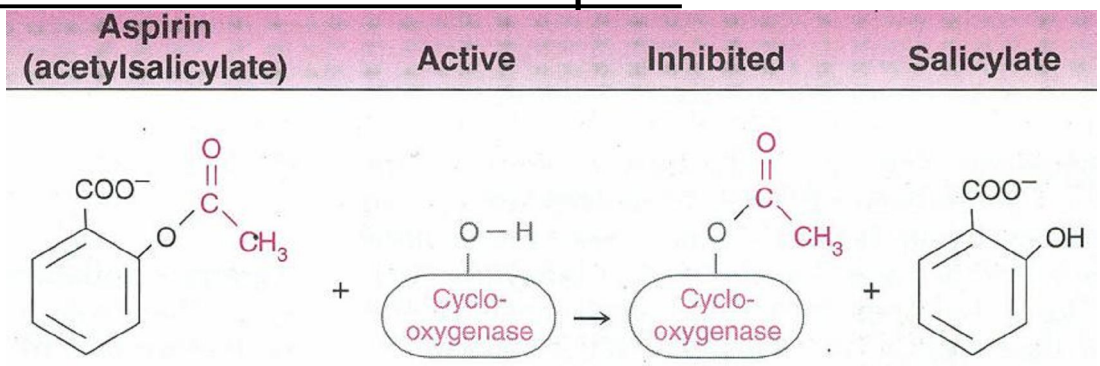
Irreversible Inhibitors

- Irreversible inhibitors generally result in the destruction or modification of an essential amino acid required for enzyme activity. Frequently, this is due to some type of covalent link between enzyme and inhibitor.

• Irreversible Inhibition – Examples

1. Aspirin



- Cyclooxygenase catalyzes the first reaction in the biosynthesis of prostglandins from arachidonate
- Aspirin (acetylsalicylate) acetylates an active serine leading to irreversible inhibition. New Cyclooxygenase must be synthesized to regain activity.
- Aspirin is used as an anti-inflammatory, antipyretic and analgesic drug and also to inhibit platelet aggregation and coronary thrombosis.

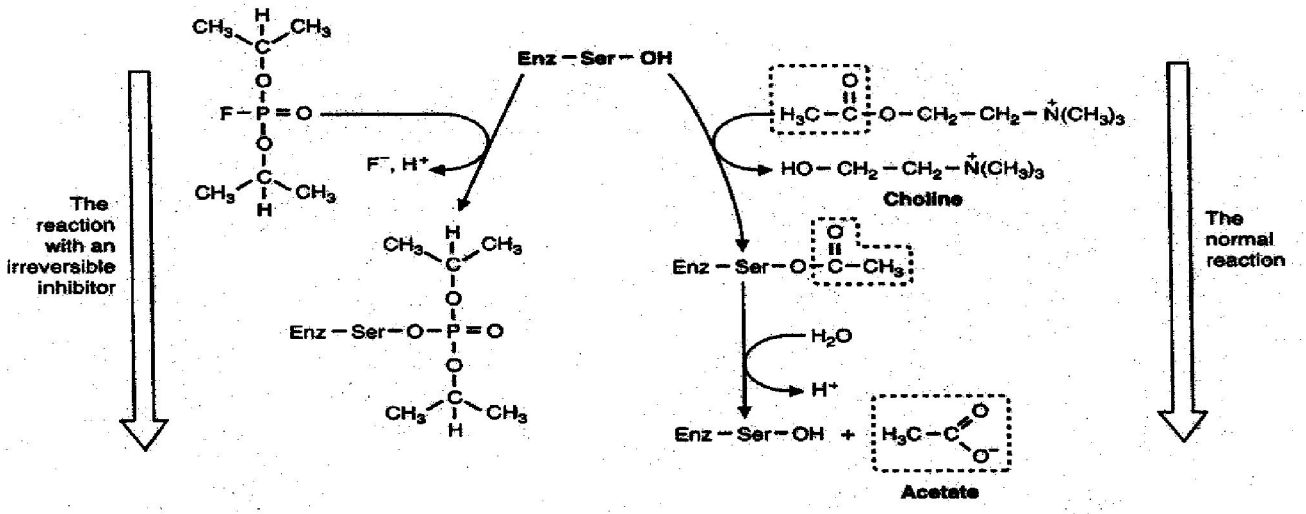
Note: The other nonsteroidal anti-inflammatory agents indomethacin and phenylbutazone are reversible inhibitors within approx. 48 hours

• Irreversible Inhibition – Examples (cont.)

2. Organophosphate nerve agents: Irreversible Acetylcholinesterase Inhibitors

• Malathion (insecticide) – converted by insects to an active irreversible inhibitor of their acetylcholinesterase.

• Sarin and Diisopropylfluorophosphate "DFP" (nerve gases)



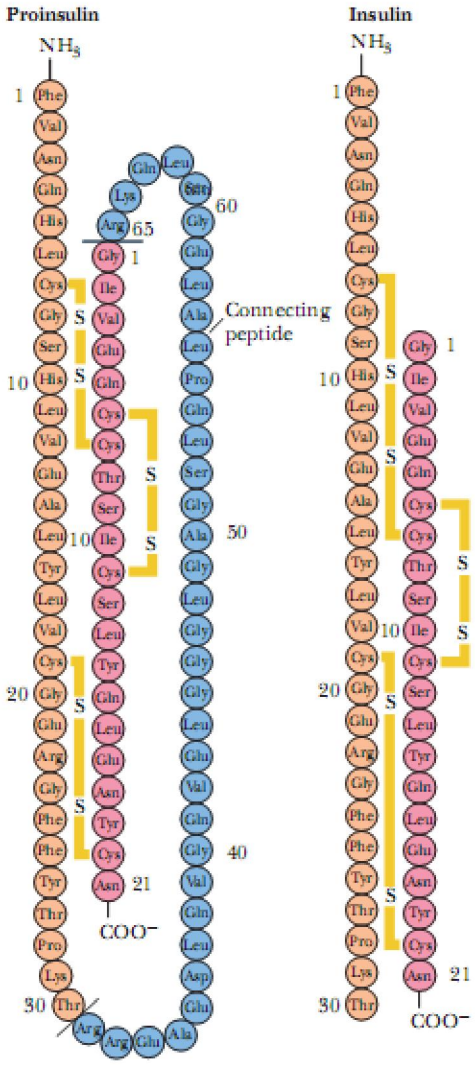
•The neurotransmitter acetylcholine must be hydrolyzed by acetylcholinesterase, thereby ceasing the neural impulse before a second impulse can be transmitted through the synapse. The result of acetylcholinesterase irreversible inhibition is paralysis in certain functions due to the failure of nerve impulses to be transmitted properly.

Zymogens: Inactive Precursor Proteins

• A clinically important mechanism of controlling enzyme activity is the case of protease enzymes involved (predominantly) in food digestion and blood clotting. Protease enzymes (enzymes that degrade proteins) like pepsin, trypsin & chymotrypsin are synthesized first as larger, inactive precursor proteins termed zymogens (pepsinogen, trypsinogen & chymotrypsinogen, respectively).

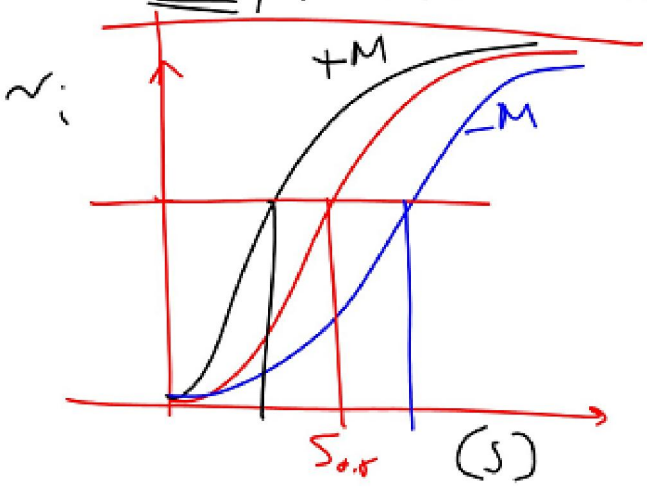
• Activation of zymogens by proteolytic cleavage result in irreversible activation. Zymogen forms allow proteins to be transported or stored in inactive forms that can be readily converted to active forms in response to some type of cellular signal. Thus, they represent a mechanism whereby the levels of a protein can be rapidly increased (post-translationally). Other examples of zymogens include proinsulin, procollagen and many blood clotting enzymes like peothrombin.

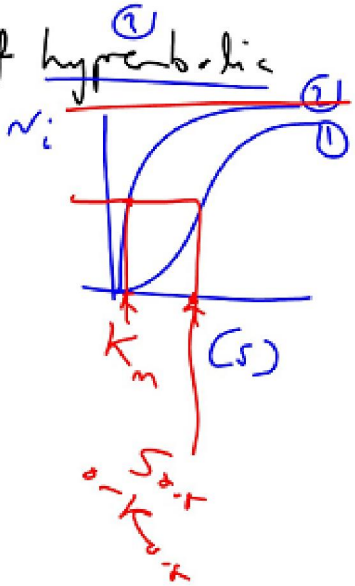
Proinsulin is an 86-residue precursor to insulin (the sequence shown here is human proinsulin).
Proteolytic removal of residues 31 to 65 (the C chain) yields insulin.
Residues 1 through 30 (the B chain) remain linked to residues 66 through 87 (the A chain) by a pair of interchain disulfide bridges.



Allosteric Enzymes

- have an allosteric site for binding of a modulator (M) (in addition to the active site)
- multimeric (larger in size) (4 or 6)
- v_i vs. [S] → sigmoidal instead of hyperbolic (1)
- Cooperativity
- Do not follow M.–M. kinetics





Allosteric Enzymes

- **Allosteric enzymes** - from the Greek *allos* for "other" and *stereos* for "site", meaning "other site". These enzymes function through reversible, non-covalent binding of a regulatory metabolite at a site other than the catalytic, active site. When bound, these metabolites do not participate in catalysis directly, but lead to conformational changes in one part of an enzyme that then affect the overall conformation of the active site (causing an increase or decrease in activity, hence these metabolites are termed allosteric activators or allosteric inhibitors).

- Allosteric enzymes differ from other enzymes in that they are generally larger in mass and are composed of multiple subunits containing active sites and regulatory molecule binding sites.

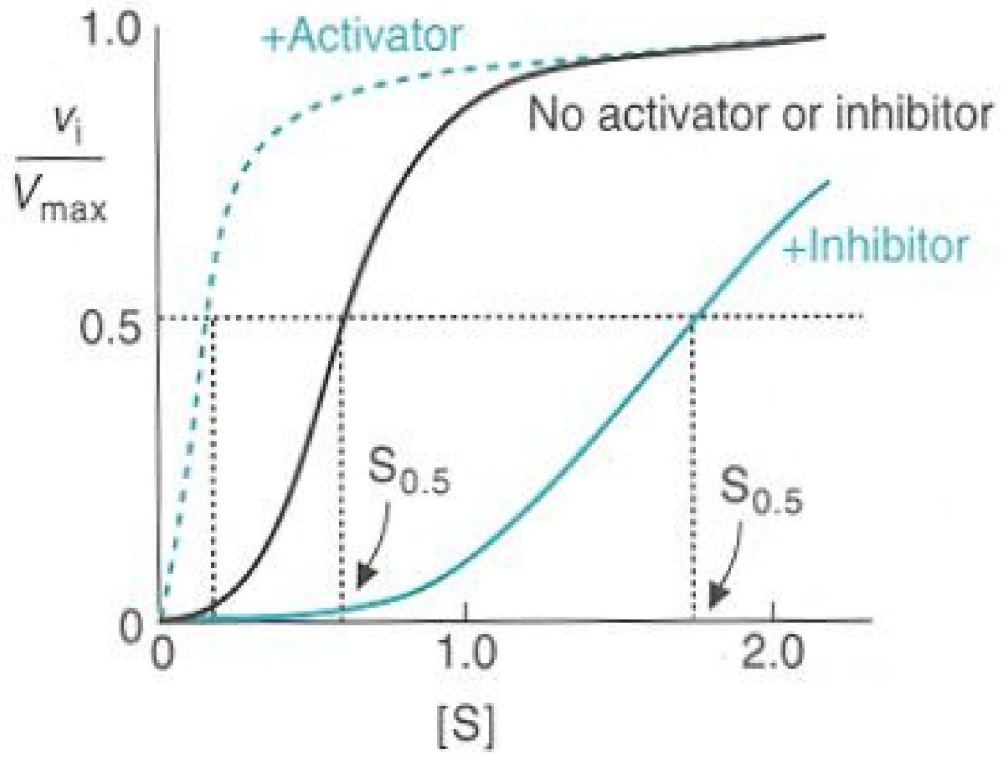
Kinetics of Allosteric Enzymes - Terms

- **Cooperativity** - in relation to multiple subunit enzymes, changes in the conformation of one subunit leads to conformational changes in adjacent subunits. These changes occur at the tertiary and quaternary levels of protein organization and can be caused by an allosteric regulator.
- **Homotropic regulation** - when binding of one molecule to a multi-subunit enzyme causes a conformational shift that affects the binding of the same molecule to another subunit of the enzyme.
- **Heterotropic regulation** - when binding of one molecule to a multi-subunit enzyme affects the binding of a different molecule to this enzyme (Note: These terms are similar to those used for oxygen binding to hemoglobin)

Allosteric Enzymes - Kinetics

- Allosteric enzymes do exhibit saturation kinetics at high [S], but they have a characteristic sigmoidal saturation curve rather than hyperbolic curve when v_o is plotted versus [S] (analogous to the oxygen saturation curves of myoglobin vs. hemoglobin). Addition of an allosteric activator (+) tends to shift the curve to a more hyperbolic profile (more like Michaelis-Menten curves), while an allosteric inhibitor (-) will result in more pronounced sigmoidal curves. The sigmoidicity is thought to result from the cooperativity of structural changes between enzyme subunits (again similar to oxygen binding to hemoglobin).
- NOTE: A true K_m cannot be determined for allosteric enzymes, so a comparative constant like $S_{0.5}$ or $K_{0.5}$ is used.

V_i vs [S] for Allosteric Enzymes



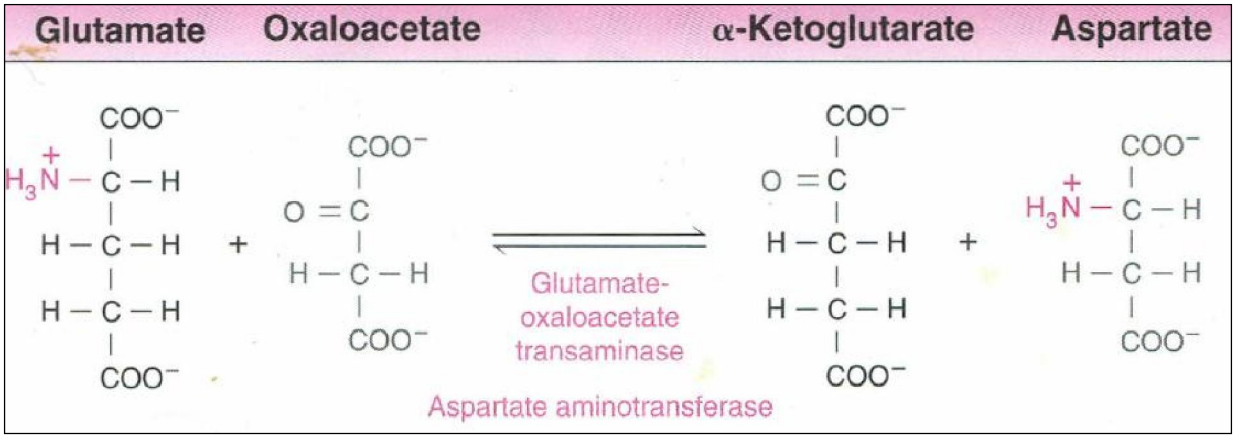
Clinical Significance of Enzymes

- The activity of enzymes in the plasma, released under pathological conditions, provides information of value in the diagnosis of various diseases.
- The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage.
- Some enzymes show relatively high activity in only one or a few tissue. Therefore, the presence of increased levels of these enzymes in plasma reflects damage to the corresponding tissue.

Clinically Significant Enzymes: Examples

1) Transaminases

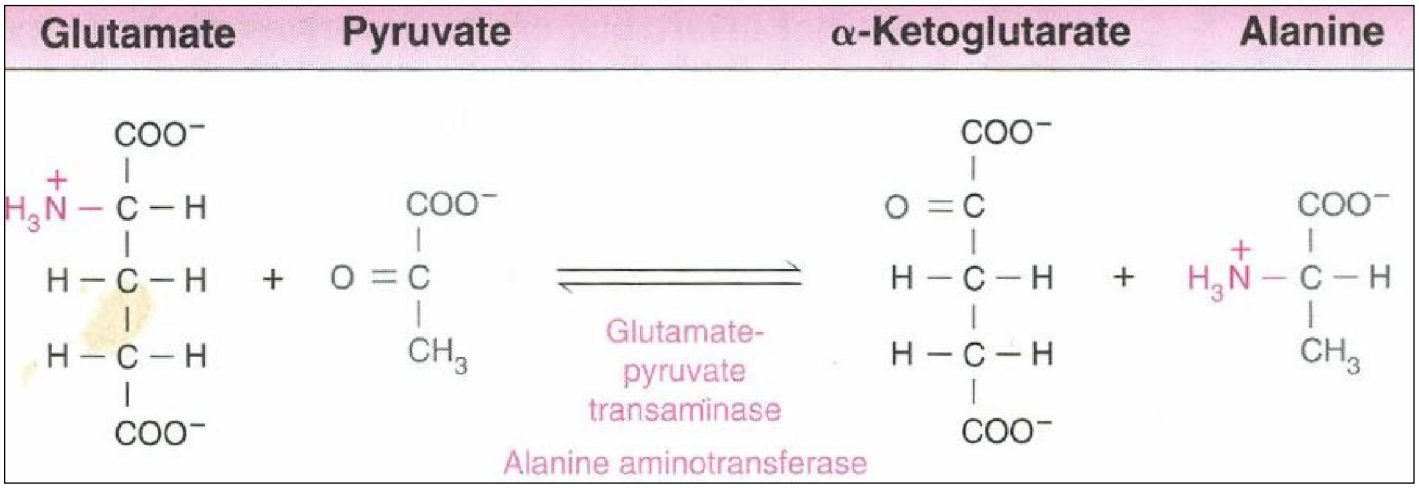
a- Glutamate-oxaloacetate transaminase (GOT), or aspertate aminotransferase (AST)



- GOT is released from many diseased cells into serum as SGOT.
- SGOT is elevated in liver disease and following a myocardial infarction.
- It can be moderately elevated (5X) in people with cirrhosis and obstructive liver disease.
- It can become very high (25X) in people with viral hepatitis.

Clinically Significant Enzymes: Examples (cont)

b- Glutamate-pyruvate transaminase (GPT), or alanine aminotransferase (ALT)



- GPT has most of the properties noted for GOT.
- SGPT is also elevated during liver disease and following a myocardial infarction.

Clinically Significant Enzymes: Examples (cont)

2) Phosphatases

a- Alkaline Phosphatase

- It catalyzes the hydrolysis of phosphate from a variety of phosphate esters. So named because of its high pH optimum (pH 8-9).
- It is often elevated in many bone disorders.
- The invasion of bone by cancer from a primary tumor located elsewhere can lead to an elevation of alkaline phosphatase.
- Obstructive liver disease also elevates alkaline phosphatase.

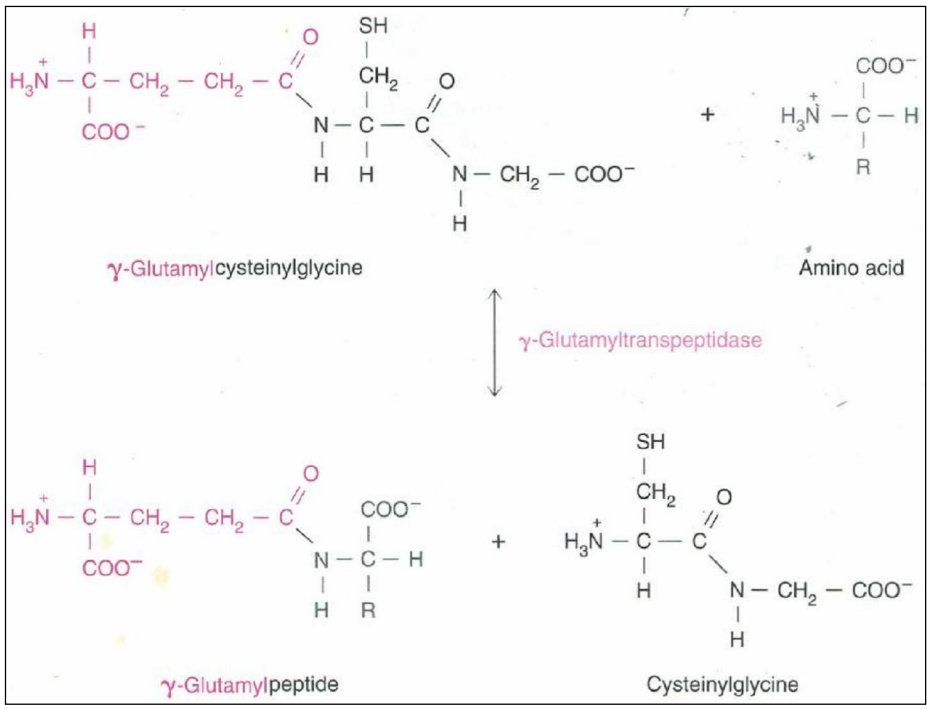
b- Acid Phosphatase

- A lysosomal enzyme that catalyzes the hydrolysis of phosphate from a variety of phosphate esters. So named because of its low pH optimum (pH 5-6).
- Elevation of acid phosphatase occurs with carcinoma of the prostate.

Clinically Significant Enzymes: Examples (cont)

3) Transferases

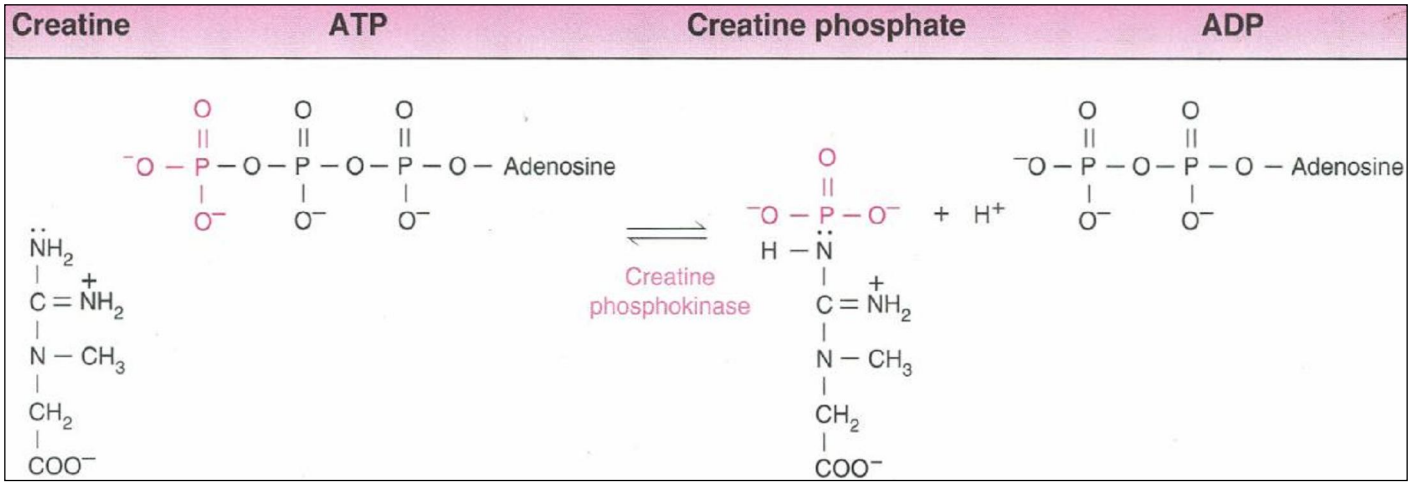
a- Gamma Glutamyl transpeptidase (GGT)



- This enzyme is a sensitive indicator of liver disease and can be elevated in **alcoholism** when there are no other serum enzyme abnormalities.

Clinically Significant Enzymes: Examples (cont)

b- Creatine phosphokinase (CPK)



- CPK is found in heart, skeletal muscle and brain.
- CPK is the first cardiac enzyme whose activity is elevated following myocardial infarction.
- CPK is also elevated in people with Duchenne muscular dystrophy.

Clinically Significant Enzymes: Examples (cont)

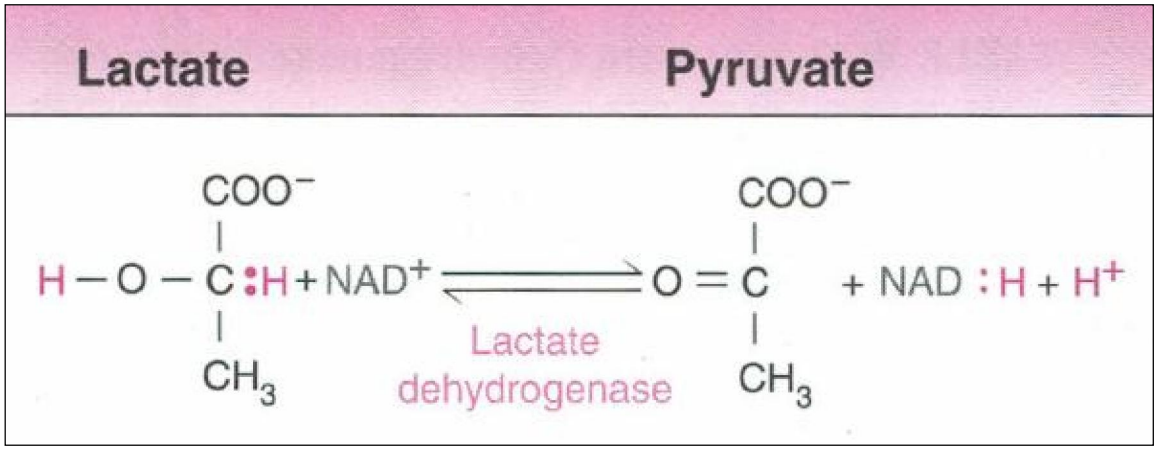
4) Amylase

- Catalyzes the hydrolysis of starch and glycogen
- Produced by the pancreas and salivary gland and is elevated during acute pancreatitis and also modest elevation in amylase can occur with inflammation of the salivary gland.

Clinically Significant Enzymes: Examples (cont)

5) Dehydrogenases

Lactate Dehydrogenase (LDH)



- LDH occurs in all human cells.
- LDH can be elevated following myocardial infarction and in many liver diseases.
- LDH activity can also be increased during hemolytic anemia when erythrocytes are degraded more rapidly than normally.

Isozymes

- Isozymes (isoenzymes) are different but closely related protein molecules that catalyze the same reaction.
- Separation of isozyme forms have mostly relied upon their different electrophoretic migration patterns.
- Isozymes can be produced from different combinations of polypeptide subunits which is the case with lactae dehydrogenase (LDH) and creatine phosphokinase (CPK).

Isozymes (cont)

1. Lactae dehydrogenase (LDH)

* LDH is a tetrameric enzyme made up of two types of subunits: H (for heart) and M (for muscle). These two subunits can be combined in five different ways (table)

Lactate Dehydrogenase Isozymes

Type	Composition*	Location
LDH_1	HHHH	Myocardium and red blood cell
LDH_2	HHHM	Myocardium and red blood cell
LDH_3	HHMM	Brain and kidney
LDH_4	HMMM	
LDH_5	MMMM	Liver and skeletal muscle

* H = heart, M = muscle.

* Establishing the tissue of origin aids in diagnosis

Isozymes (cont)

2. Creatine phosphokinase (CPK)

- Two gene products correspond to CPK:

M (for muscle) and B (for brain).

- CPK occur as a dimer, and three isozyme forms therefore are possible:

  CPK_1 (BB) that occurs in brain, CPK_2 (MB) that occurs in heart, and CPK_3 (MM) that occurs in muscle.

- The activity of plasma CPK_2 is the cornerstone for the diagnosis of a myocardial infarction because of its abundance in heart and absence from other cells. It may be elevated after 4 hours, and its activity may increase two- to ten-fold after 16-24 hours.

Hemoglobin & Myoglobin

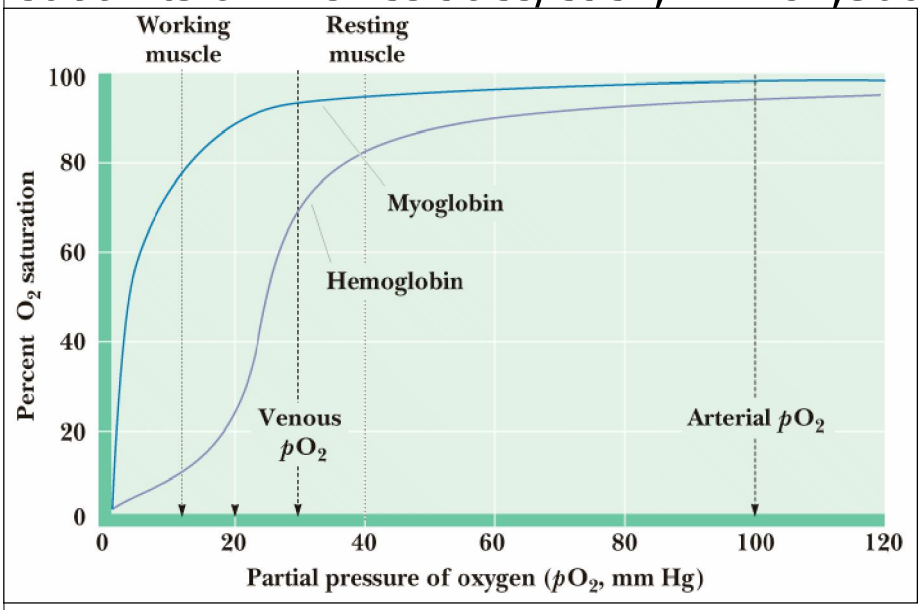
Oxygen Binding Proteins

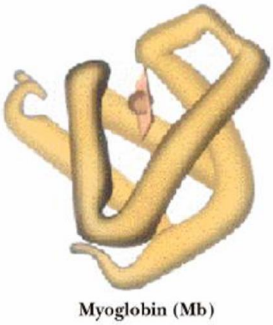
- Two very similar proteins, but different functions:
- Myoglobin (Mb) binds oxygen to store it temporarily in tissues
- Hemoglobin (Hb) transports oxygen from lungs to tissues, so its binding affinity must change in the two locations

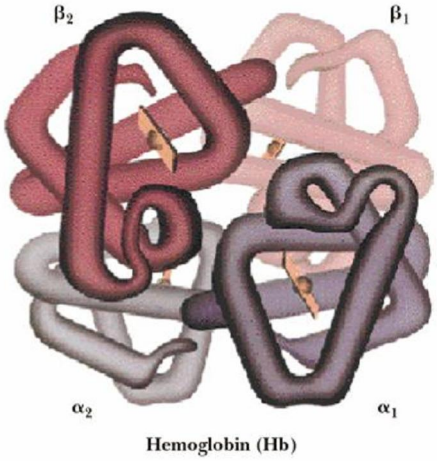
- Both have heme (iron-porphyrin) as a prosthetic group which serves as the binding site of oxygen.

Hemoglobin & Myoglobin (cont.)

- Myoglobin (Mb) is monomeric; Hemoglobin (Hg) is tetrameric ($\alpha_2\beta_2$)
- Mb: 153 a. acid residues, MW 17,200
- Hb: 2 α subunits of 141 residues/each, 2 β subunits of 146 residues/each, MW 64,500




Myoglobin (Mb)

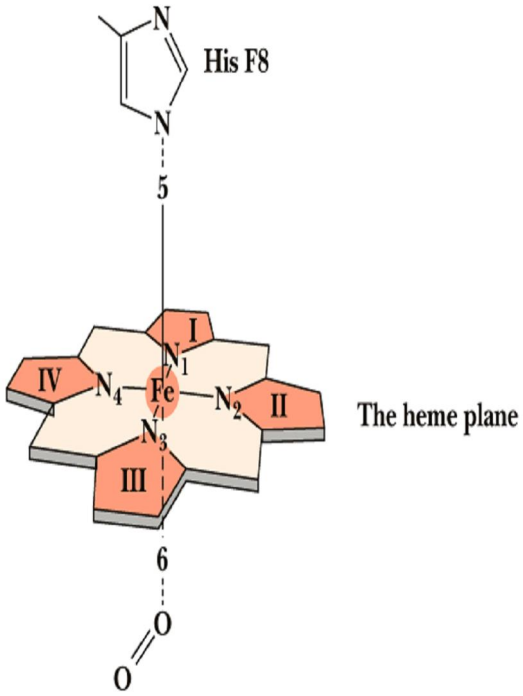


Hemoglobin (Hb)

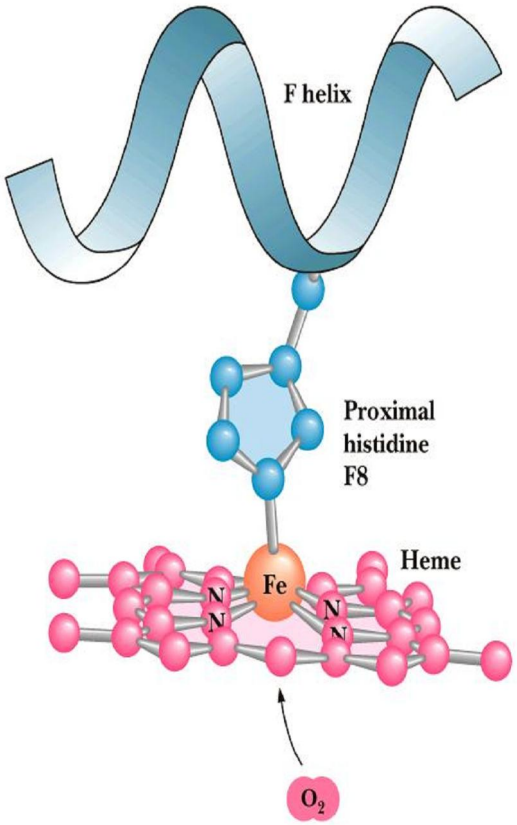
Hemoglobin Function

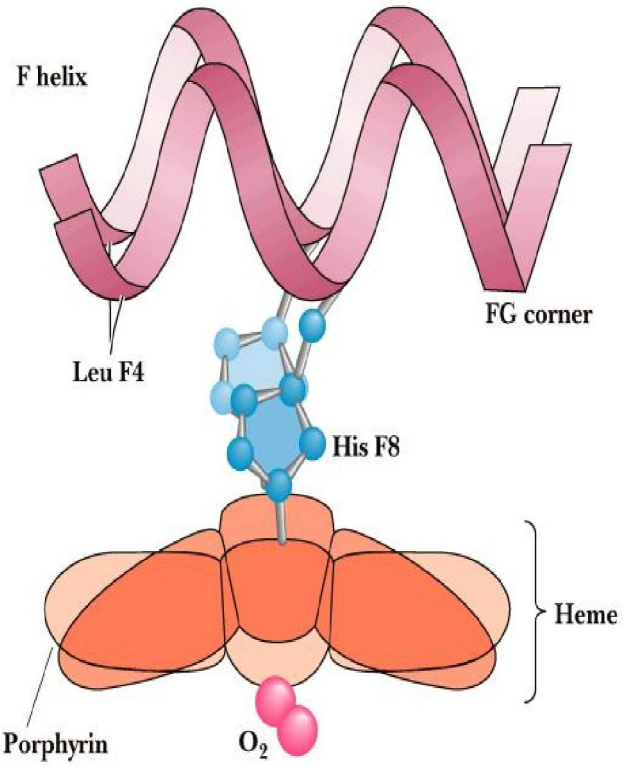
Hb must bind oxygen in lungs and release it in capillaries

- When a first oxygen binds to Fe in heme of Hb, the heme Fe is drawn into the plane of the porphyrin ring
- This initiates a series of conformational changes that are transmitted to adjacent subunits
- Adjacent subunits' affinity for oxygen increases
- This is called positive cooperativity



Hemoglobin Function (cont.)

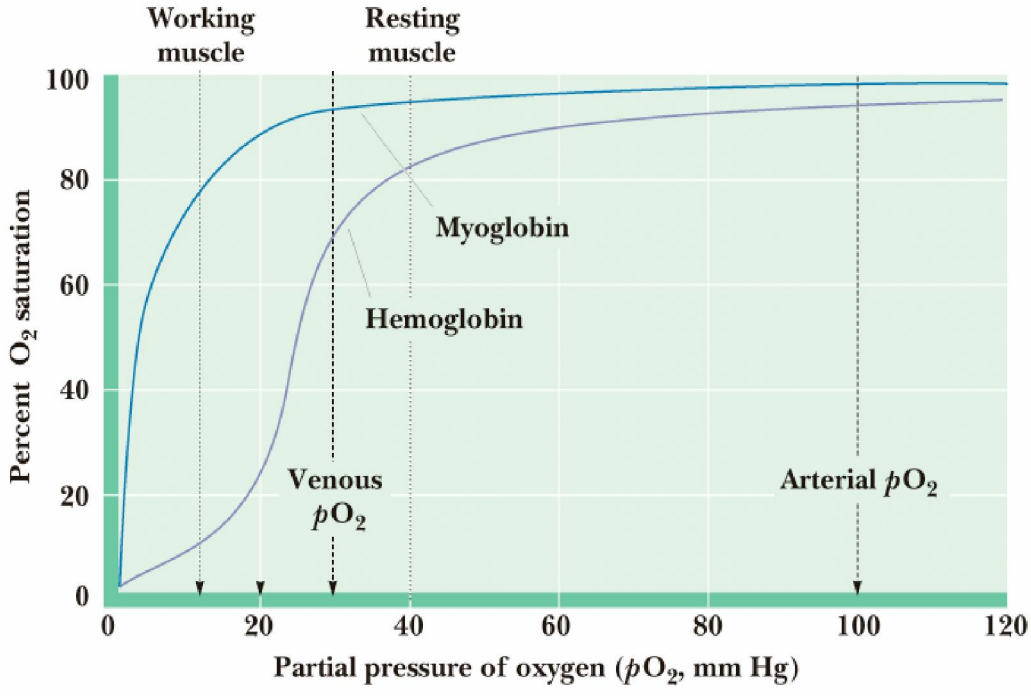




Binding of Oxygen by Hb

The Physiological Significance

- Hb must be able to bind oxygen in the lungs and to release oxygen in capillaries
- If Hb behaved like Mb, very little oxygen would be released in capillaries
- The sigmoid, cooperative oxygen binding curve of Hb makes this possible!



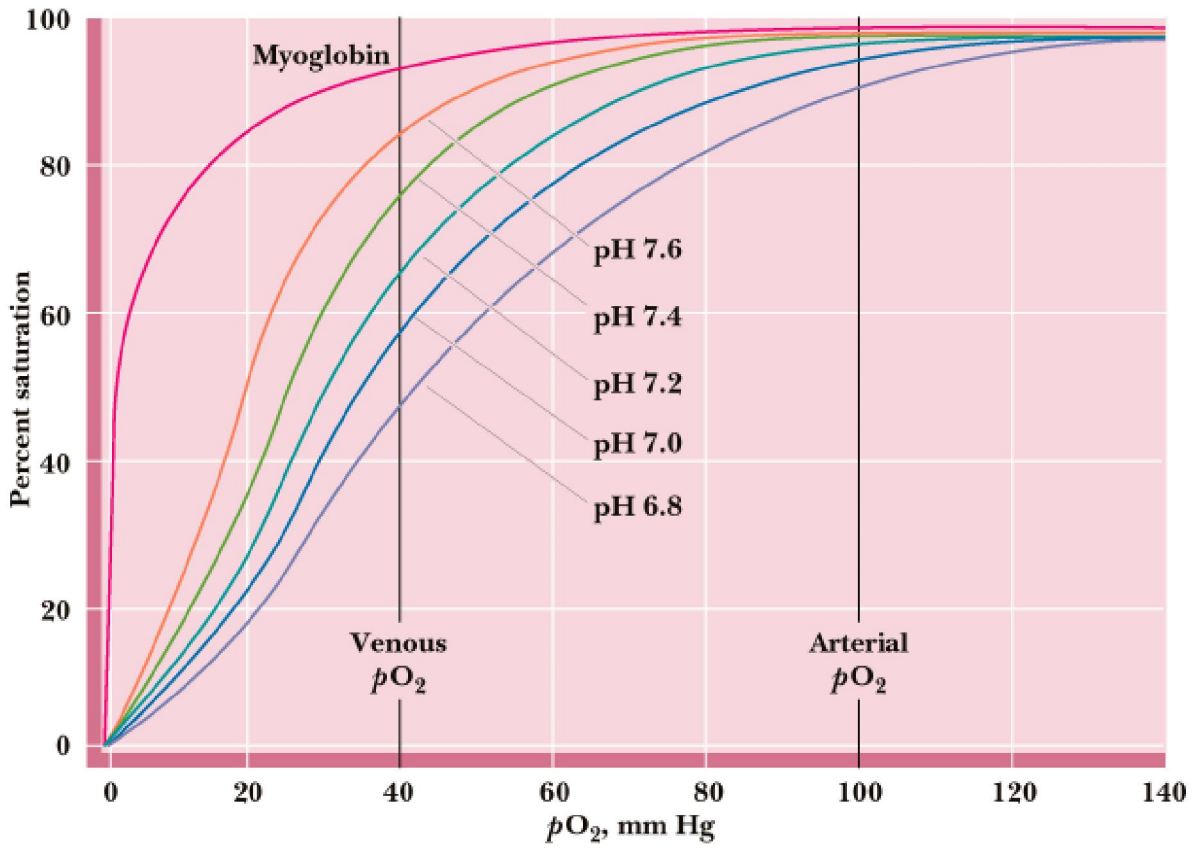
The Bohr Effect

Competition between oxygen and H^+

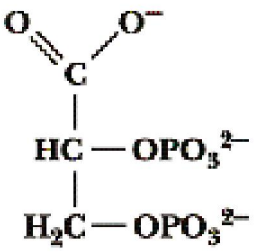
- Discovered by Christian Bohr
- Binding of protons diminishes oxygen binding
- Binding of oxygen diminishes proton binding
- Important physiological significance

Carbon dioxide diminishes oxygen binding

- Hydration of CO_2 in tissues and extremities leads to proton production
- These protons are taken up by Hb as oxygen dissociates
- The reverse occurs in the lungs



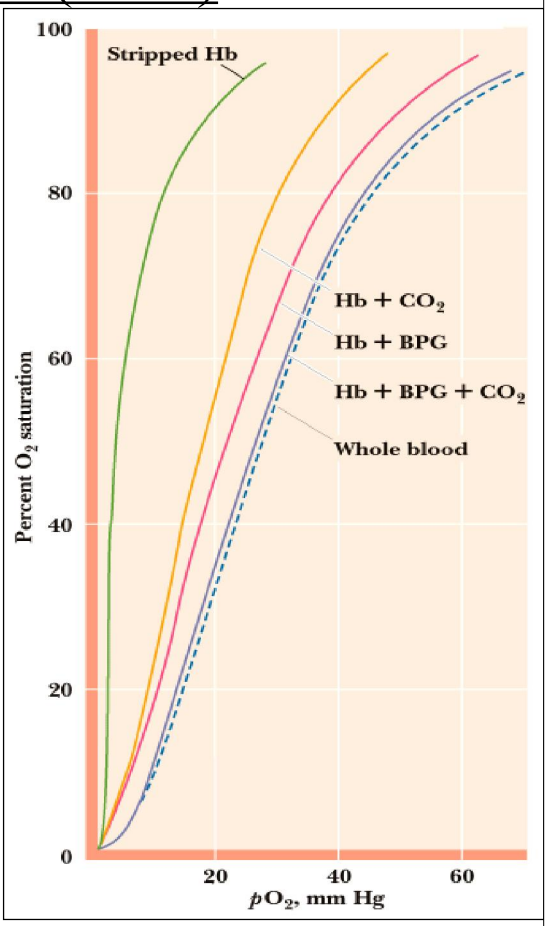
2,3-Bisphosphoglycerate (BPG)



An Allosteric Effector of Hemoglobin

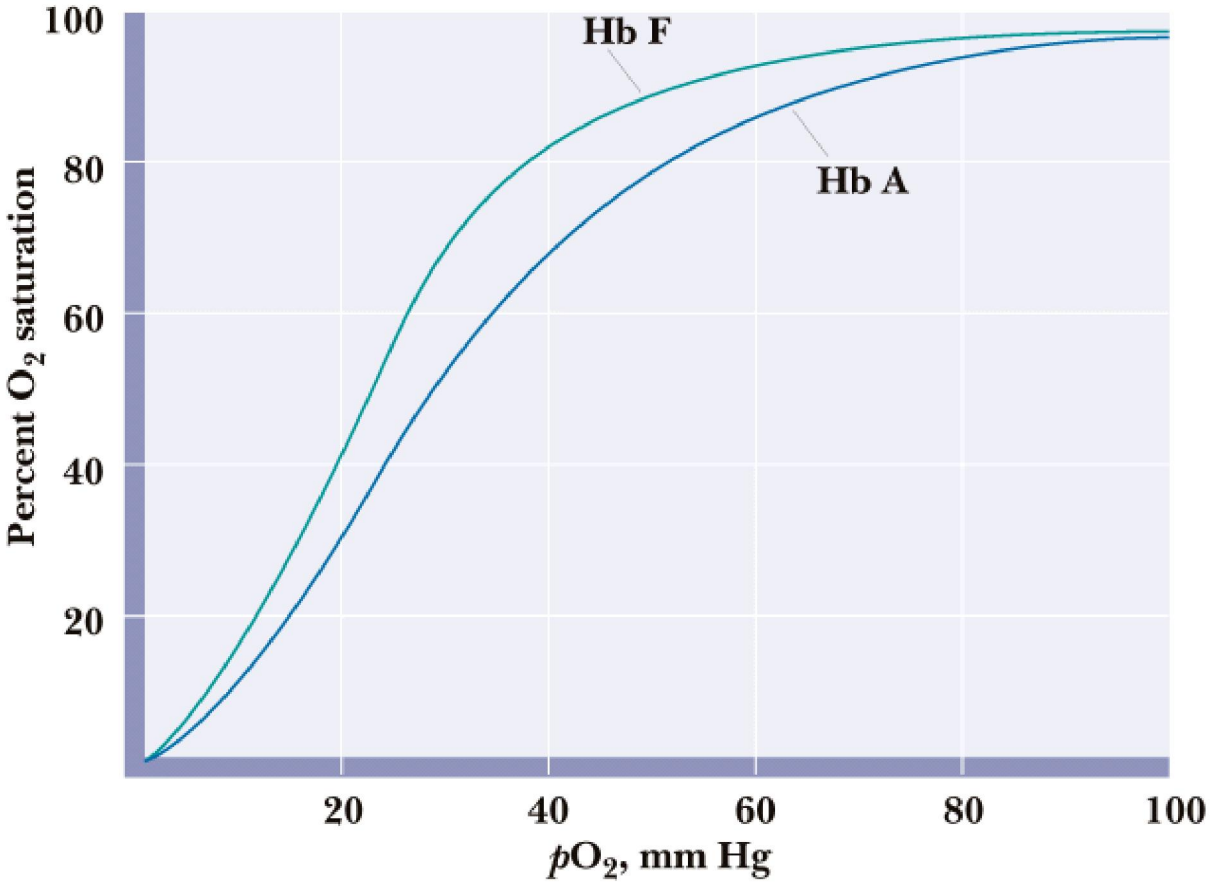
BPG is a highly –ve molecule that bind to a highly +ve (2 Lys, 4 His, 2 N-termini) allosteric site in the central cavity of Hb

- The sigmoid binding curve is only observed in the presence of 2,3-BPG
- [BPG] increases in high altitudes, so O_2 affinity to Hb decreases ($p50$ increases) resulting in enhancement of more O_2 unloading in tissues.



Fetal Hemoglobin

- In fetal hemoglobin (Hb F), the β-chains are replaced by γ- chains. They have Ser instead of His at position 143.
- The BPG binding cavity has two less positive charges, so BPG binding to Hb is less tightly.
- Therefore oxygen binds more tightly.
- By having a higher affinity for oxygen, fetal hemoglobin is able to receive oxygen from the hemoglobin of the maternal blood.


100
80
60
40
20
Percent O_2 saturation
Hb F
Hb A
20
40
60
80
100
pO_2, mm Hg