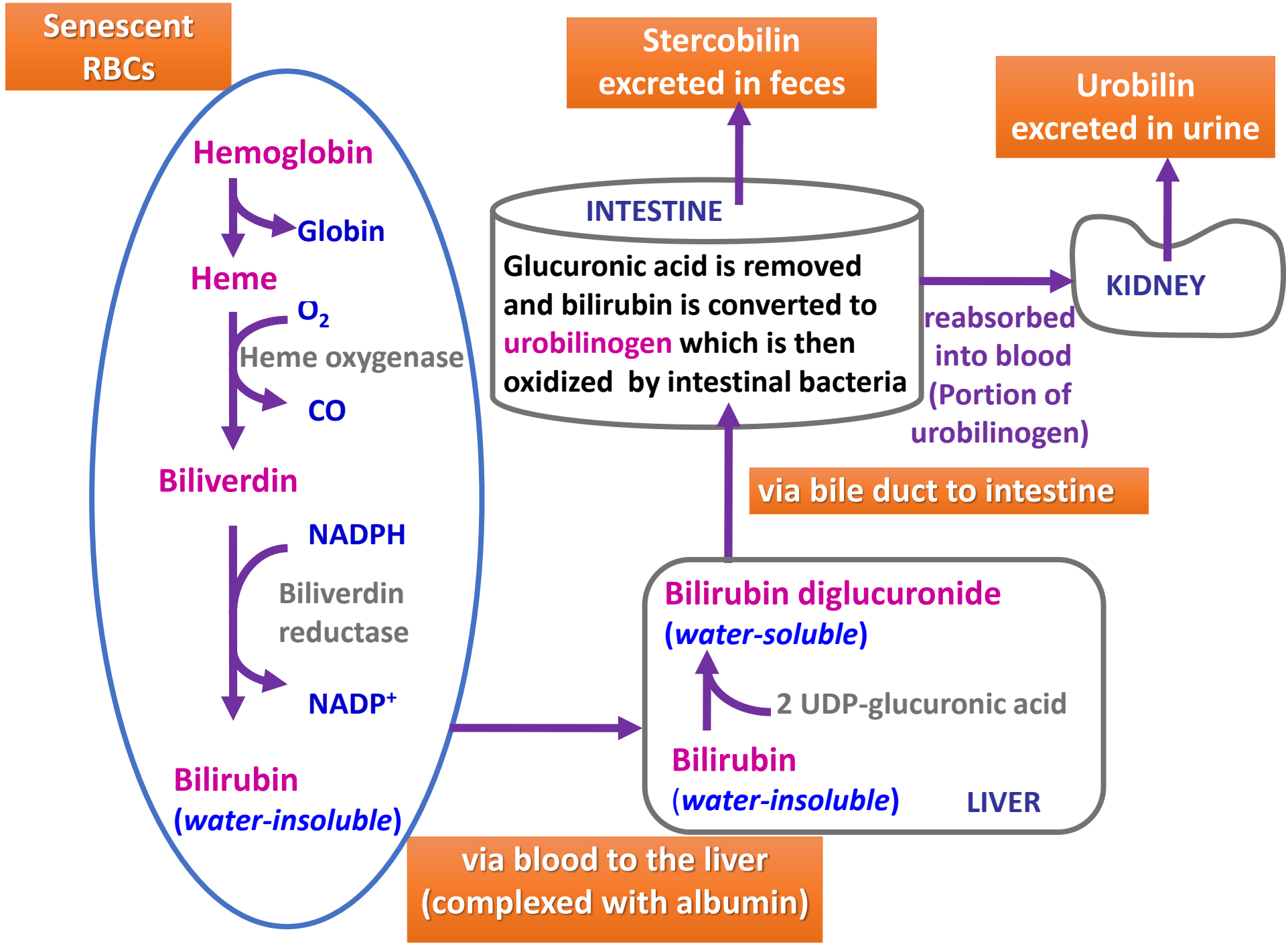


Figure 6.1 **(a)** Normal red blood cell (RBC) breakdown.

Catabolism of hemoglobin



Conjugated vs unconjugated bilirubin
Why is this important?

Unconjugated bilirubin

- Toxic to tissues
- Not soluble in aqueous solutions
- Tightly complexed to albumin
- Cannot be excreted in the urine even when blood levels are high

Conjugated bilirubin

- Water-soluble
- Non-toxic
- Loosely bound to albumin
- Excreted in urine (bilirubinuria)

What is Jaundice?

- Jaundice describes the yellowing of sclera, skin and mucosal membranes due to increased circulating bilirubin in the plasma

- This becomes clinically evident when serum bilirubin reaches about **80-100 μmol/l.**

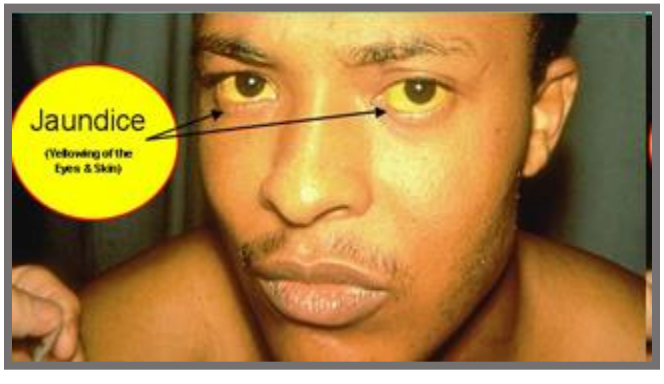
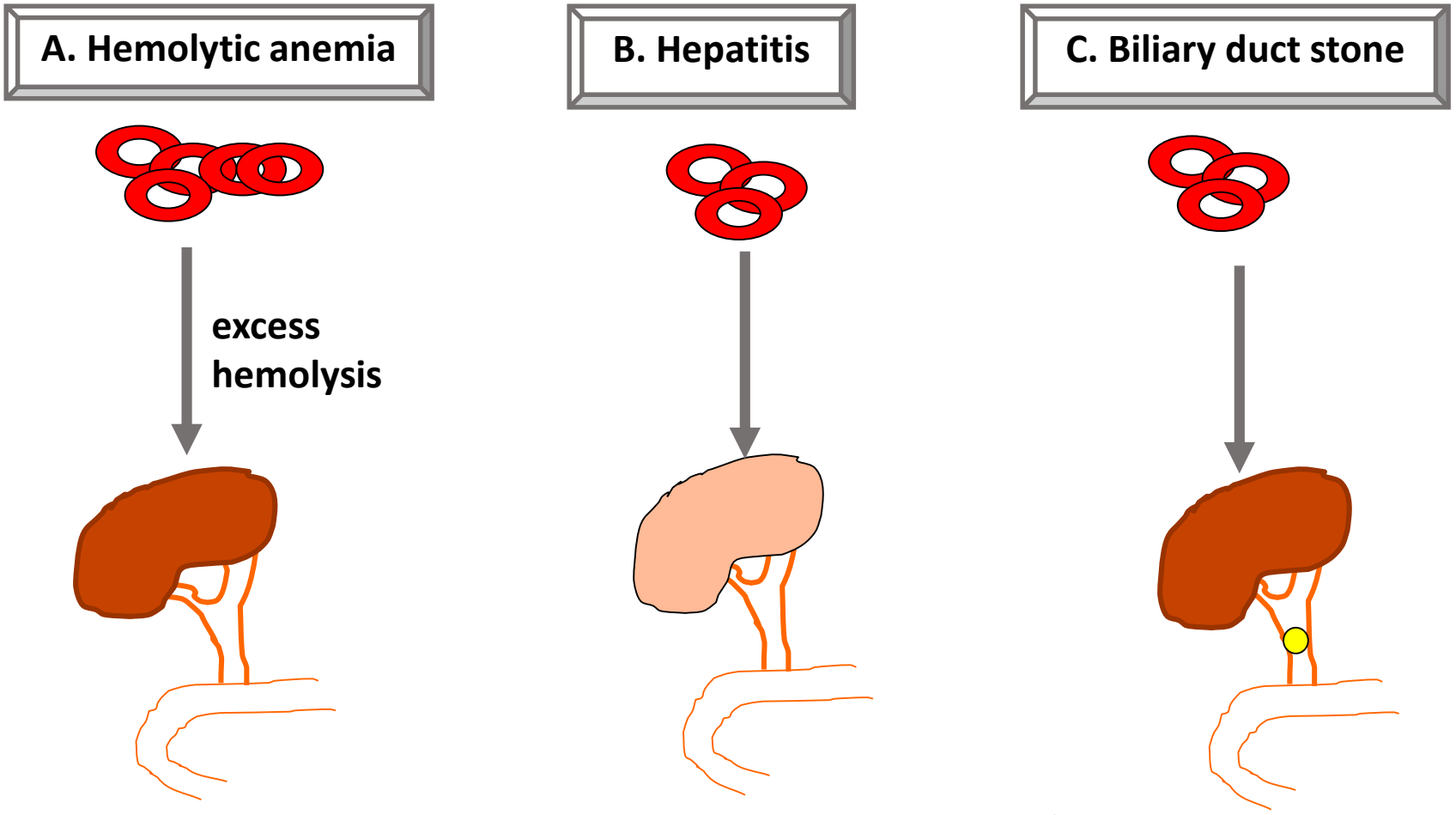
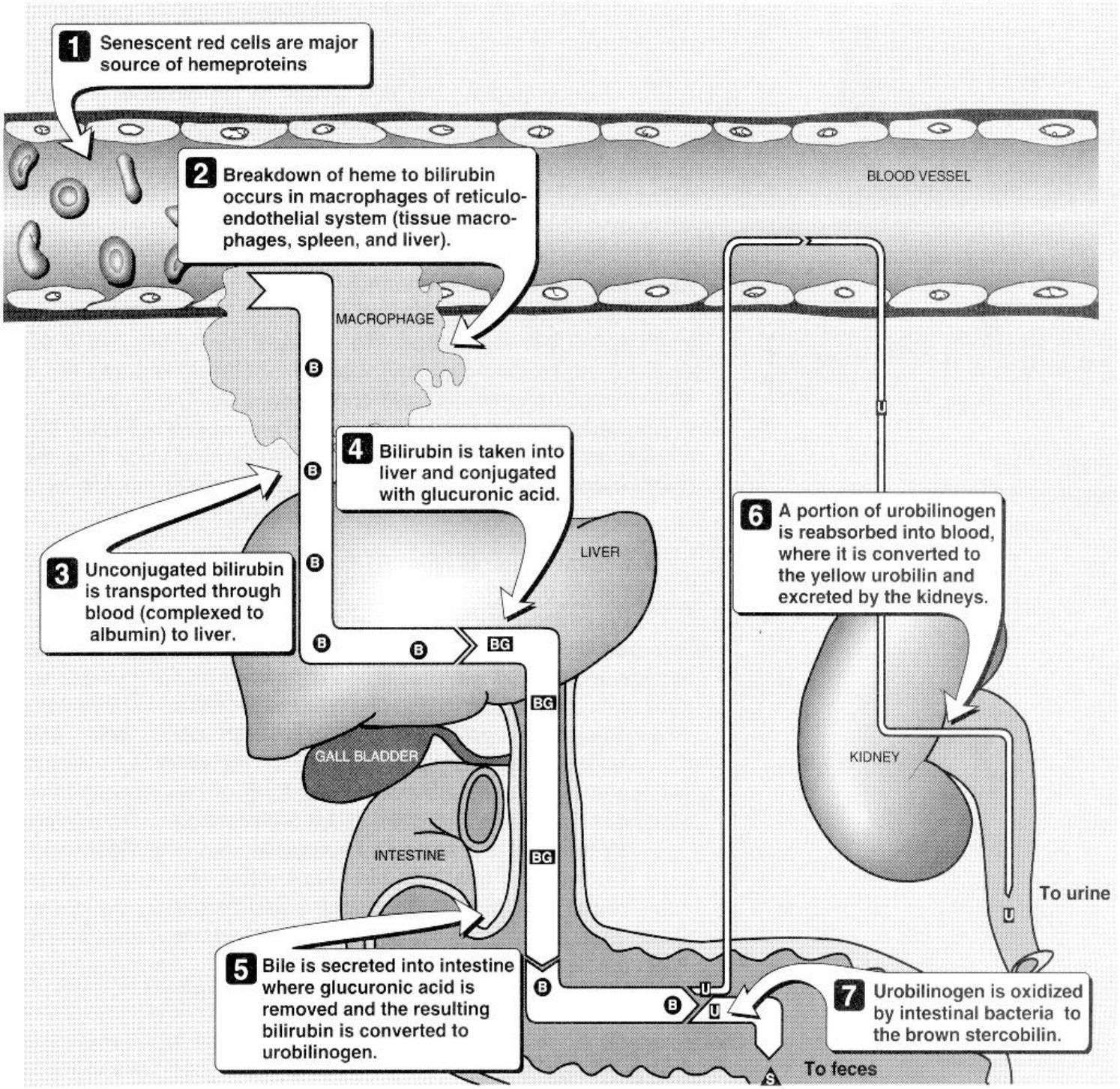
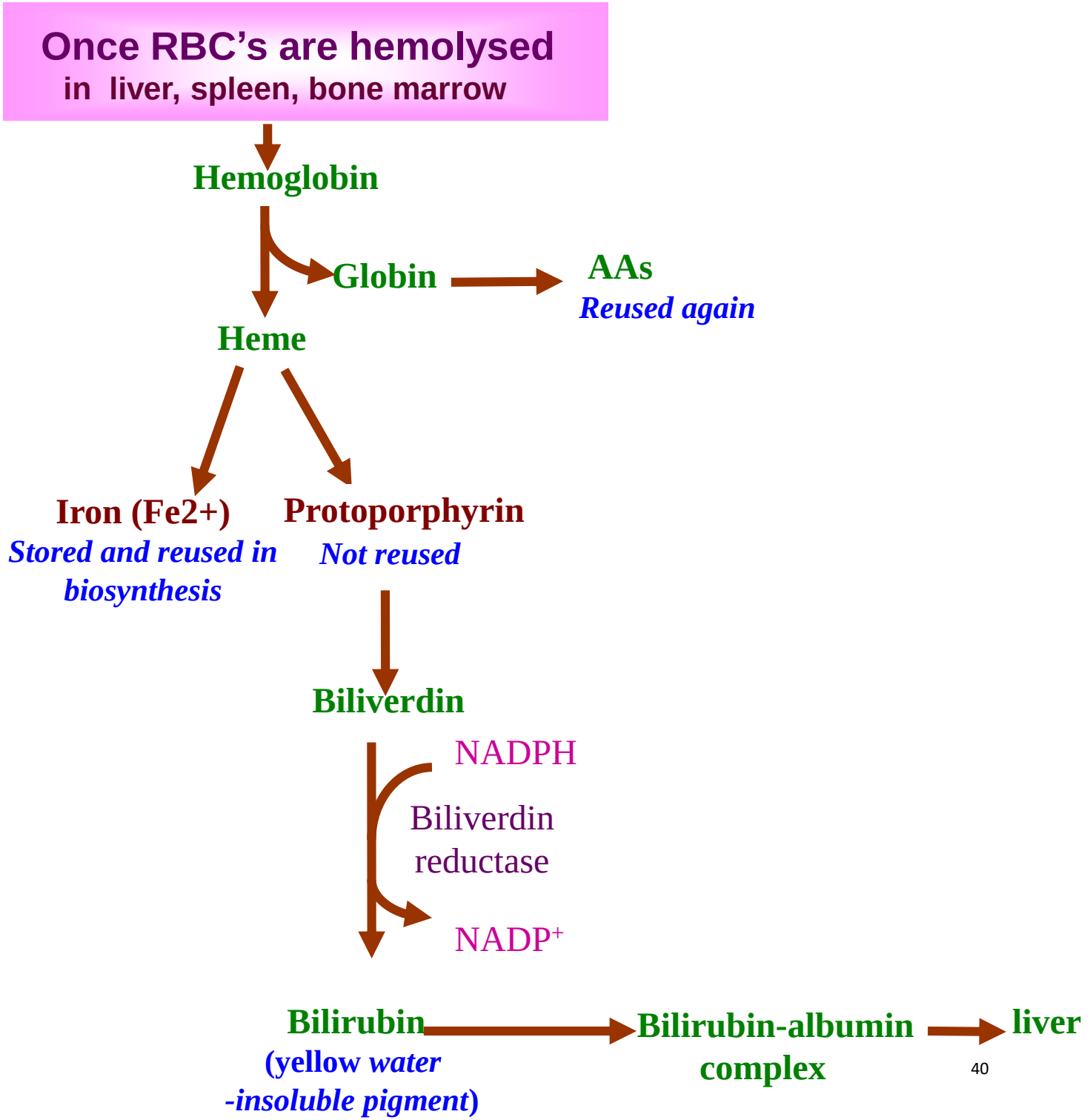


Figure : Examples of hyperbilirubinemia




1 Senescent red cells are major source of hemeproteins
2 Breakdown of heme to bilirubin occurs in macrophages of reticulo-endothelial system (tissue macrophages, spleen, and liver).
BLOOD VESSEL
MACROPHAGE
3 Unconjugated bilirubin is transported through blood (complexed to albumin) to liver.
4 Bilirubin is taken into liver and conjugated with glucuronic acid.
LIVER
6 A portion of urobilinogen is reabsorbed into blood, where it is converted to the yellow urobilin and excreted by the kidneys.
KIDNEY
GALL BLADDER
INTESTINE
To urine
5 Bile is secreted into intestine where glucuronic acid is removed and the resulting bilirubin is converted to urobilinogen.
7 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces
B
BG
U
S


Once RBC's are hemolysed
in liver, spleen, bone marrow
Hemoglobin
Globin
AAs
Reused again
Heme
Iron (Fe2+)
Stored and reused in biosynthesis
Protoporphyrin
Not reused
Biliverdin
NADPH
Biliverdin reductase
NADP+
Bilirubin
(yellow water -insoluble pigment)
Bilirubin-albumin complex
liver