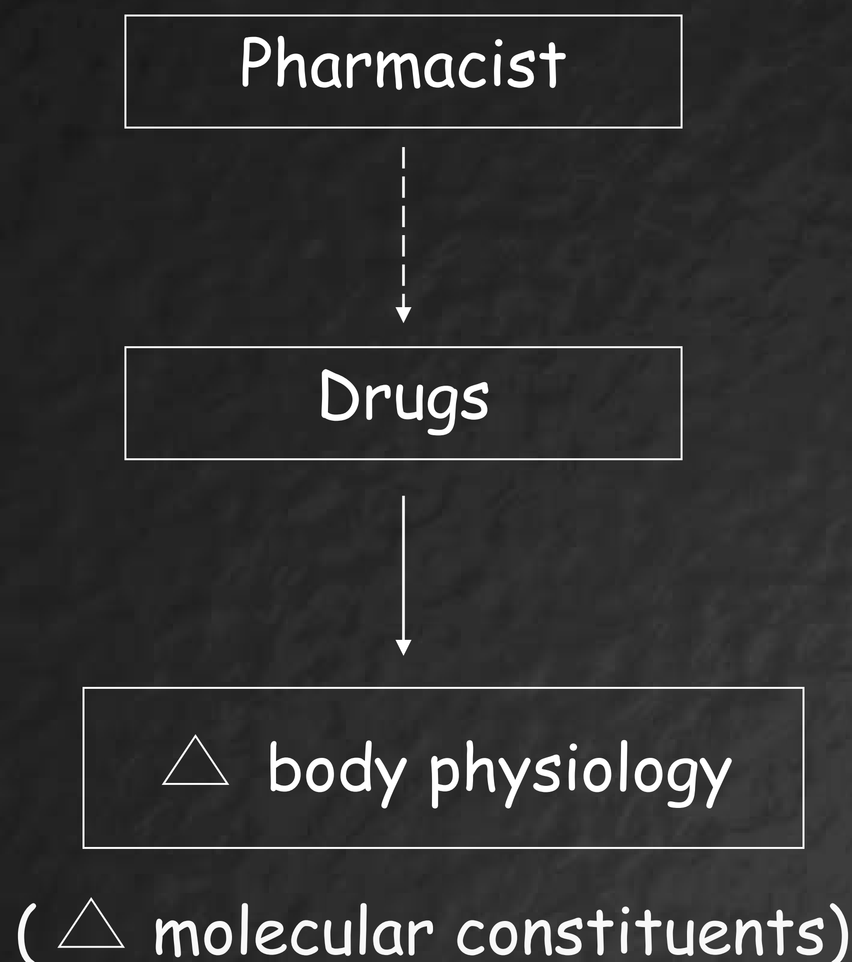
# Why?


Pharmacist
Drugs
△ body physiology
( △ molecular constituents)

# Mechanistic levels of response:

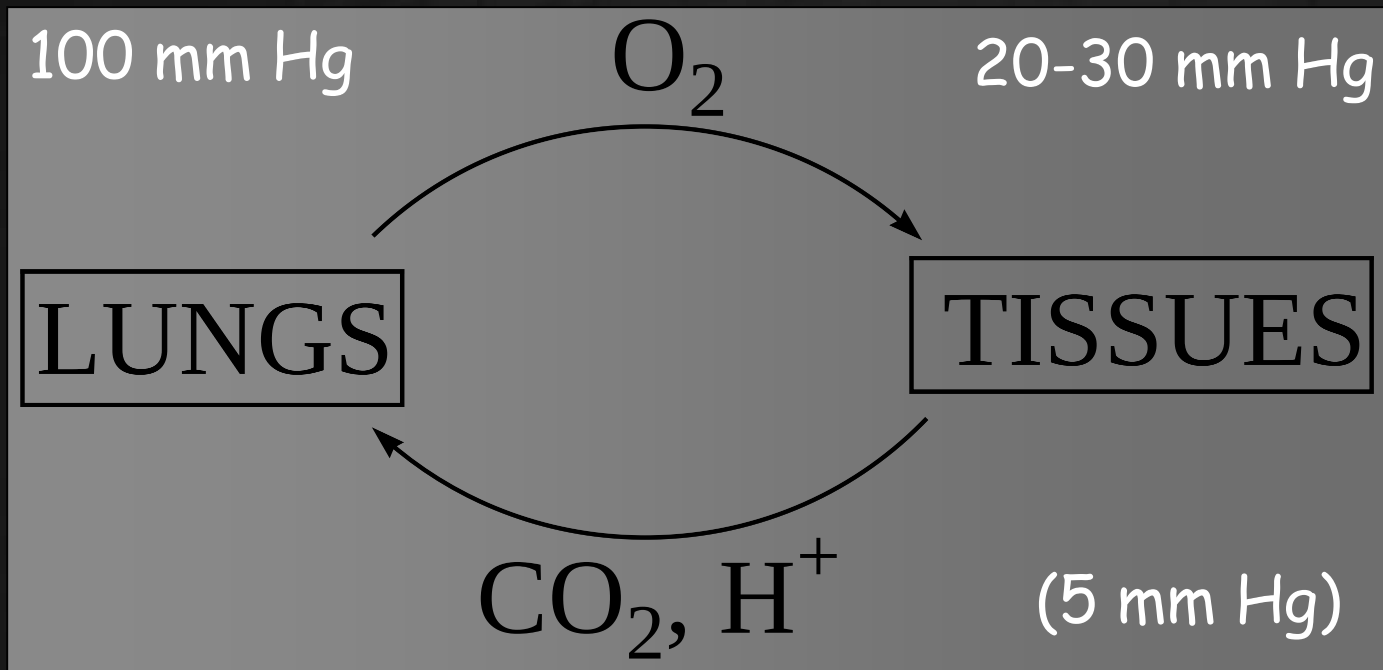
- Altered patient response
- physiologic systems
- Vascular system
- tissues / organs
- blood, muscle, liver
- cellular processes
- erythrocytes
- (proteins)
- hemoglobin
  myoglobin

# UNIT OVERVIEW:
# ERYTHROCYTES AND OXYGEN DELIVERY

1. Biology of erythrocytes / vasculature
2. Hemoglobin and Myoglobin function
3. Energy metabolism in erythrocytes
4. 2,3 Diphosphoglycerate
5. Drugs / toxins which affect erythrocyte function

Recommended reading:
(Devlin) pp. 393-410, (Stryer) pp. 269-274

# Primary RBC function: transport of $O_2$ / $CO_2$



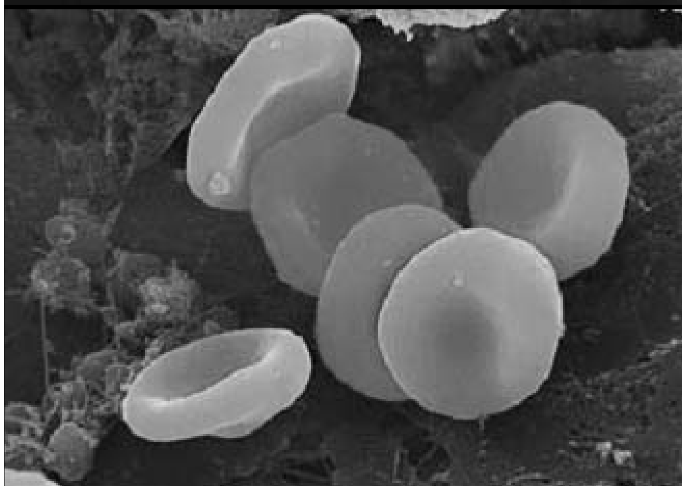

*Blood is a colloid, in addition to RBC's, blood also contains:

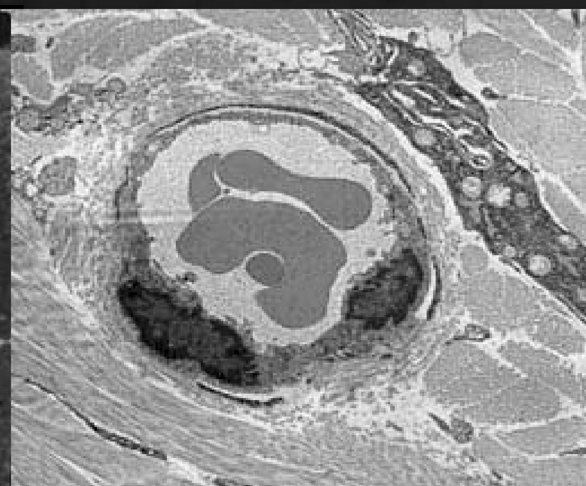
Additional cell types: leucocytes (WBC's), platelets (clotting)

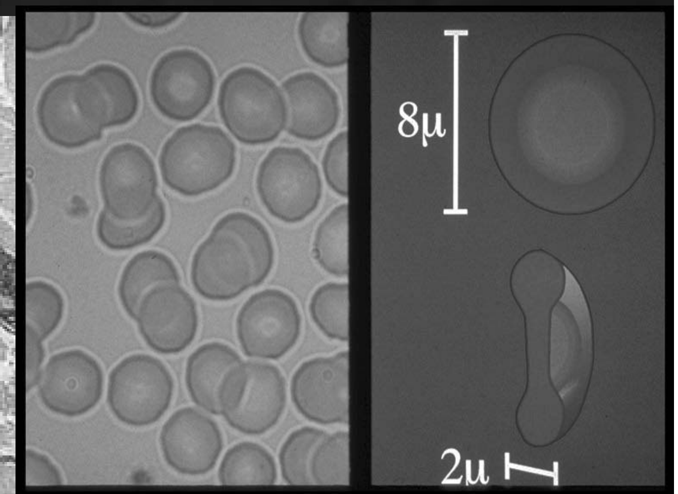
Free proteins: albumin, globulins (Ig), ferritins (transport), enzymes (clotting), hormones

Other non-cellular components: electrolytes

# Erythrocytes:







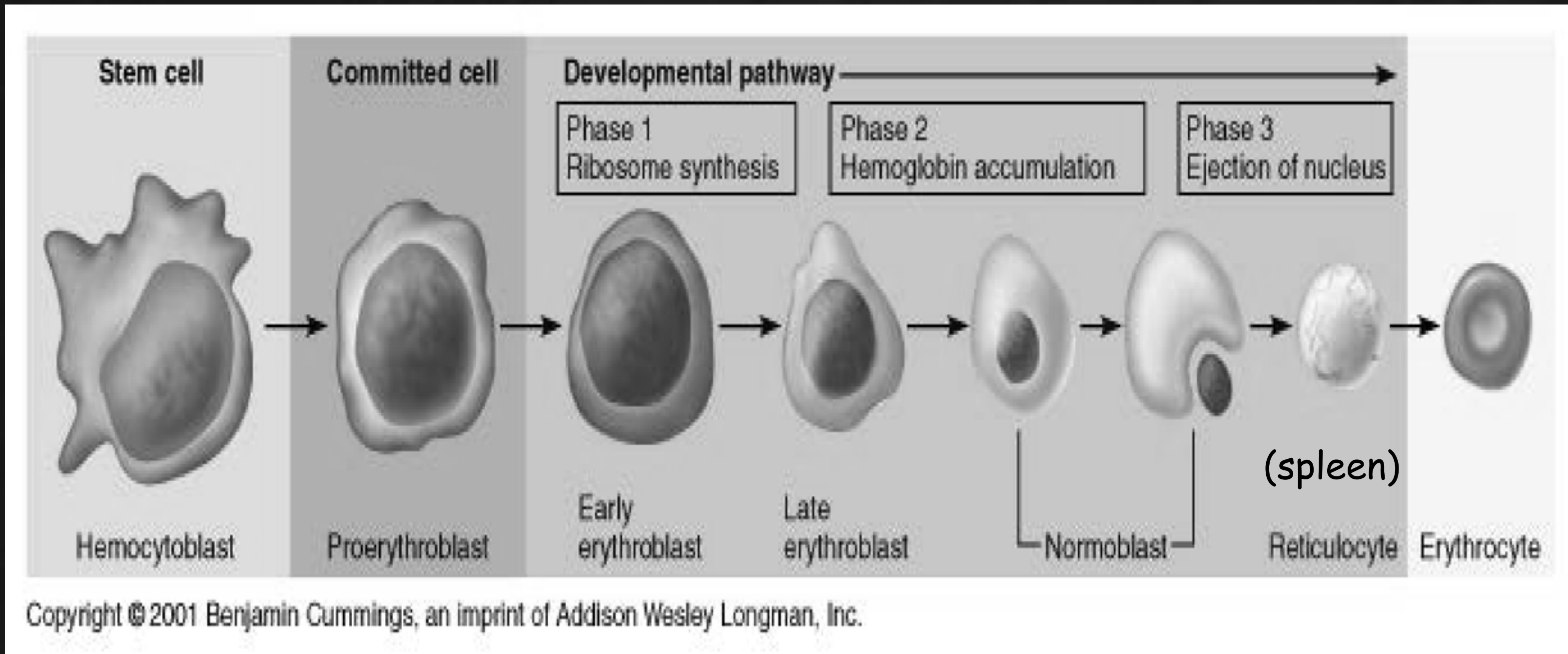

From Tina Carvalho (MicroAngela)

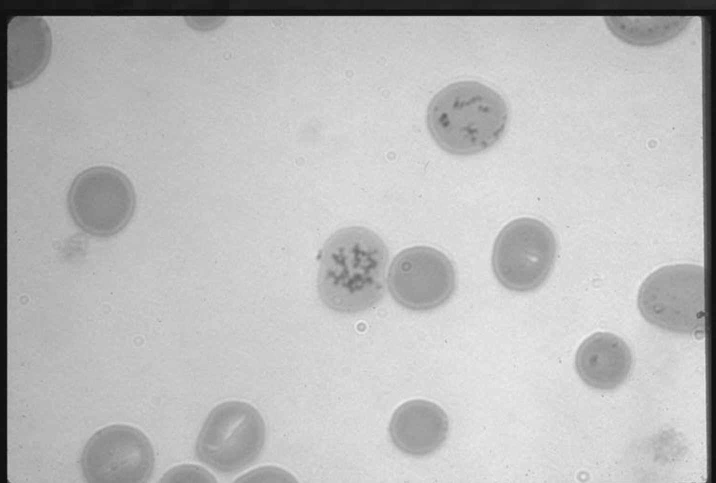
Key erythrocyte features:

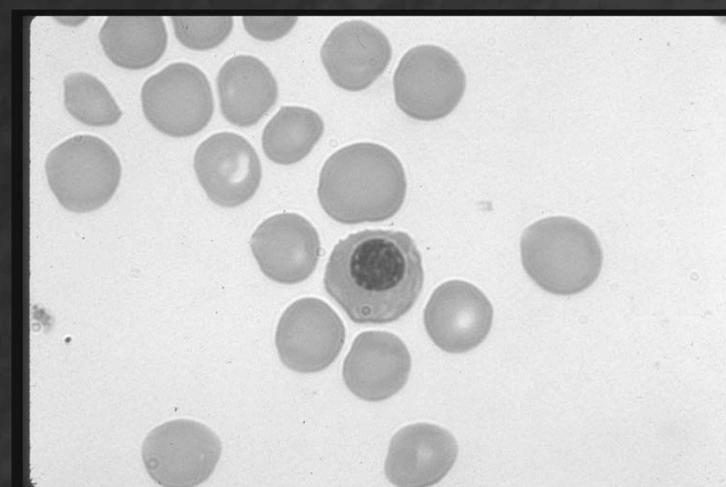
- 95% of cellular protein is hemoglobin (35% by weight)
- humans: 300x $10^6$ Hb molecules/RBC, 4.2-5.8 x $10^6$ RBC's/cc blood
- hematocrit 35-50%, produce / destroy 2.5 x $10^6$ RBC's/sec
- RBC's harbor variety of membrane transporters (glucose) on cell surface

| RBC Parameters | Normal Values |
|---|---|
| | |
| Hematocrit | |
| Females | 35-47% |
| Males | 40-52% |
| Hemoglobin | |
| Females | 12.0-16.0 gm/dl |
| Males | 13.5-17.5 gm/dl |
| | |
| MCV | 80-100 fl |
| Reticulocyte Count | 0.2-2.0% |

# Erythrocyte development:

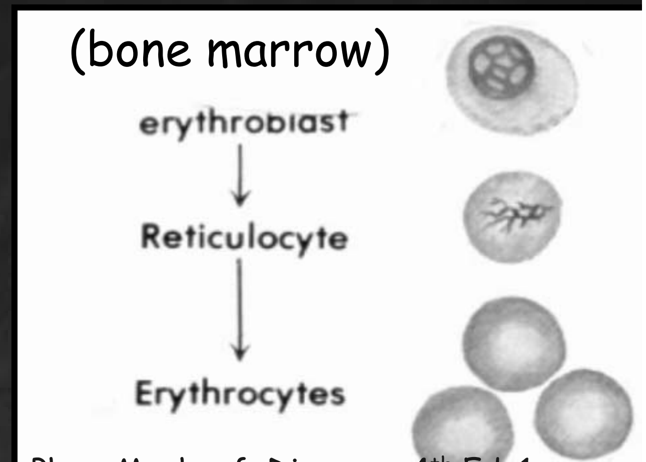




# BIOLOGY OF ERYTHROCYTES/VASCULATURE

* Fetal development – RBC's produced in liver. Bone marrow production commences at 4 mo. in humans. Adult – erythrocyte production occurs only in bone marrow.



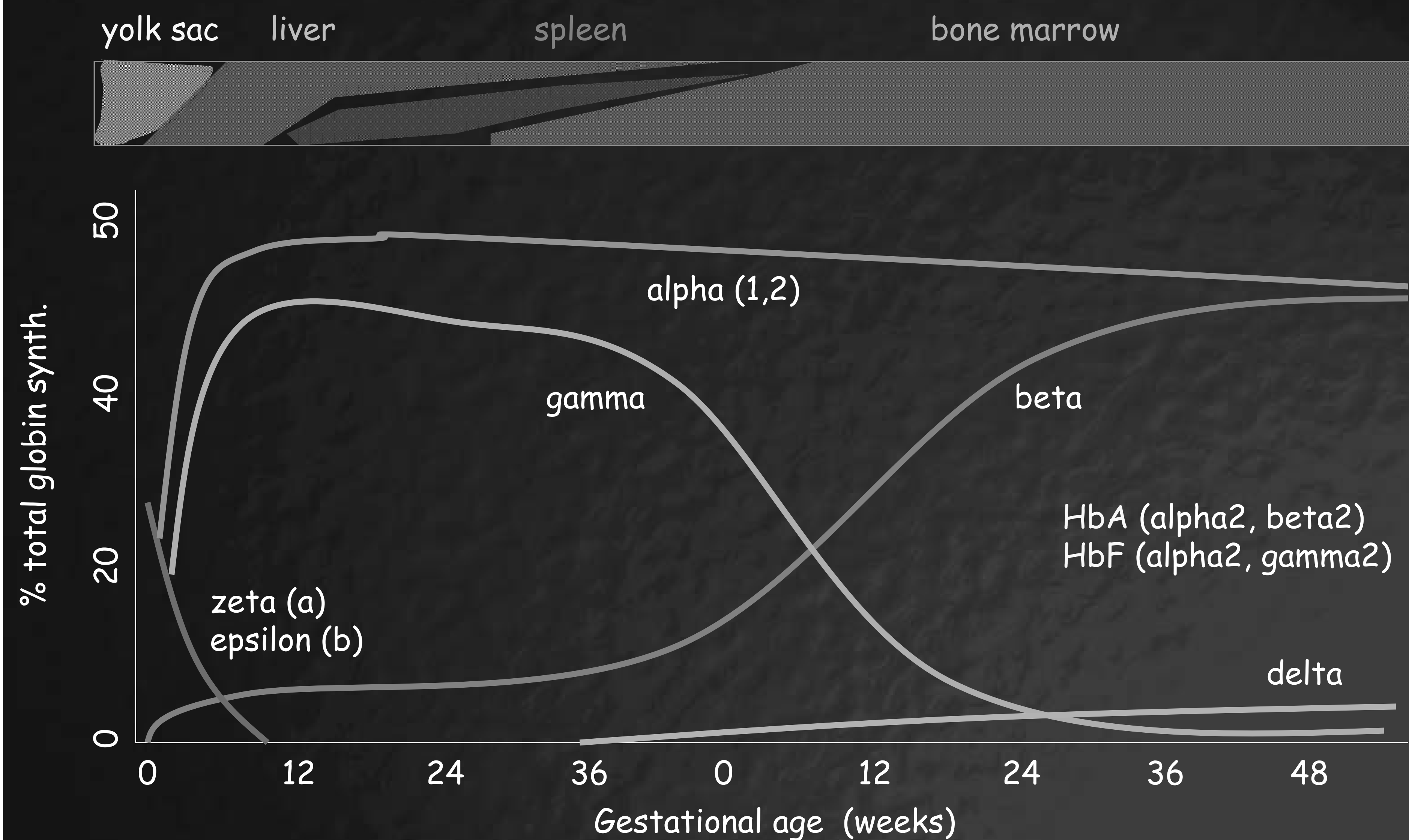
Phys. Mech. of Disease: 4th Ed. 1

* Mitochondria, nuclei and endoplasmic reticulum lost as reticulocytes mature into adult erythrocytes. Thus NO mito. Respiration.

* Therefore NO gene transcription or protein translation occurs in RBC's – all proteins within the erythrocyte must be produced at the time of genesis.

* No mitochondrial respiration, thus low ATP formation. Energy requirements of the cell must be met largely through GLYCOLYSIS.

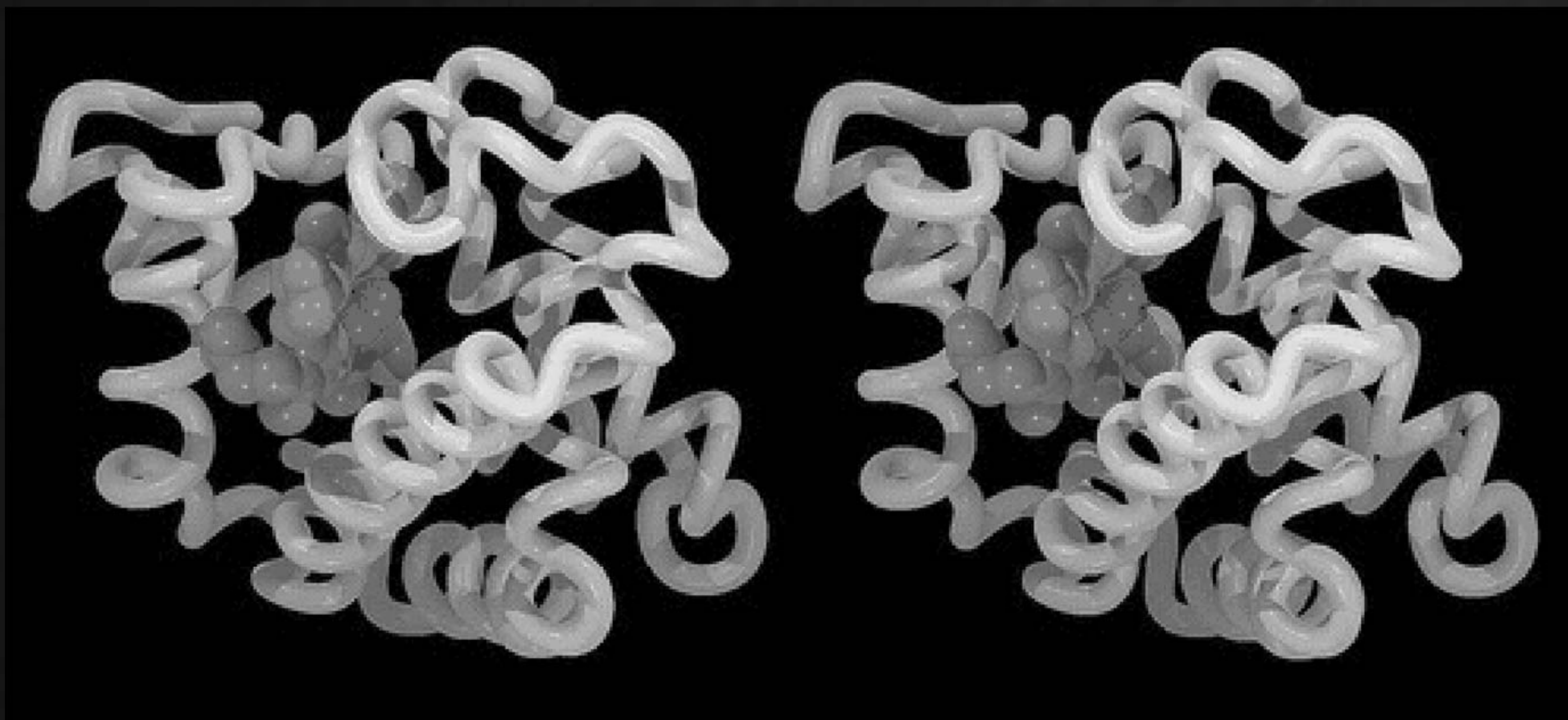
# Globin synthesis during development:



# HEMOGLOBIN and MYOGLOBIN

- Physical structure of hemoglobin
- Developmental expression of globin genes
- Mechanisms of $O_2$ regulation
- Allosterism and important conformational changes
- Regulation by external agents

# Structure of Myoglobin:



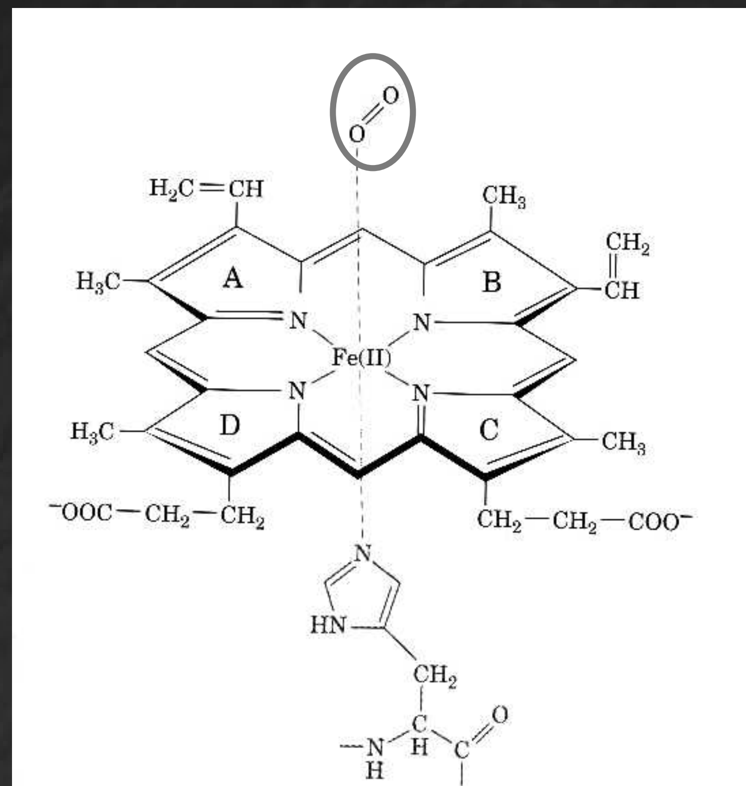
# Hemoglobin / Myoglobin: Heme-containing proteins


pyrrole ring

The iron atom in heme can form 6 bonds.

Catabolism:
Fe ⟶ reused (Tf)
Globin ⟶ peptidase (AA)
Heme ⟶ Bilirubin

*Heme* - a cyclic tetrapyrrole
(Fe(II)-protoporphyrin IX)

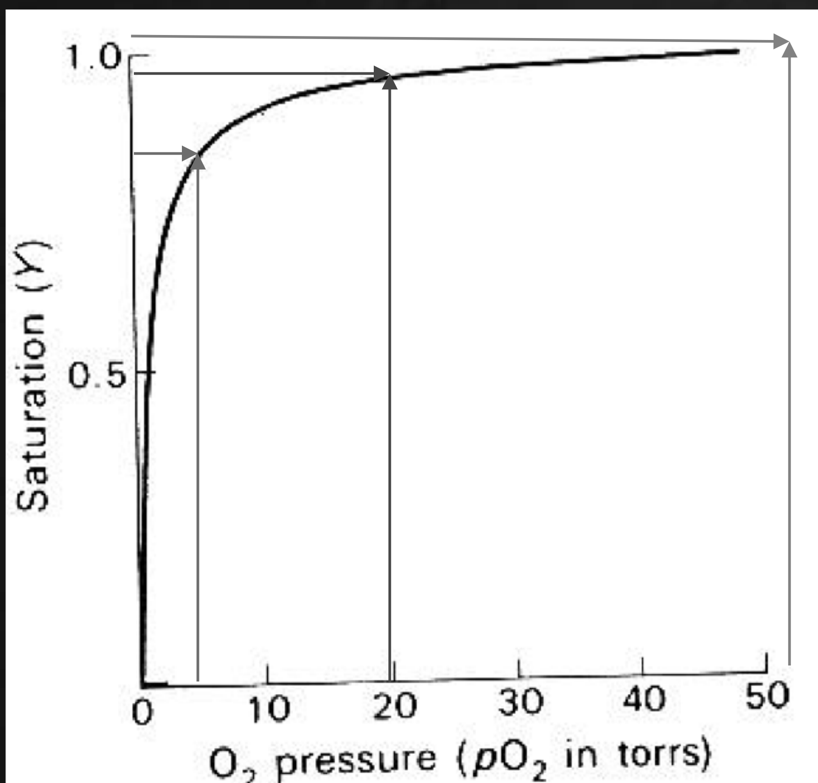



$O_2$ binding to heme of Mb or Hb is reversible

Devlin Fig. 9.31, 9.32, pyrrole - Harpers Fig. 7-1

# Myoglobin (Mb) $O_2$ binding curve:

Myoglobin: single chain protein, 1 heme/protein, first x-ray solution structure solved. Because it contains only a single subunit, it does NOT display cooperativity or allosterism (hyperbolic O2 curve).



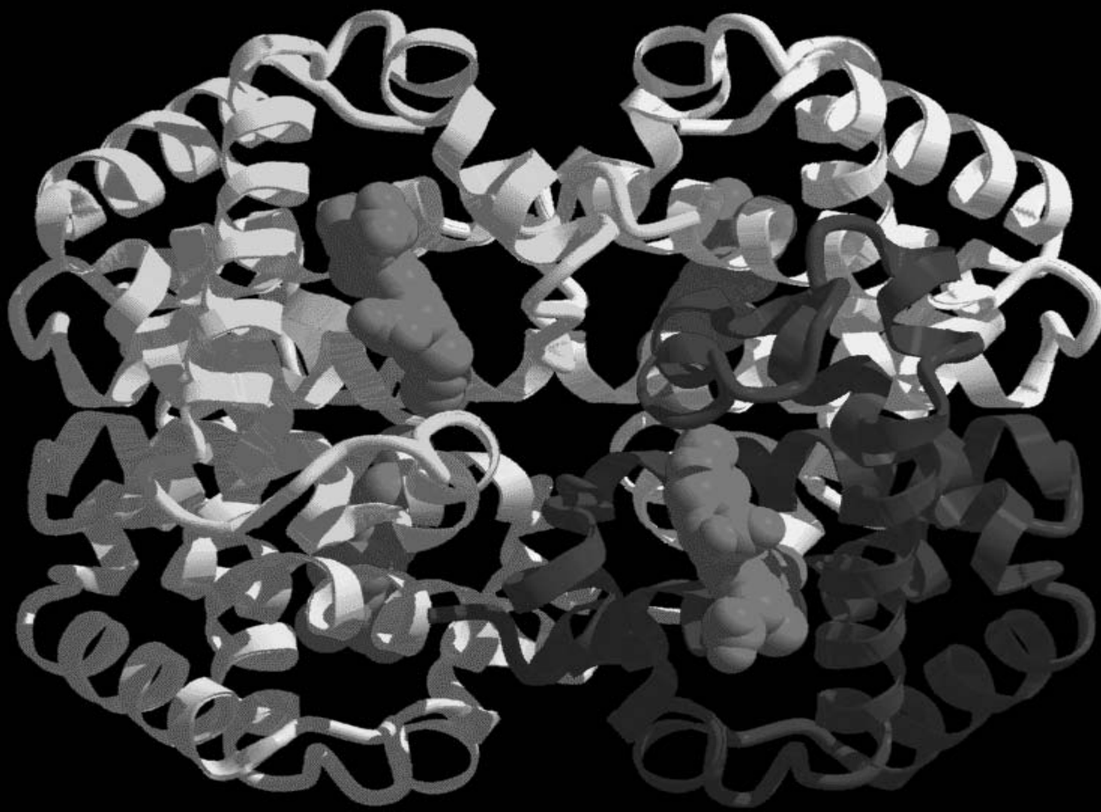

$P_{50}$ Mb: 2.8 torr

*low $P_{50}$ = high $O_2$ affinity*

$pO_2$ lungs: 100 mm Hg
tissues: 20 mm Hg
working muscle: 5 mm Hg
~1,100 m. years ago

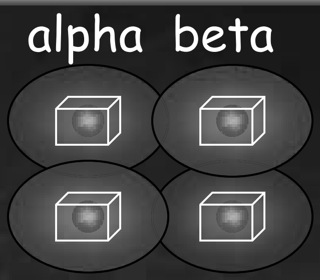
modified from Stryer Fig 10.17, see also Devlin 9.35

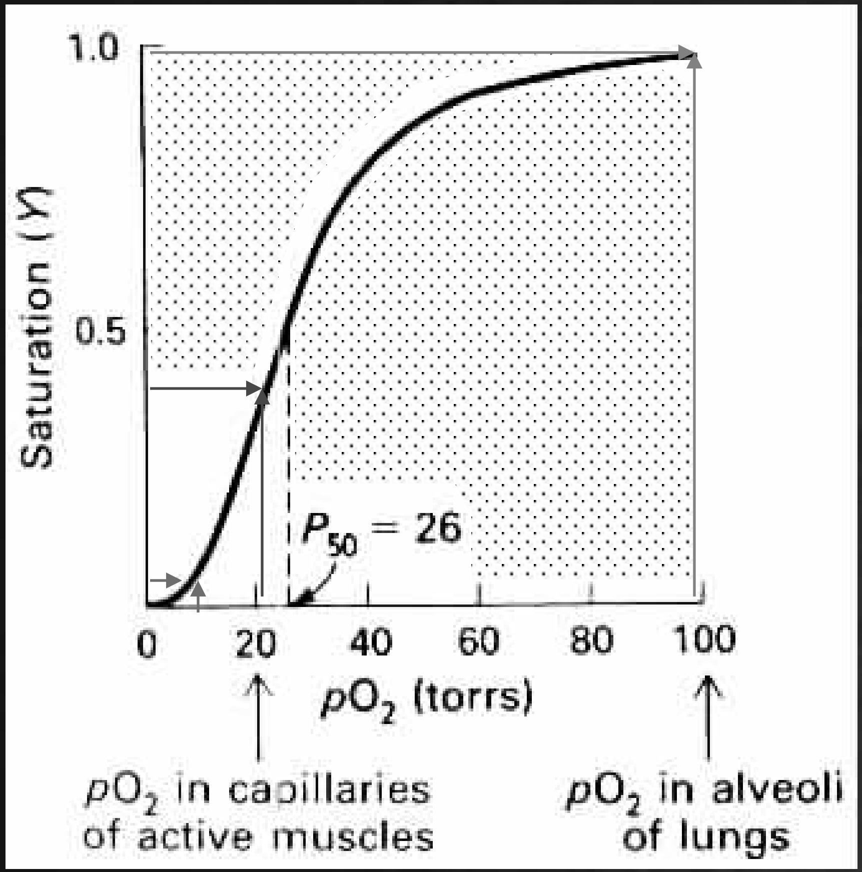
# Hemoglobin Structure:



# Hemoglobin (Hb) $O_2$ binding curve:

Hb: tetrameric protein (~2-500 m years)
4 subunits and heme groups/protein




Devlin 9.35

The tetrameric structure of Hb imparts it with several important properties:

Allosterism:
The binding of a ligand ($O_2$) at one site affects the binding of other ligands at distal sites. Thus Hb exhibits sigmoidal $O_2$ kinetics.

Positive cooperativity:
The affinity of Hb for the 4th $O_2$ is 100x greater than for the first, due to conformation changes in Hb.

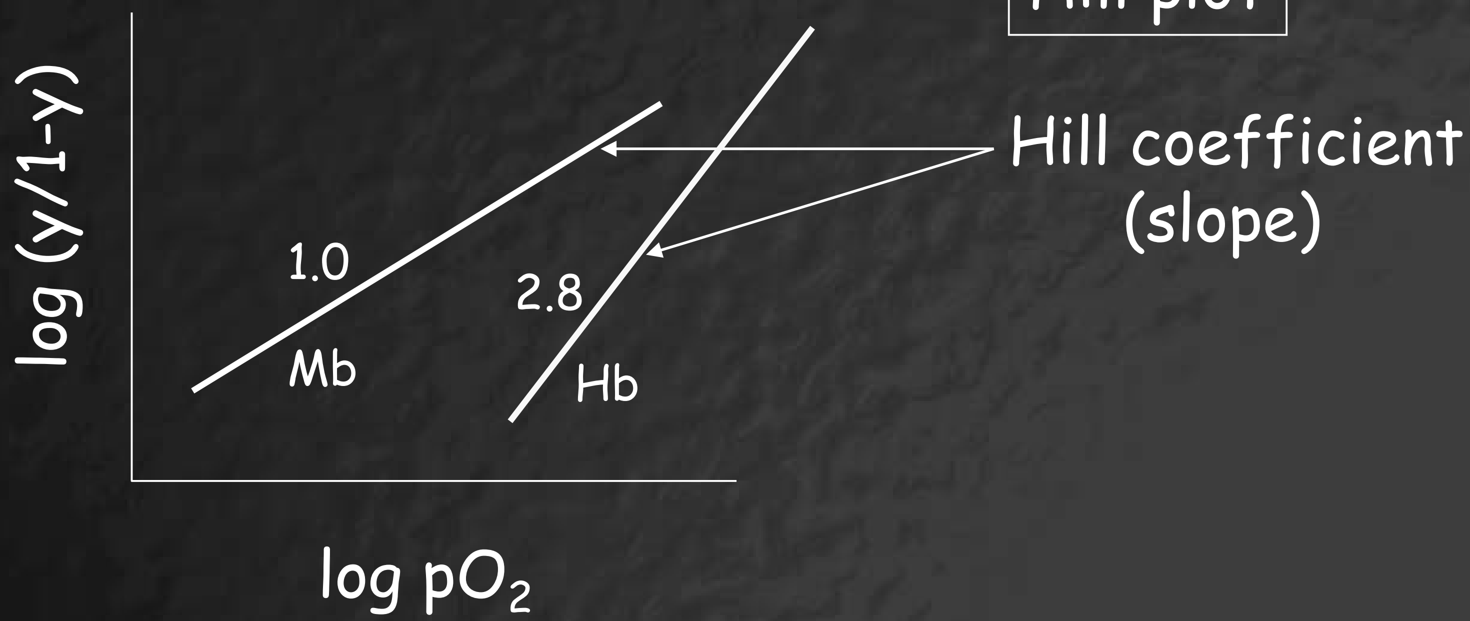
# Measures of cooperativity, Hill plot:

$$Y = \frac{\text{number of binding sites occupied}}{\text{total number of binding sites}}$$

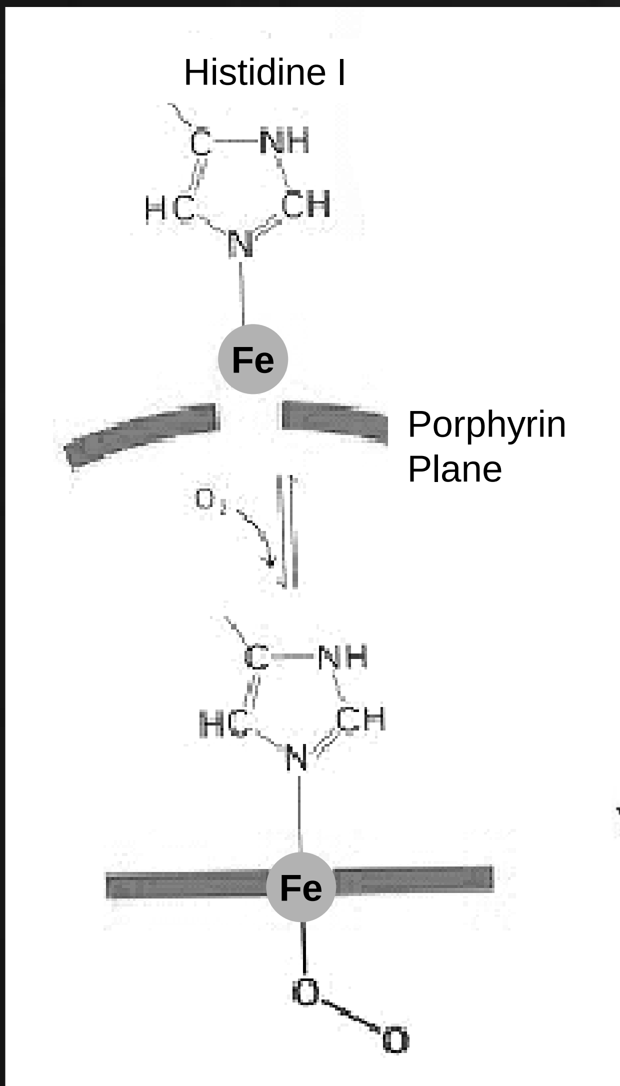
$$Y/1\text{-}Y = pO_2 / pO2^{(50)}$$

$$\log Y/1\text{-}Y = \log pO2 - \log pO2^{(50)}$$

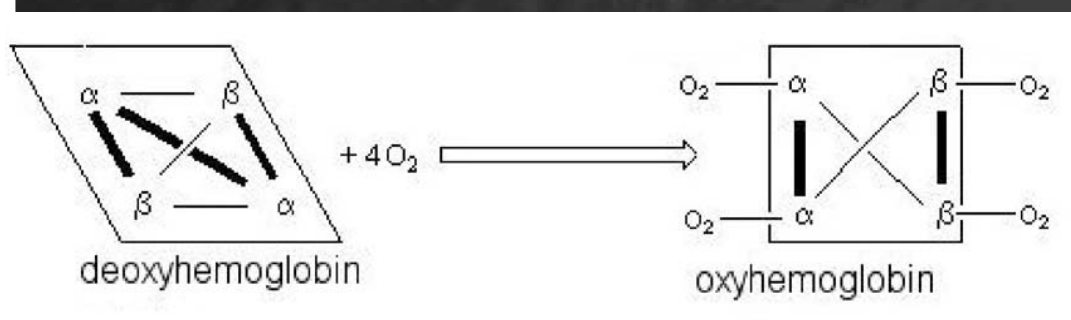
Hill plot



# Perutz mechanism:




On the basis of the X-ray structure of oxy- and deoxyhemoglobin, Perutz formulated a mechanism for hemoglobin oxygenation. Perutz postulated that hemoglobin has 2 stable conformational states; the dexoy "T"-state, and the fully oxygenated "R"-state. The conformation of subunits in T-state hemoglobin differ from those in the R-state. O2 binding initiates a series of coordinated movements that result in a shift from the T to the R state in a few microseconds.




R = relaxed = oxy state, T = tense = deoxy state

# Oxygen Binding Site of Hemoglobin:

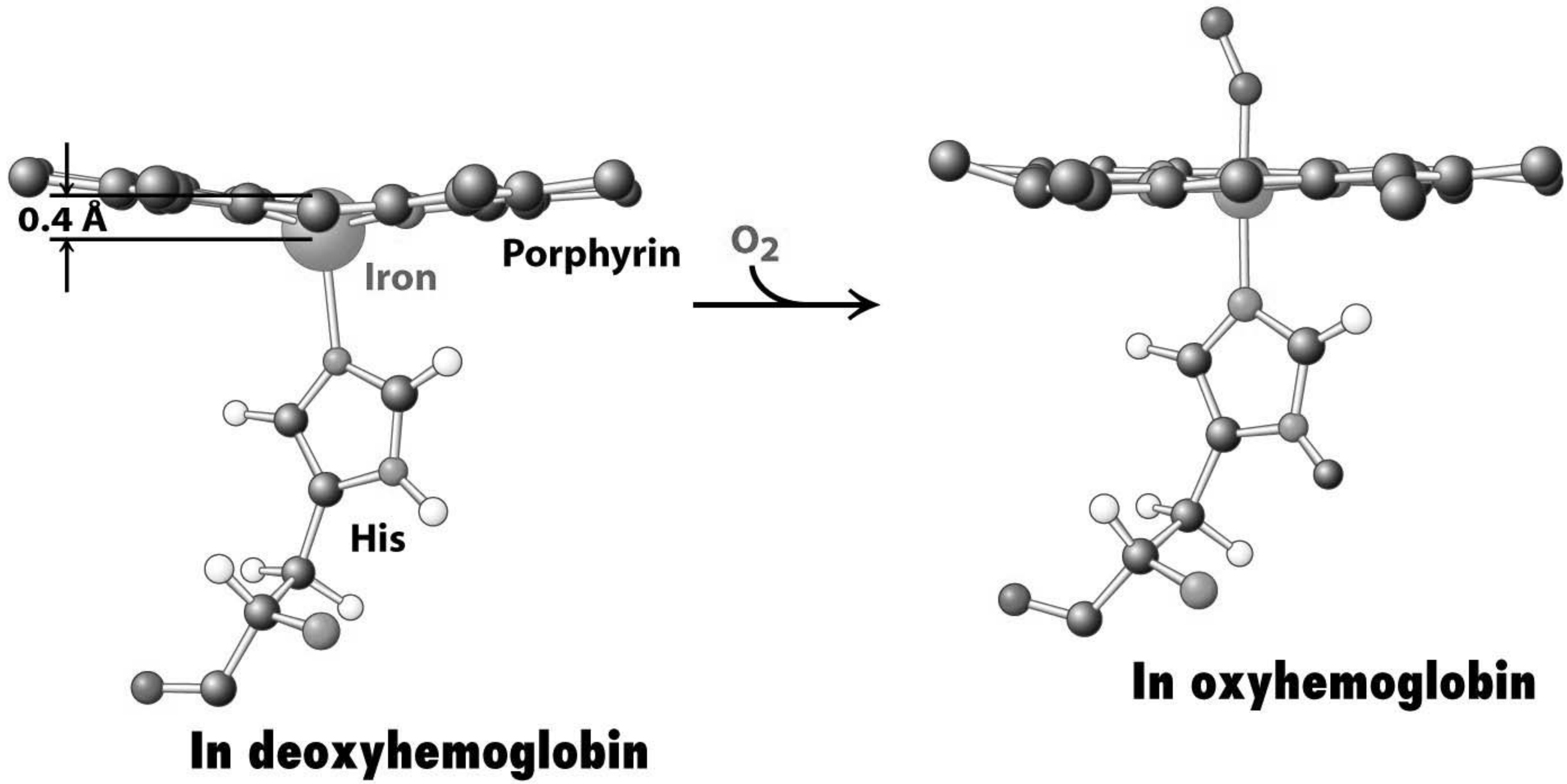



**Figure 7-2**
***Biochemistry, Sixth Edition***

# Oxygen Binding Site of Hemoglobin:

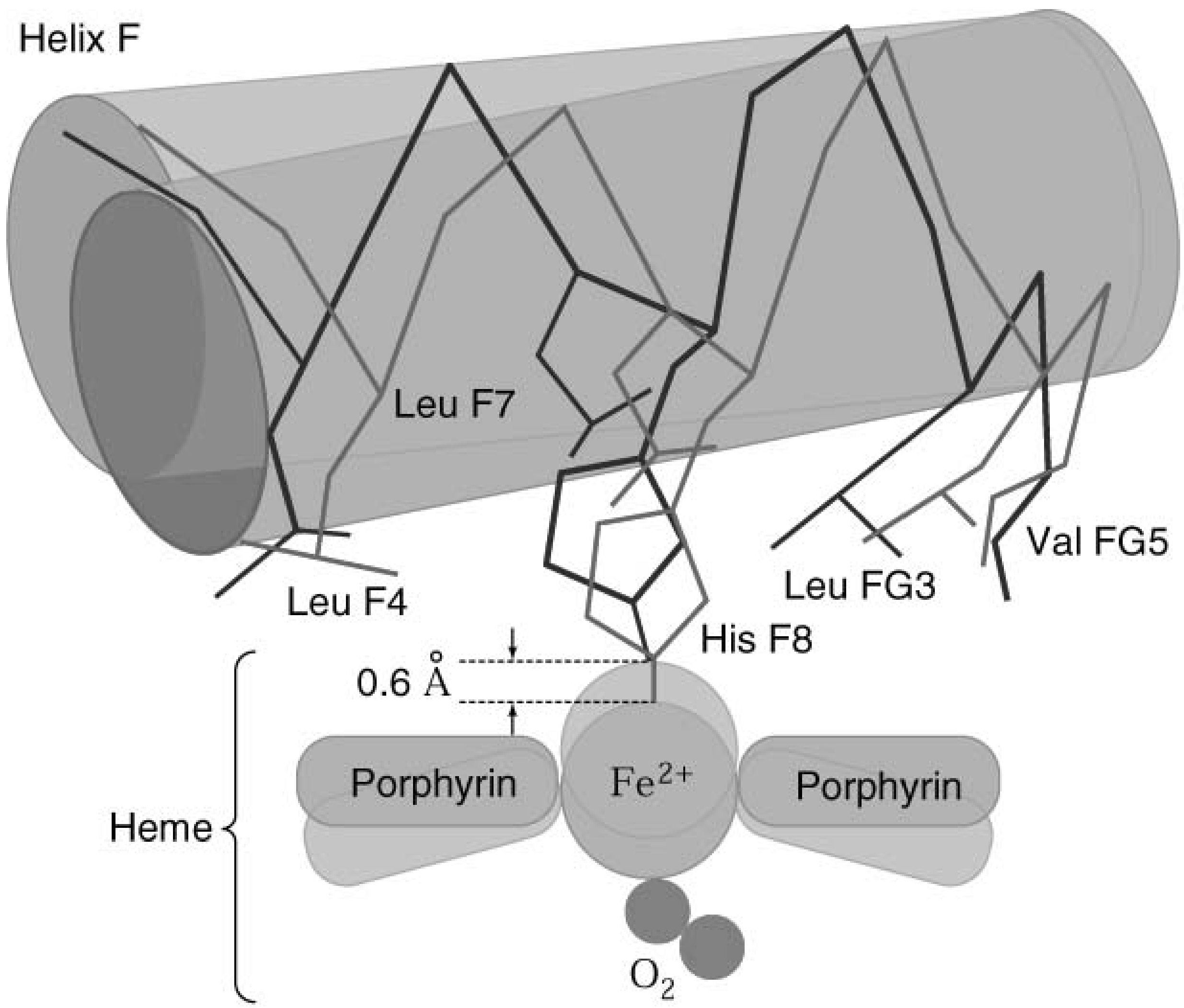
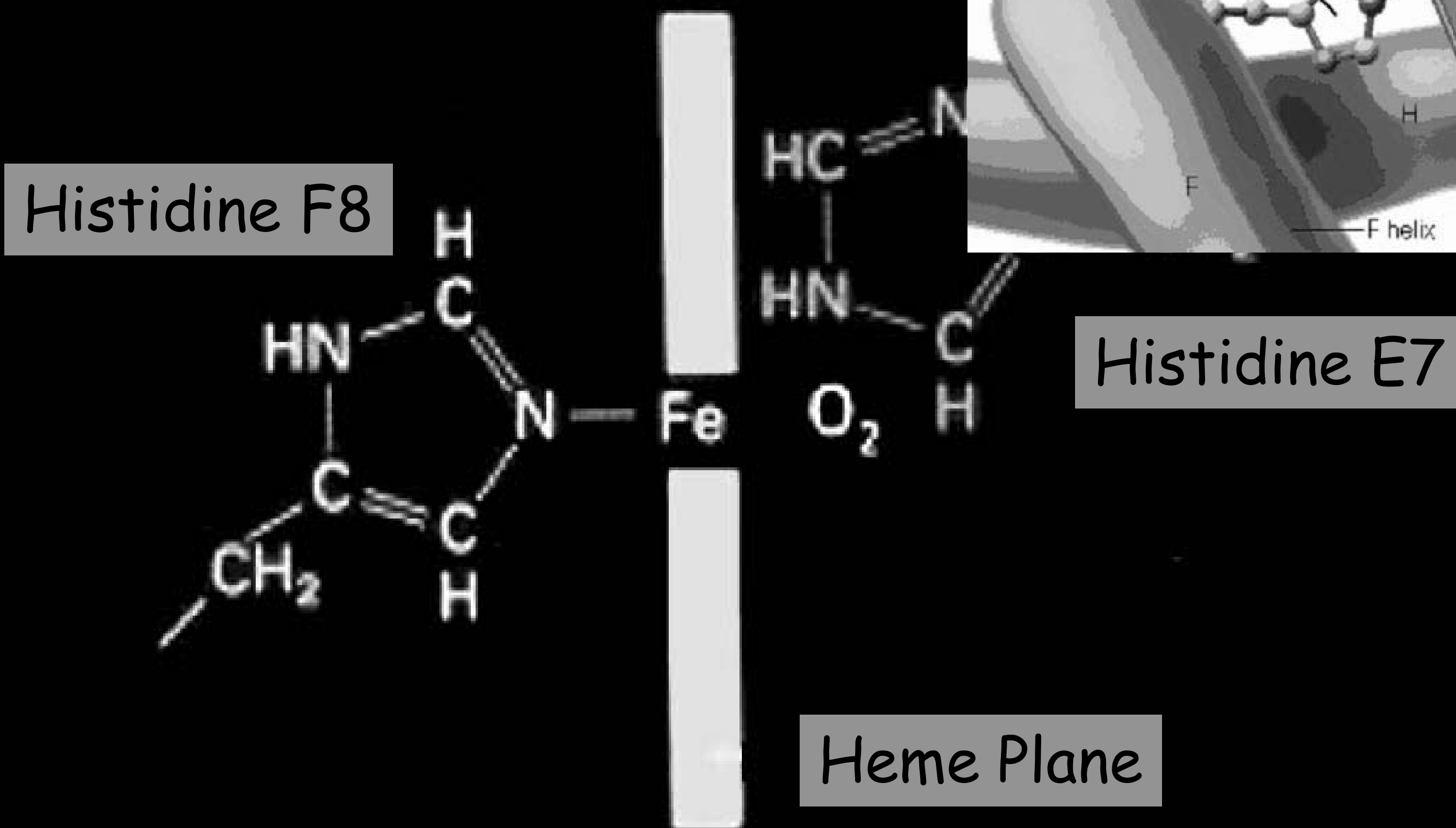
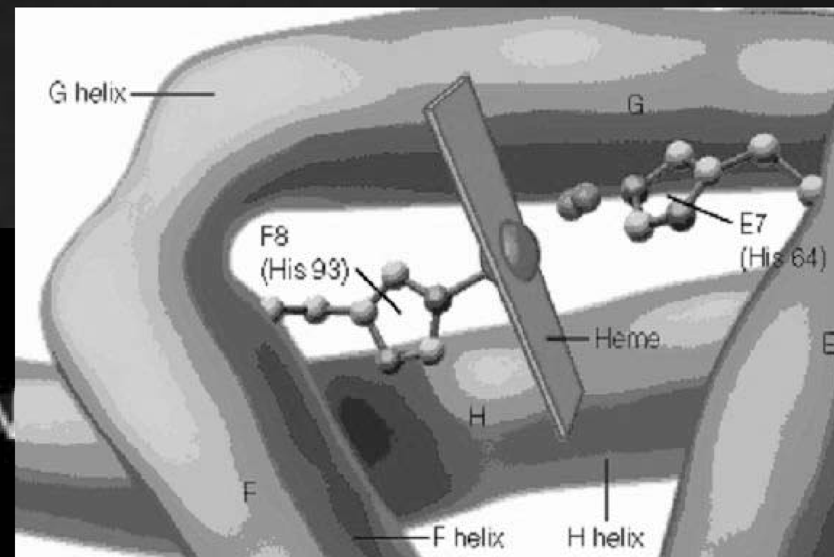



Figure 7-9. Movements of the heme and the F helix during the T → R transition in hemoglobin.

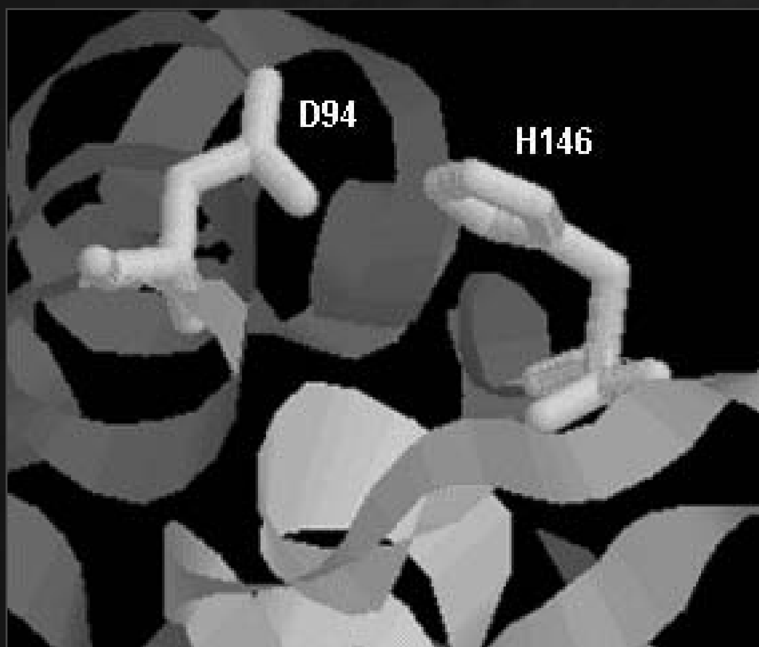
# Oxygen Binding Site of Hemoglobin:

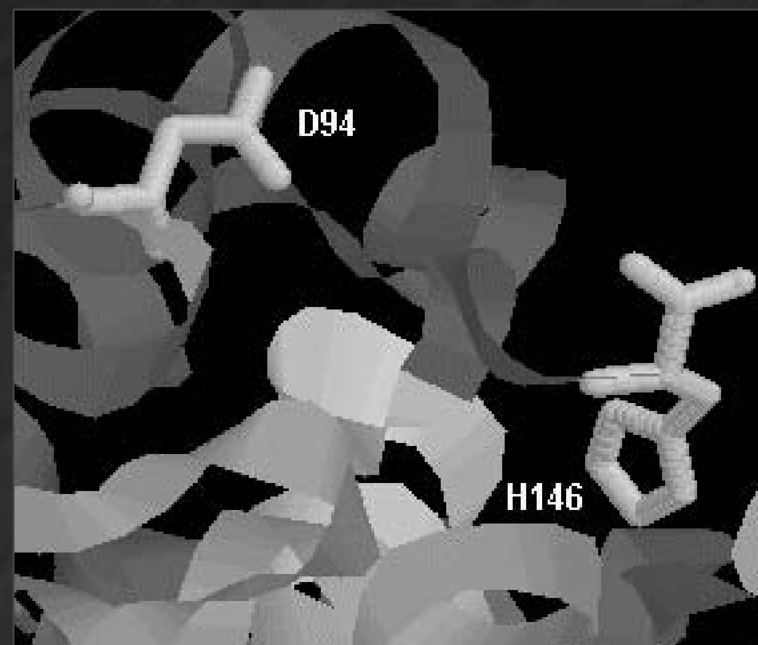


# Structural states of Hb:

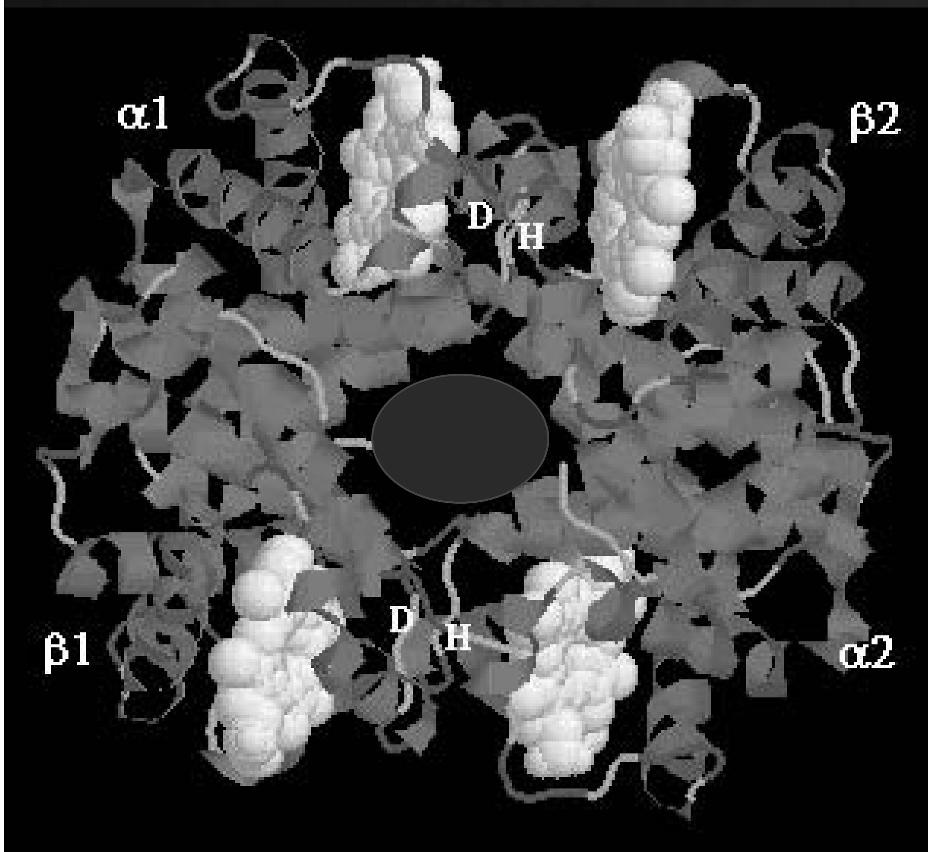
Deoxy Hb (T state) ⇌ Oxy Hb (R state)

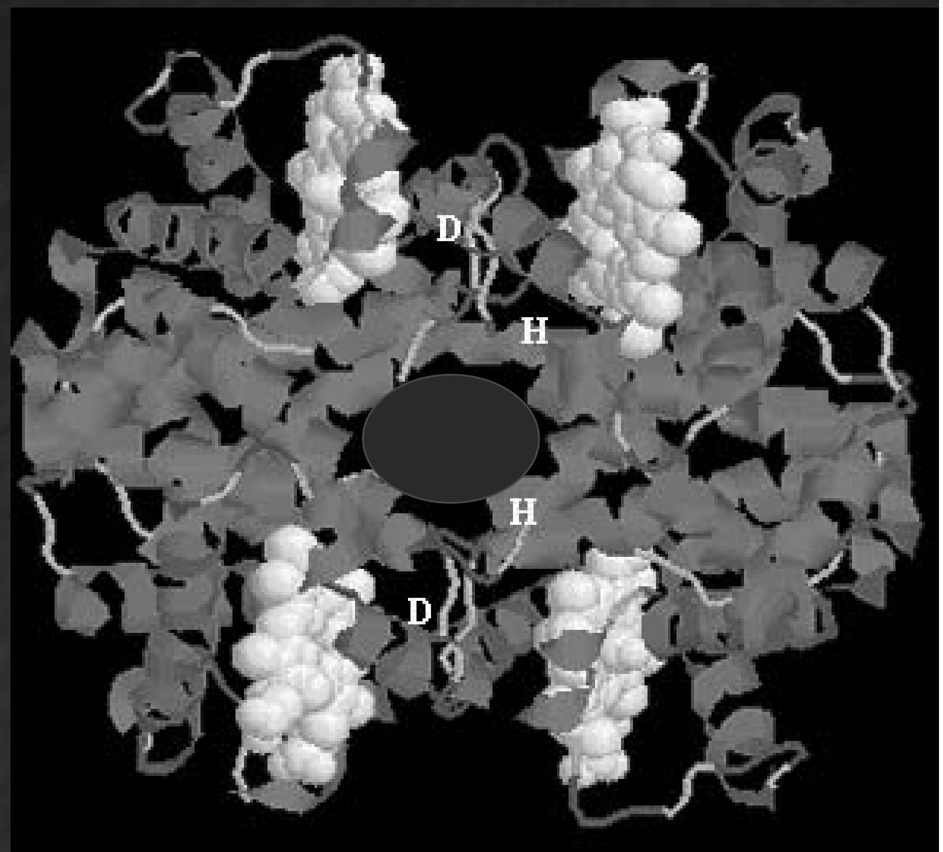


# Structural states of Hb:

Deoxy Hb (T state) ⇄ Oxy Hb (R state)



# The Bohr effect:

Blood pH: 7.35-7.5

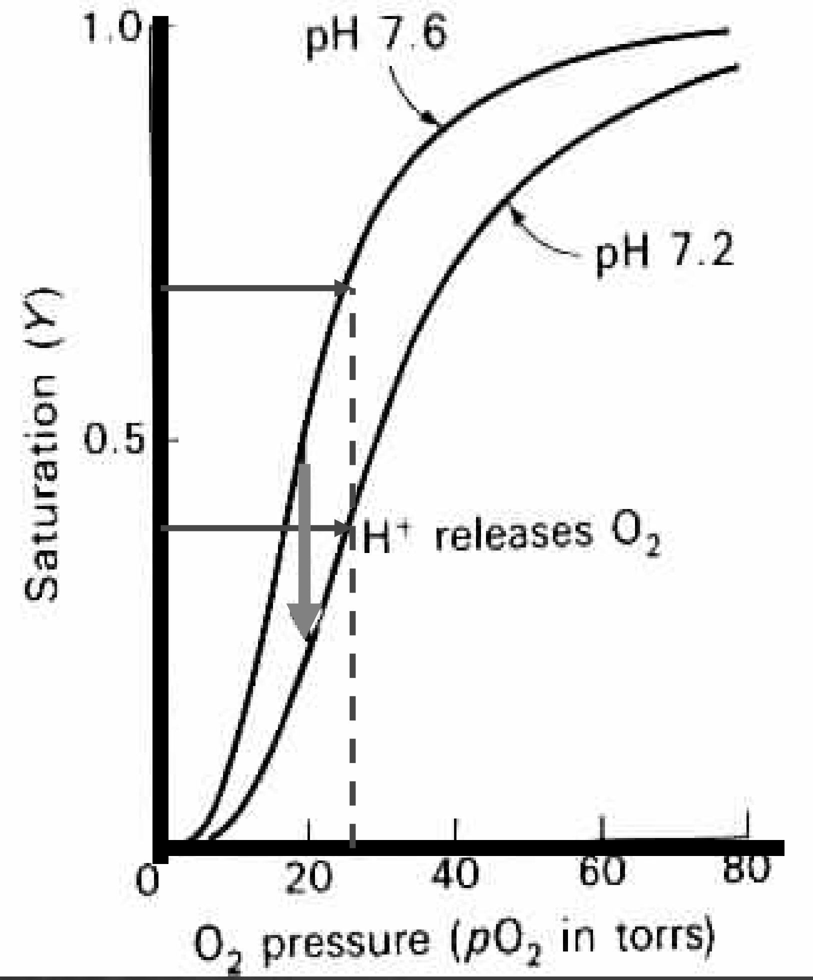
$CO_2 + H_2O$

↓ carbonic anhydrase

T-Hb ← $H_2CO_3$ Carbonic acid

↓

$H^+ + HCO_3^-$ Bicarbonic ion




$CO_2$ and $H^+$ produced during metabolism causes ↓ pH in RBCs, resulting in protonation of some amino acid groups in Hb. These effects decrease the affinity of Hb for $O_2$ in RBCs (protons bind to the T form of hemoglobin thus stabilizing it).

See Devlin 9.42, and Stryer Fig. 10.25