Enzymes
Part III: regulation III

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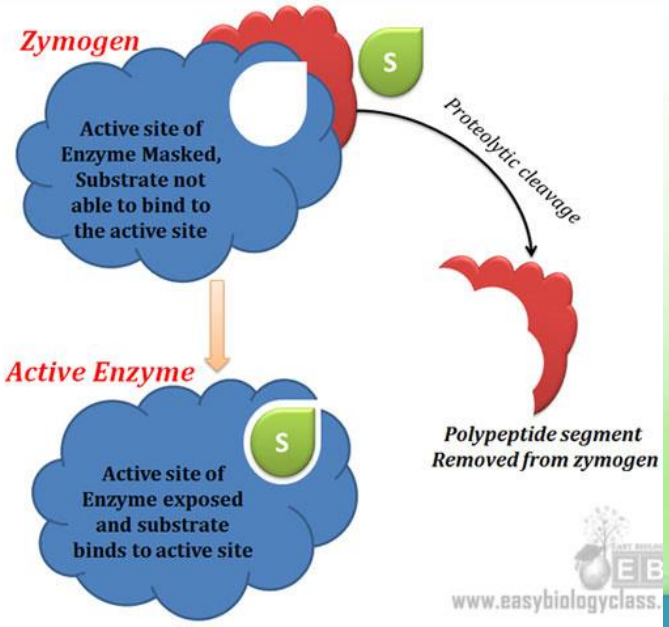
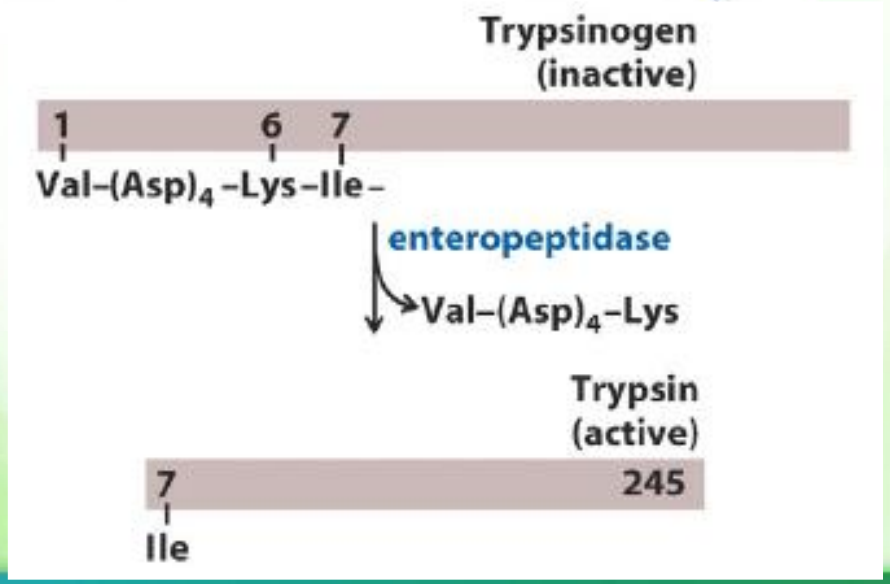
Irreversible covalent modification (proteolytic activation)

Zymogens

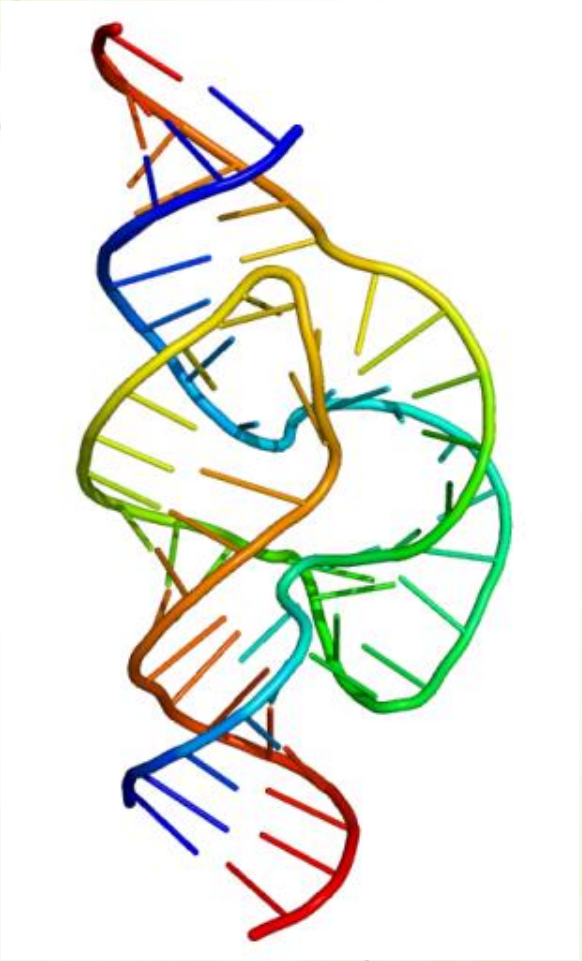
- Zymogens or proenzymes are inactive precursors of enzymes.
- Activation is done by irreversibly removing part of the enzyme (usually known as the pro region present at the N-terminus).
- Examples: digestive enzymes such as chymotrypsin, trypsin, and pepsin that get activated when food is ingested.
  - Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus.





An exception to enzymes: *Ribozymes*

- Ribozymes are enzymes made of both protein and RNA part (only a few).
- For some, catalysis is performed by RNA.
- Example include those involved in RNA splicing reactions in those responsible for protein synthesis in ribosomes.
- The catalytic efficiency of RNAs is less than that of protein enzymes, but can be enhanced and stabilized by the presence of protein subunits.



Nonspecific Inhibitors

Regulation of enzyme amount

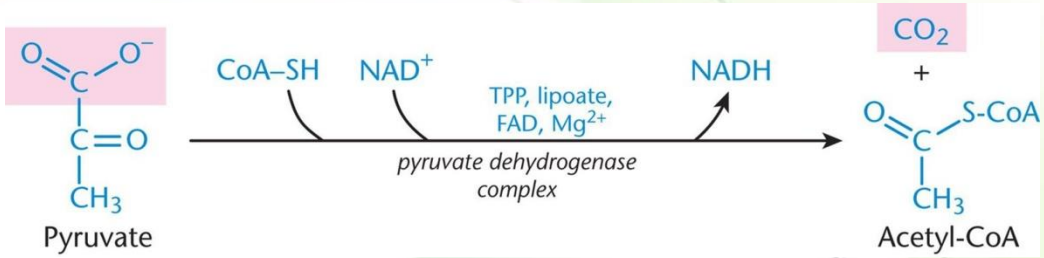
- Three mechanisms:
  - Synthesis of isozymes
  - Enzyme synthesis at the gene level
  - Enzyme degradation by proteases

- They are comparatively slow mechanisms for regulating enzyme concentration (hours-weeks).

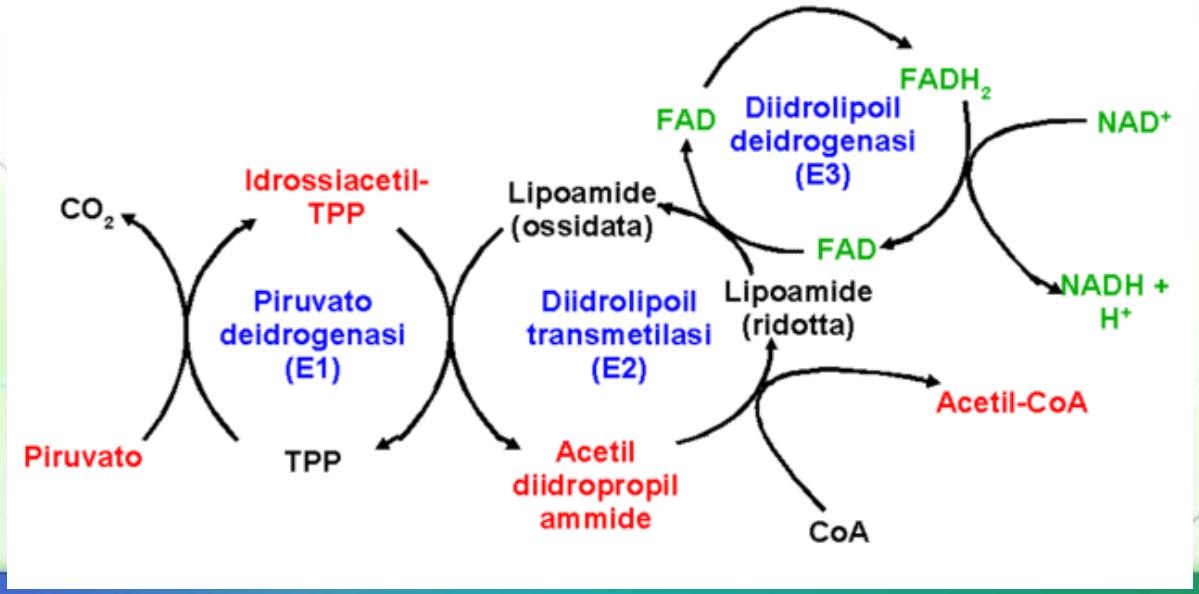
Compartmentalization

- Compartmentalization reduces the area of diffusion of both enzyme and substrate and increasing the probability that they meet and collide.
  - Example 1: lysosomal enzymes
  - Example 2: fatty acid metabilism
    - Synthesis occurs in cytosol, whereas break-sown is mitochondrial.

Enzyme complexing

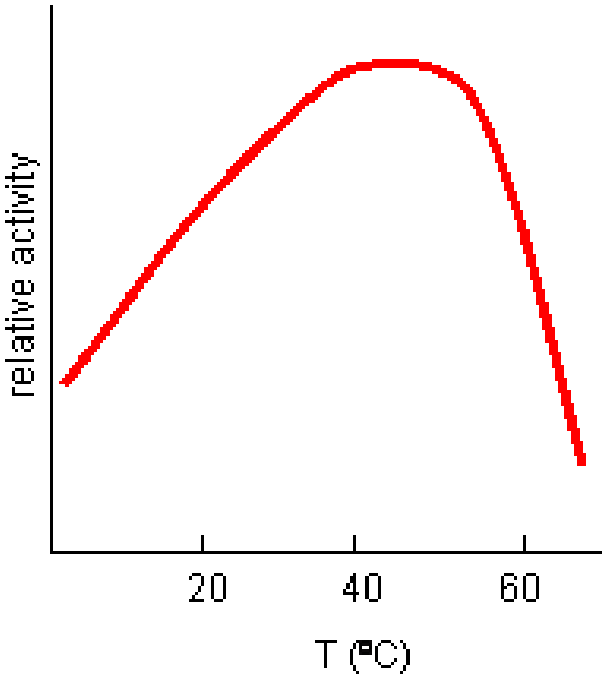


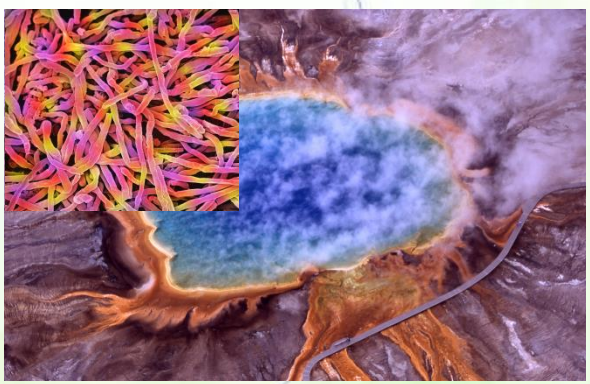
- Formation of a complex of multiple enzymes also reduces diffusion.
- Example: Pyruvate dehydrogenase (mitochondria) is composed of 3 enzymes: decarboxylation, oxidation, & transfer of the acyl group to CoA.



Temperature

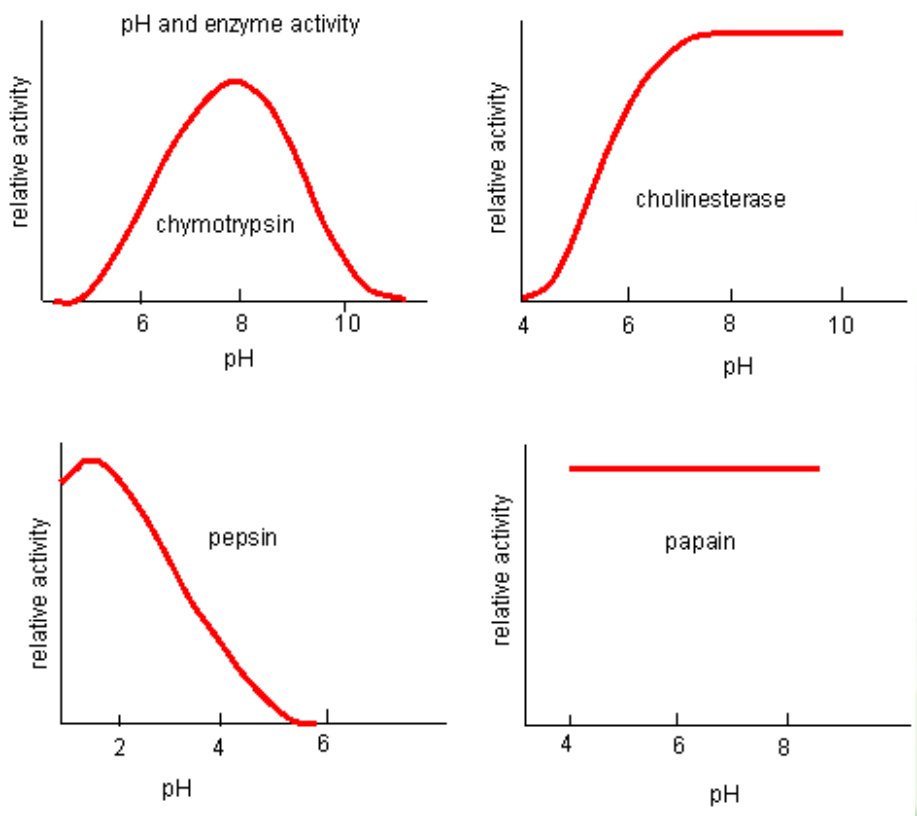
- Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates.
- However, high temperatures lead to protein denaturation.
- Each enzyme has an optimal temperature.
- For thermophilic bacteria, the optimal temperature is as high as 65°C.





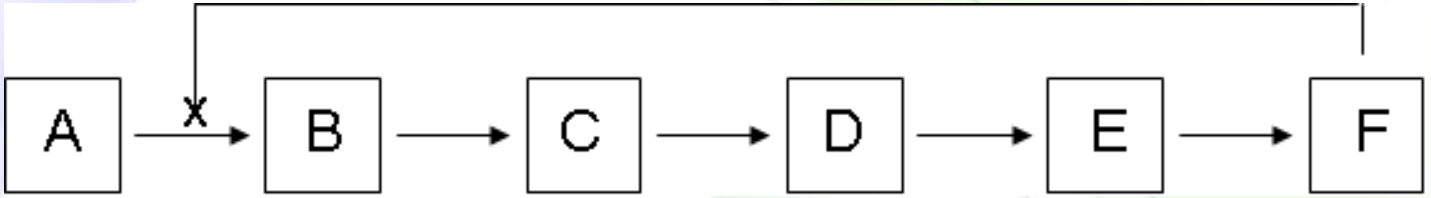
pH

- pH alters binding of substrate to enzyme (K_M) by altering the protonation state of the substrate and/or altering the conformation of the enzyme.
- The effect of pH is enzyme-dependent.

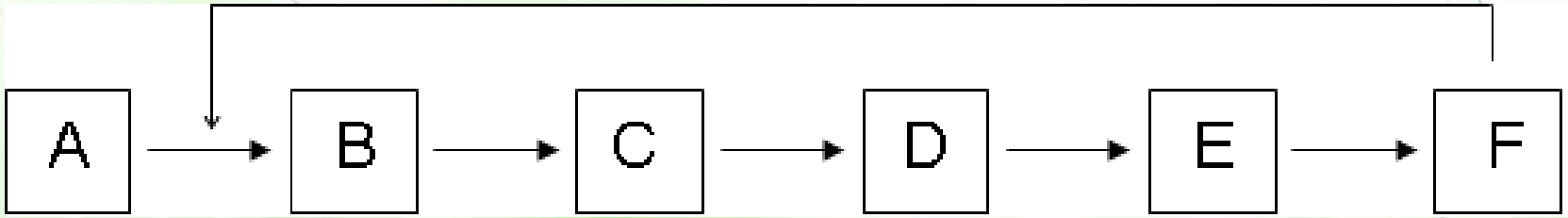


Modes of regulation

Feedback regulation

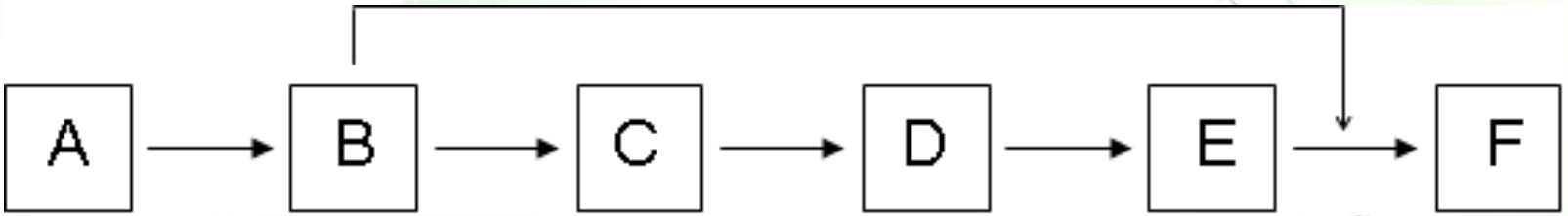


- Feedback inhibition or negative feedback regulation: an enzyme present early in a biochemical pathway is inhibited by a late product of pathway.
- Positive feedback regulation: a product stimulates the activity of an enzyme.



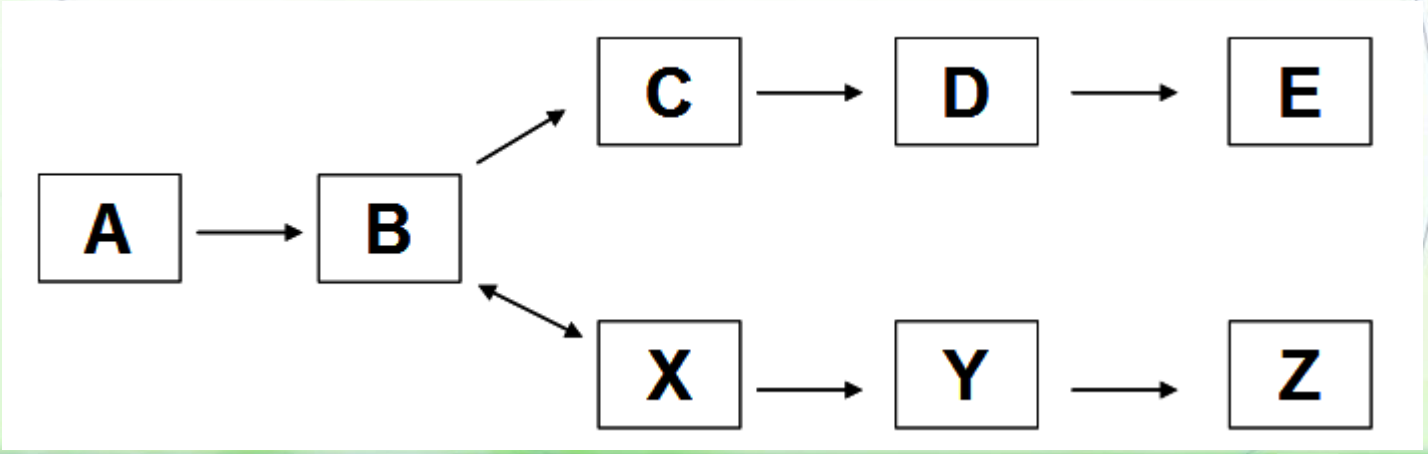
Feed-forward regulation

- Feed-forward regulation: a substrate produced early in a pathway activates an enzyme downstream of the same pathway.



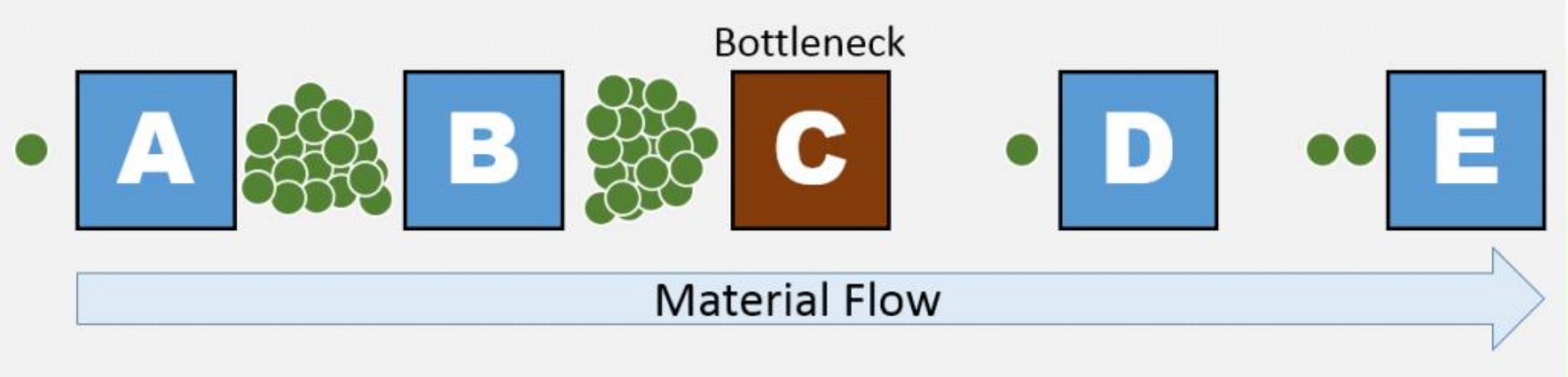
A committed step

- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return
- Committed steps are exergonic reaction
- For example, the committed step for making product E is (B → C), not (A → B)



Rate-limiting reactions

- Rate-limiting reactions sow down rate of reactions because:
  - requirement for high amount of energy
  - strict regulation of enzymes
  - high Km values of enzyme towards its substrate
- These reactions are also usually, but not necessarily, committed steps.



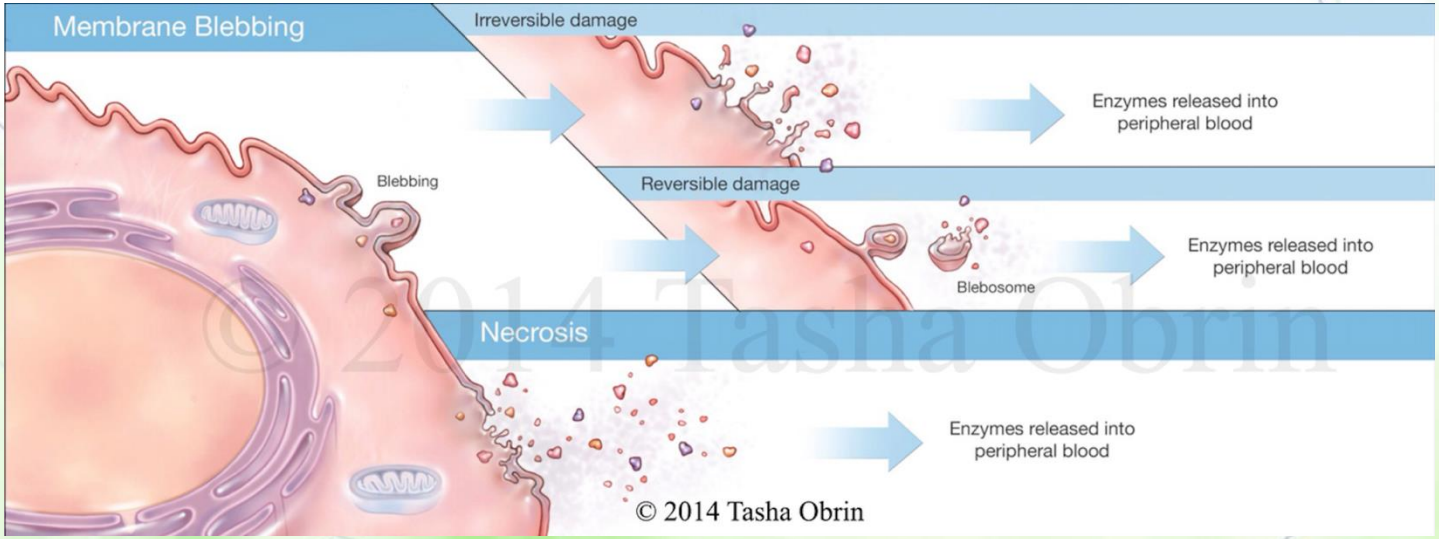
Enzymes in disease diagnosis

Concept

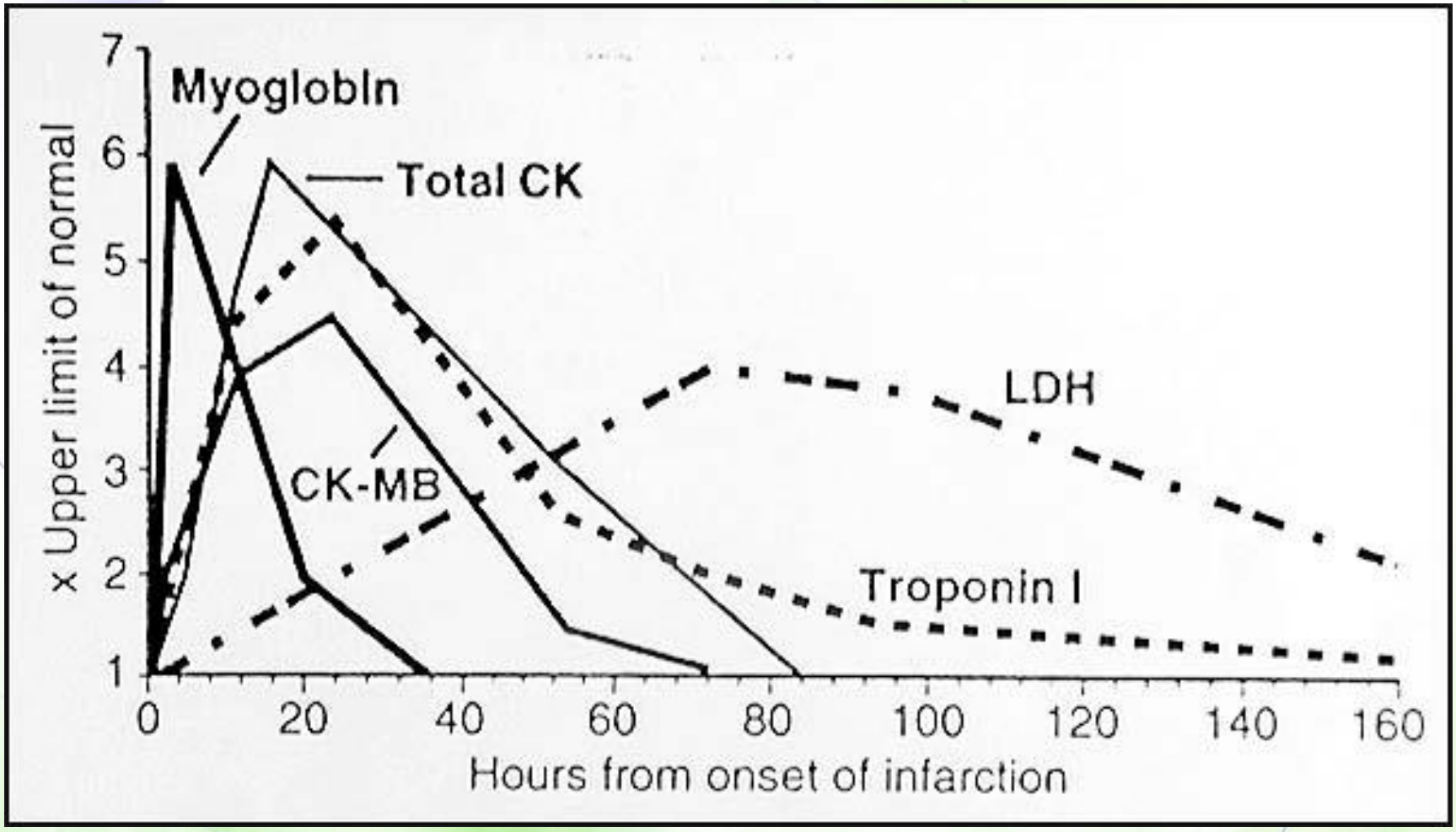
- The presence of enzymes in serum indicates that tissue or cellular damage.
- The measurement enzyme amount in serum is of diagnostic significance.
- Examples:
  - The amino transferases: alanine transaminase, ALT and aspartate aminotransferase, AST
  - lactate dehydrogenase, LDH
  - creatine kinase, CK (also called creatine phosphokinase, CPK)

AST and ALT

- The typical liver enzymes measured are AST and ALT.
- ALT is predominantly in hepatocytes.
- The ratio of ALT/AST is diagnostic.
  - Liver disease/damage (not of viral origin) < 1.
  - Viral hepatitis > 1.

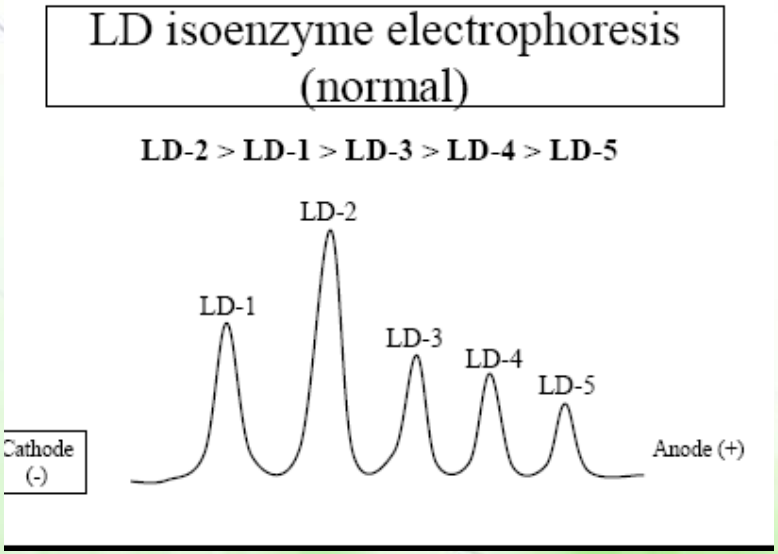


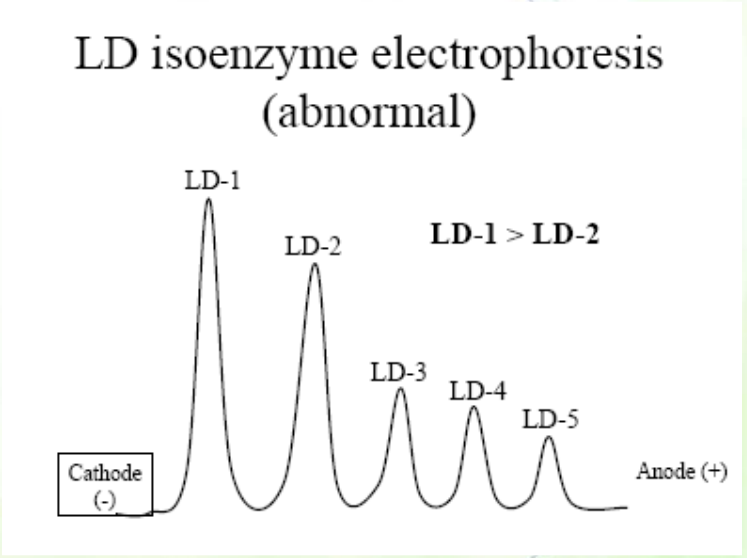
Protein profile in myocardial infarction



LDH

- A comparison of serum levels of LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks).
- Normally, this ratio is less than 1.
- Following an acute myocardial infarct, the LDH ratio will be more than 1.





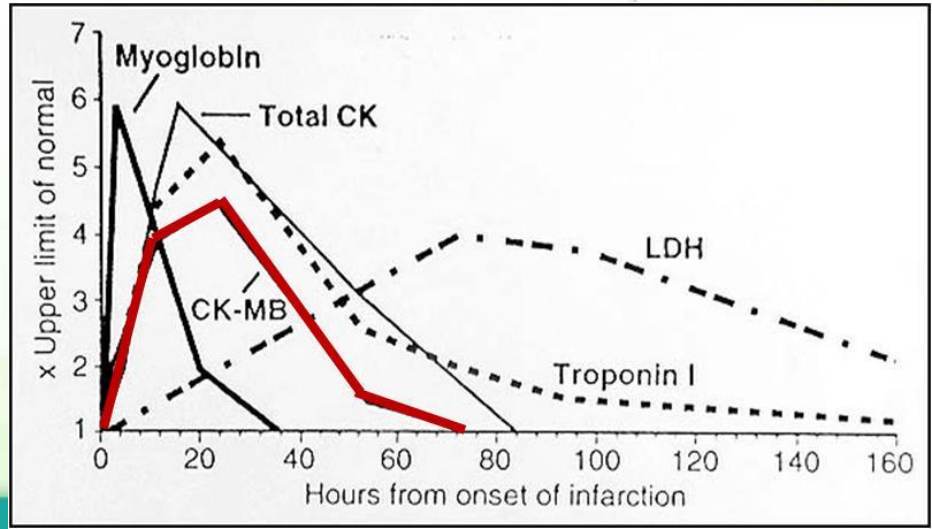
CPK

- CPK is found primarily in heart and skeletal muscle as well as the brain.
- Three tissue-specific isozymes of CPK:
  - CPK3 (CPK-MM) is the predominant isozyme in muscle.
  - CPK2 (CPK-MB) accounts for about 35% of the CPK activity in cardiac muscle, but less than 5% in skeletal muscle.
  - CPK1 (CPK-BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle.

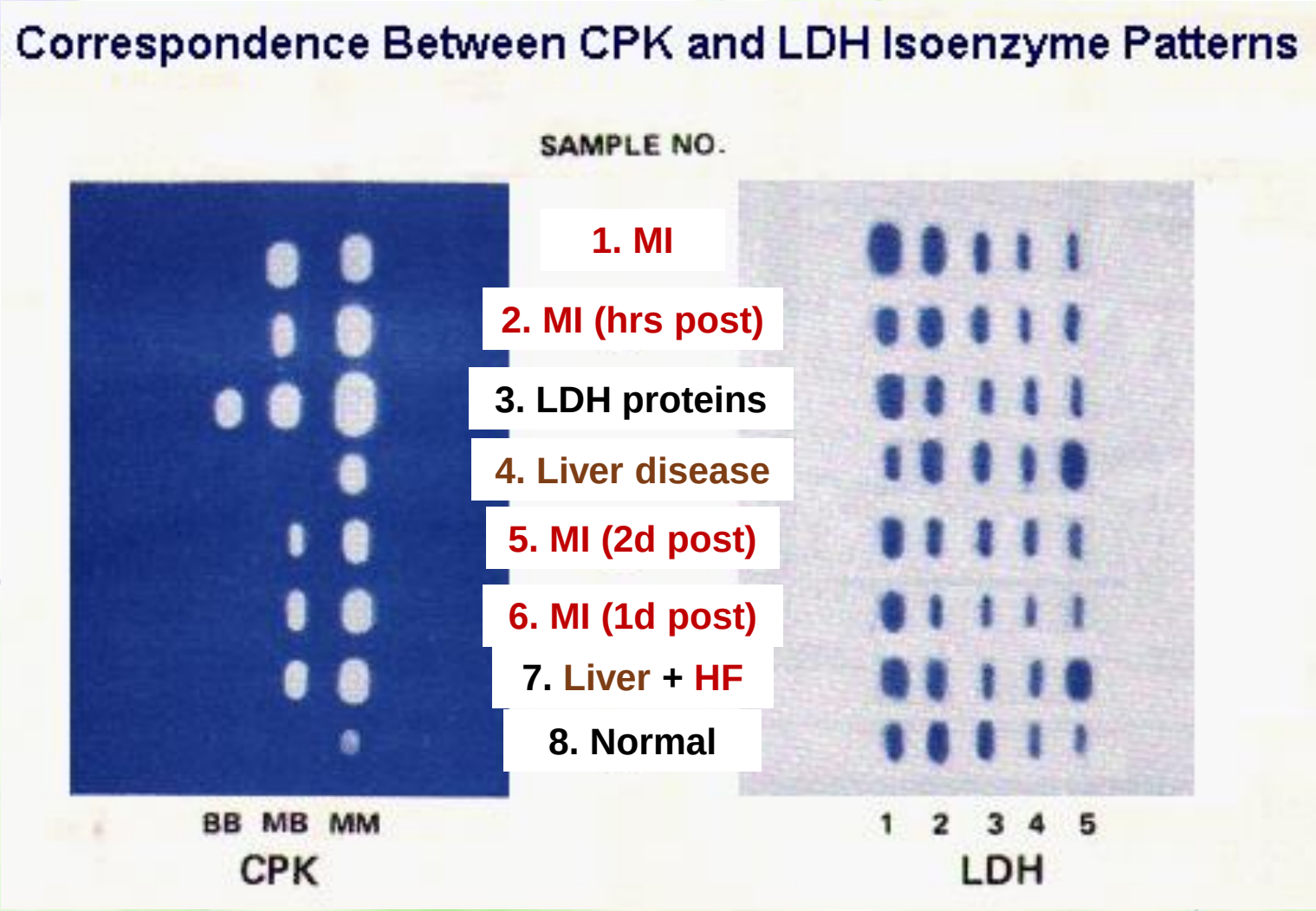
Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

CPK and myocardial infarction

- Since most of the released CPK after a myocardial infarction is CPK-MB, an increased ratio of CPK-MB to total CPK may be diagnosis of an acute infarction, but an increase of total CPK in itself may not.
- The CPK-MB is also useful for diagnosis of reinfarction because it begins to fall after a day and disappears in 1 to 3 days, so subsequent elevations are indicative of another event.



Example


Correspondence Between CPK and LDH Isoenzyme Patterns
SAMPLE NO.
1. MI
2. MI (hrs post)
3. LDH proteins
4. Liver disease
5. MI (2d post)
6. MI (1d post)
7. Liver + HF
8. Normal
BB MB MM
CPK
1 2 3 4 5
LDH

Interpretation

- Sample #3 represents results for a control.
- Sample #8 results are from a normal specimen.
- Sample# 1 MI patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the LDH flip and the high relative activity of the MB isoenzyme.
- Sample# 2 MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated with a high relative MB isoenzyme activity.
- Sample# 6 MI patient (the 1st day post MI); CK activity is definitely elevated with a high relative MB isoenzyme activity and the LDH flip is evident.
- Sample# 5 MI patient (2 days post MI) so that CK has almost returned to normal activity and the LDH flip is definite.
- Sample# 7 MI patient with complications of heart failure and passive liver congestion or the patient was involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.
- Sample# 4 a patient with liver disease. Although the LDH isoenzyme pattern is indistinguishable from muscle disease or injury, the absence of at least a trace of CK-MB isoenzyme is inconsistent with the muscle CPK isoenzyme distribution as is the apparently normal total activity.

Troponins in MI

- Troponin levels rise within four to six hours after the beginning of chest pain or heart damage, and stay elevated for at least one week.
- This long elevation allows detection of a myocardial infarction that occurred days earlier, but prevents detection of a second infarction if it occurred only days after the first.