بسم الله الرحمن الرحيم

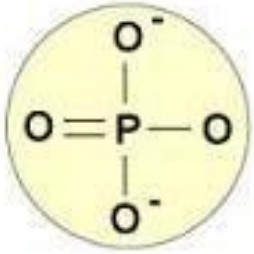
BioChemistry | FINAL 15

Enzymes pt.7

Written by : Jannat Nasri

Reviewed by : NST

Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION



Phosphate group

- **Why is it effective?**

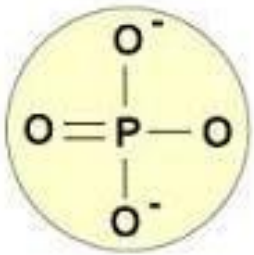
- **Adds two negative charges: new electrostatic interactions and accordingly conformation** (That's why phosphorylation is very effective in regulating enzymes, These negative charges allow for further interactions, such as hydrogen bonding or electrostatic interactions.)

- **Can form three or more hydrogen bonds: specific interactions with hydrogen-bond donors**

- **Can take place in less than a second or over a span of hours**

- **Often causes highly amplified effects**

- Now, the question arises: does phosphorylation lead to activation or inhibition of enzymes?



Phosphate group

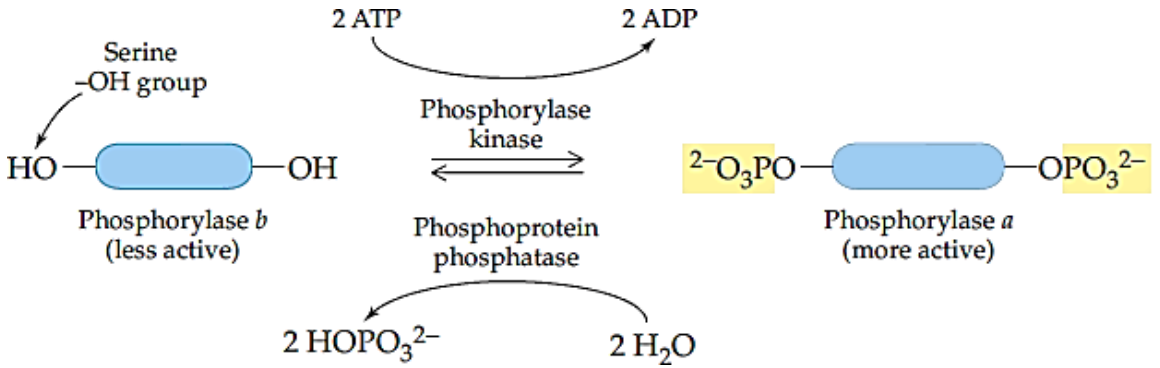
The answer is that it can do both, depending on the specific enzyme. This is because cells have a complex metabolism, sometimes contradictory.

For example, if we want to synthesize glycogen, we need to activate glycogen synthesis while simultaneously inhibiting glycogen breakdown.

Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

- **Rapid and transient regulation of enzyme activity - REVERSIBLE**
- **Phosphorylation: (Ser, Thr, & Tyr)**
  (phosphorylation involves adding a phosphate group to one of the amino acids: serine, threonine, or tyrosine.)
  - ✓ **Mostly, ATP is the donor**
  - ✓ **Kinases vs. phosphatases**

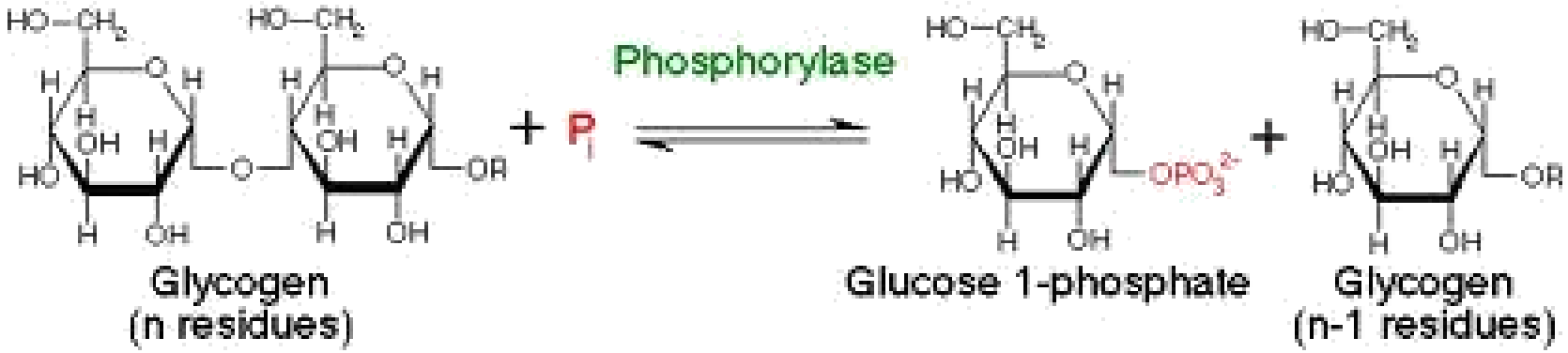
The enzymes responsible for adding these phosphate groups are called kinases, and they belong to the transferase family. On the other hand, the enzymes that remove the phosphate group are called phosphatases, and they are part of the lyases family.



Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

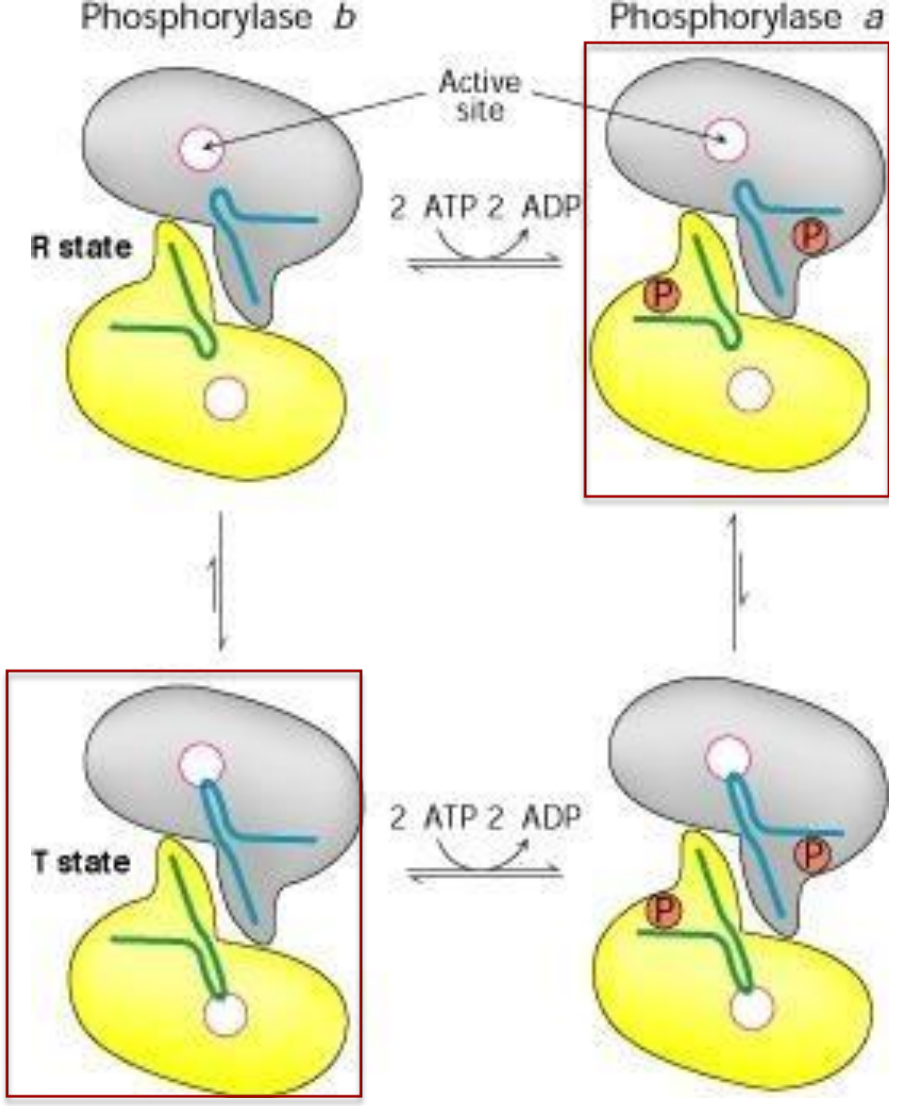
- ✓ **Phosphorylation does not lead always to activation of enzymes**
- ✓ **Glycogen phosphorylase- reaction (two forms; a & b). Ser is away from the active site**

Glycogen phosphorylase reaction. This enzyme is allosteric and exists in two forms: A and B. The A form is more active than the B form. The A form is phosphorylated and has two phosphate groups. We can switch between the B and A forms using specific enzymes: to convert phosphorylase B into phosphorylase A, we use glycogen phosphorylase kinase, and to go back from A to B, we use glycogen phosphorylase phosphatase



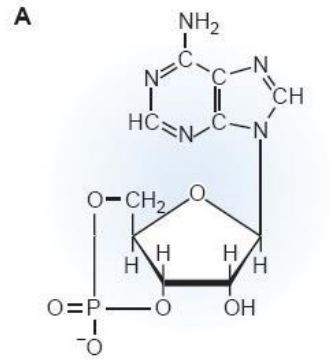
The two forms of the enzyme

- Both phosphorylase *b* and phosphorylase *a* exist as equilibria between an active R state and a less-active T state
- Phosphorylase *b* is usually inactive because the equilibrium favors the T state
- Phosphorylase *a* is usually active because the equilibrium favors the R state

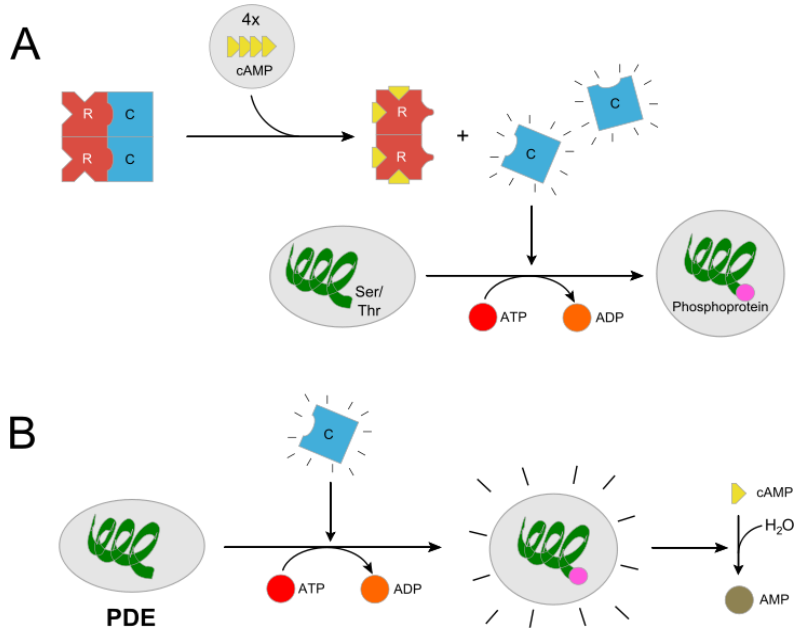


The transition of phosphorylase b between the T and the R state is controlled by the energy charge of the muscle cell.

Protein kinase A (PKA)



- Protein kinase A (PKA): refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP)
- cAMP: referred to as a hormonal 2nd messenger
- Either dedicated or not
- Has several functions in the cell, including regulation of glycogen, sugar, & lipid metabolism



- Adrenaline (epinephrine) → ↑cAMP → activates protein kinase A → phosphorylates & activates glycogen phosphorylase kinase → phosphorylates & activates glycogen phosphorylase
- Phosphorylation cascade

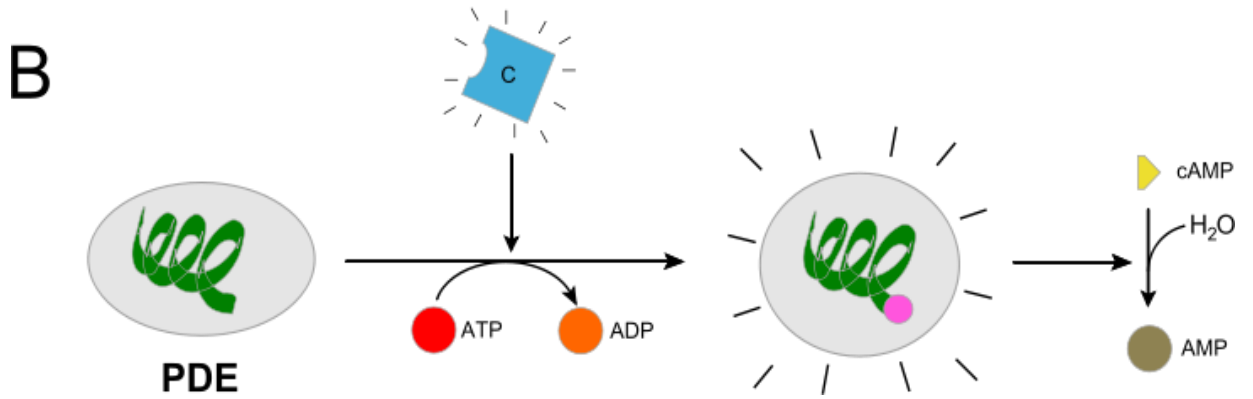
- When the body faces a stressful situation, or when there's an increase in hormones like adrenaline (epinephrine) or glucagon, these hormones bind to their receptors on the cell membrane. This activates a G protein, which in turn stimulates adenylate cyclase. Adenylate cyclase converts ATP into cyclic AMP, or cAMP. The reason we use cAMP instead of just AMP is that cAMP is more stable.

- Once cAMP is produced, it binds to Protein Kinase A, which has two regulatory subunits and two catalytic subunits. The regulatory subunits have four binding sites for cAMP. When cAMP binds, it causes the regulatory subunits to release the catalytic subunits. These catalytic subunits then go on to phosphorylate other proteins.

- In this scenario, one of the proteins phosphorylated is glycogen phosphorylase kinase, which then activates glycogen phosphorylase. This leads to the breakdown of glycogen into glucose. At the same time, glycogen synthesis is inhibited. This entire process is known as the phosphorylation cascade.

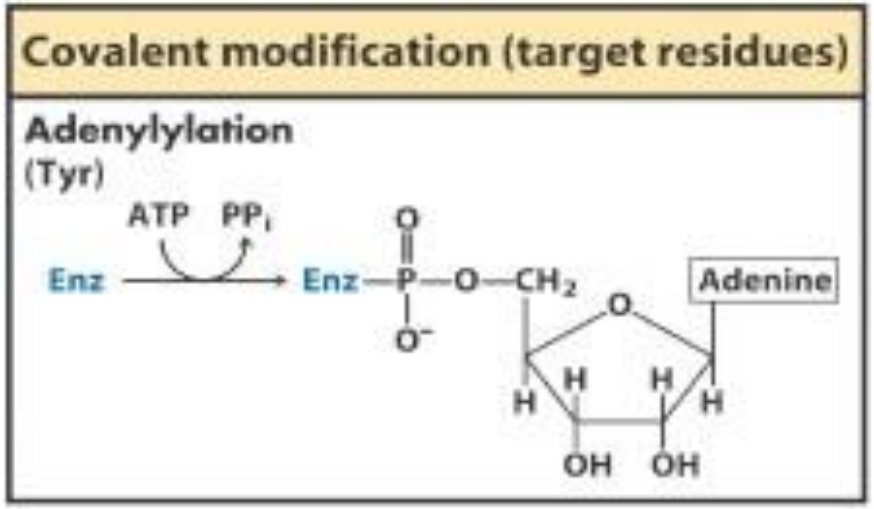
Protein kinase A (PKA)

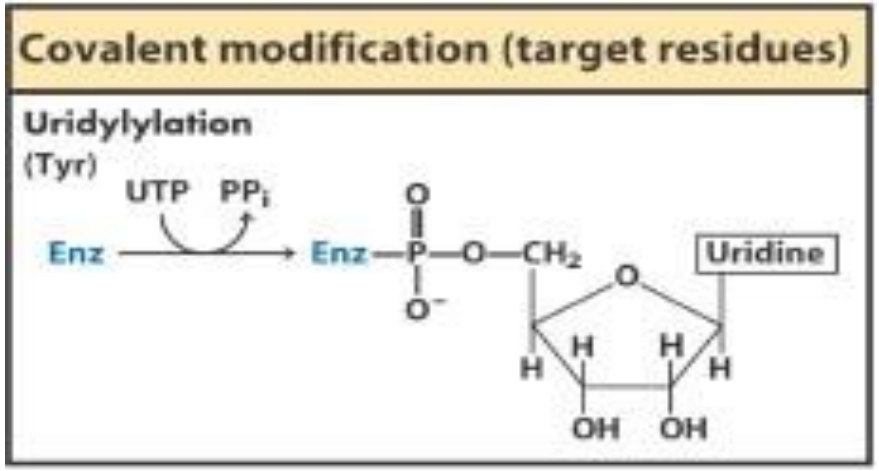
Additionally, one of the catalytic subunits of Protein Kinase A will also phosphorylate an enzyme called phosphodiesterase, or PDE. When PDE is phosphorylated, it converts cAMP into AMP, effectively reducing cAMP levels and providing a feedback inhibition to turn off Protein Kinase A activity.



Other covalent modifiers

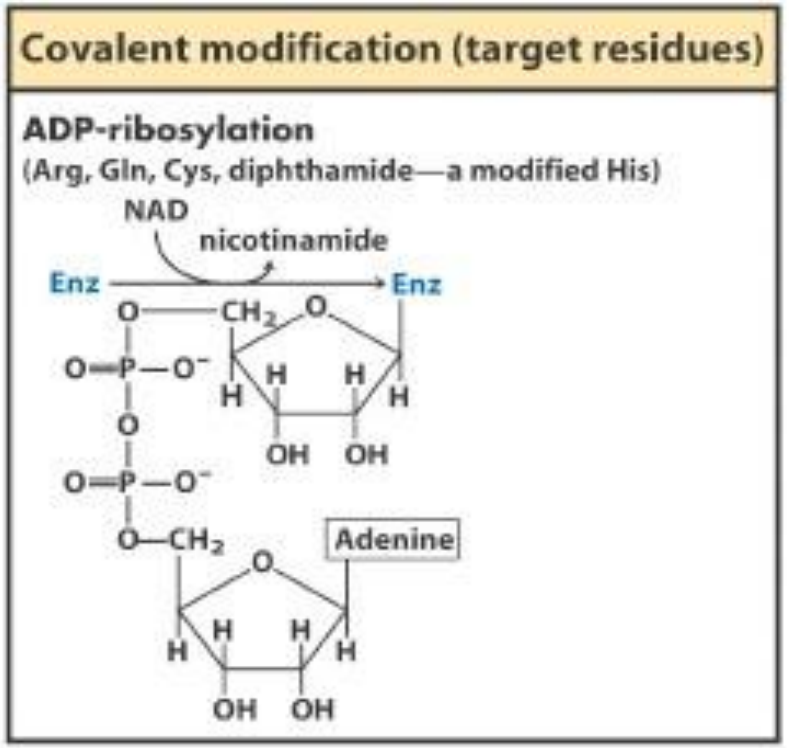
- Adenylylation (addition of adenylyl group). AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes.
- Uridylylation (addition of uridylyl group).

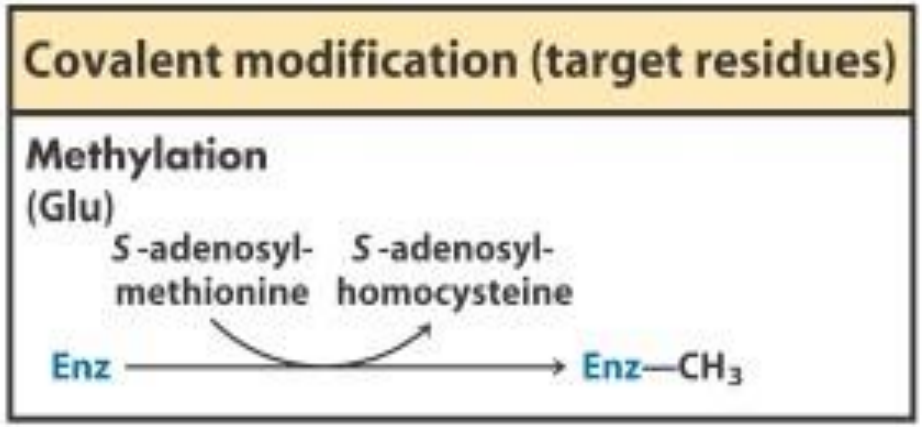




Other covalent modifiers

- ADP-ribosylation: inactivates key cellular enzymes
- Methylation: masks a negative charge & add hydrophobicity on carboxylate side chains
- Acetylation: masks positive charges when added to lysine residues

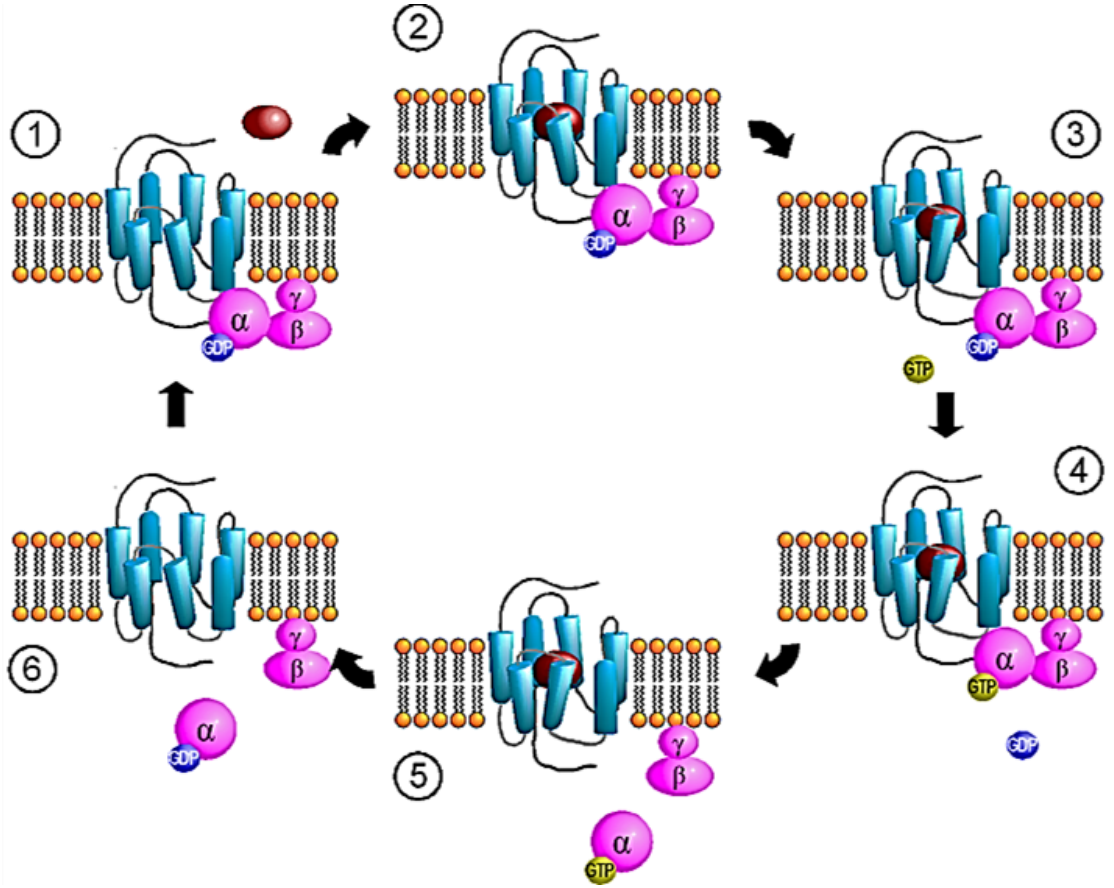




Conformational Changes from Protein–Protein Interactions

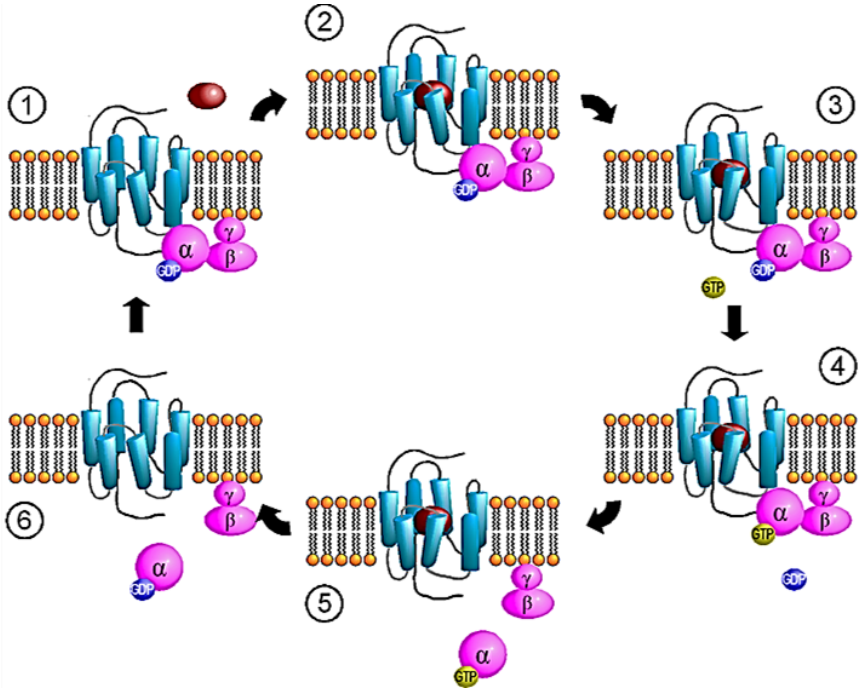
- **G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors**

So, as we've learned in physiology, a G protein is a trimeric protein that's membrane-bound and consists of three subunits: alpha, beta, and gamma. The alpha subunit attaches tightly to the membrane through a covalent bond with fatty acids, and similarly, the beta and gamma subunits also anchor to the membrane.



Conformational Changes from Protein–Protein Interactions

- When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'
- α-Subunit can be stimulatory or inhibitory

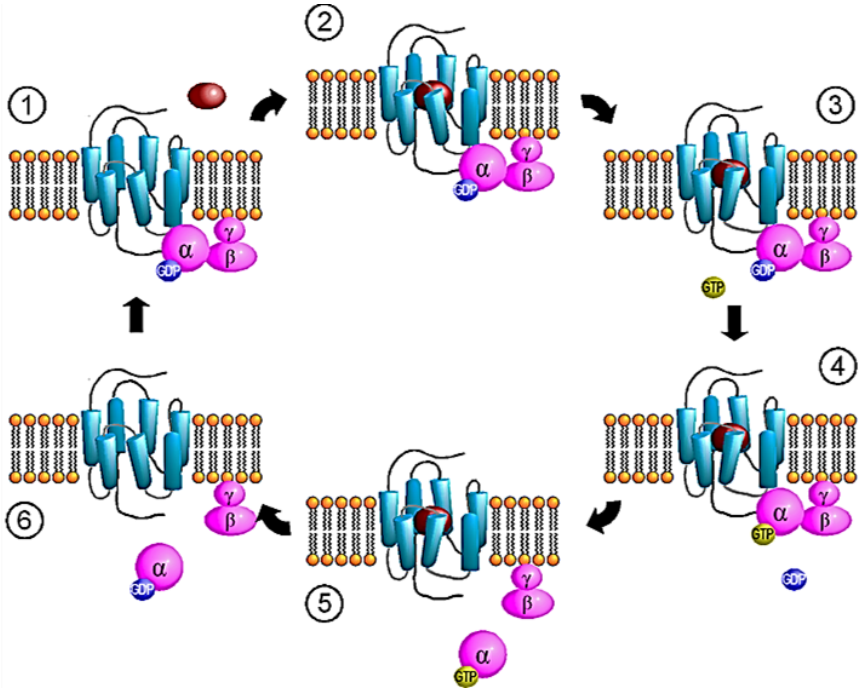


When a hormone binds to its receptor on the cell membrane, it causes a conformational change in the G protein. This change leads to the alpha subunit detaching from the beta-gamma dimer. Initially, the alpha subunit is bound to GDP, but once the hormone binds, the alpha subunit's affinity for GDP decreases and its affinity for GTP increases. This causes the alpha subunit to bind GTP and undergo a conformational change, allowing it to detach from the beta-gamma subunits and move on to activate other proteins.

Conformational Changes from Protein–Protein Interactions

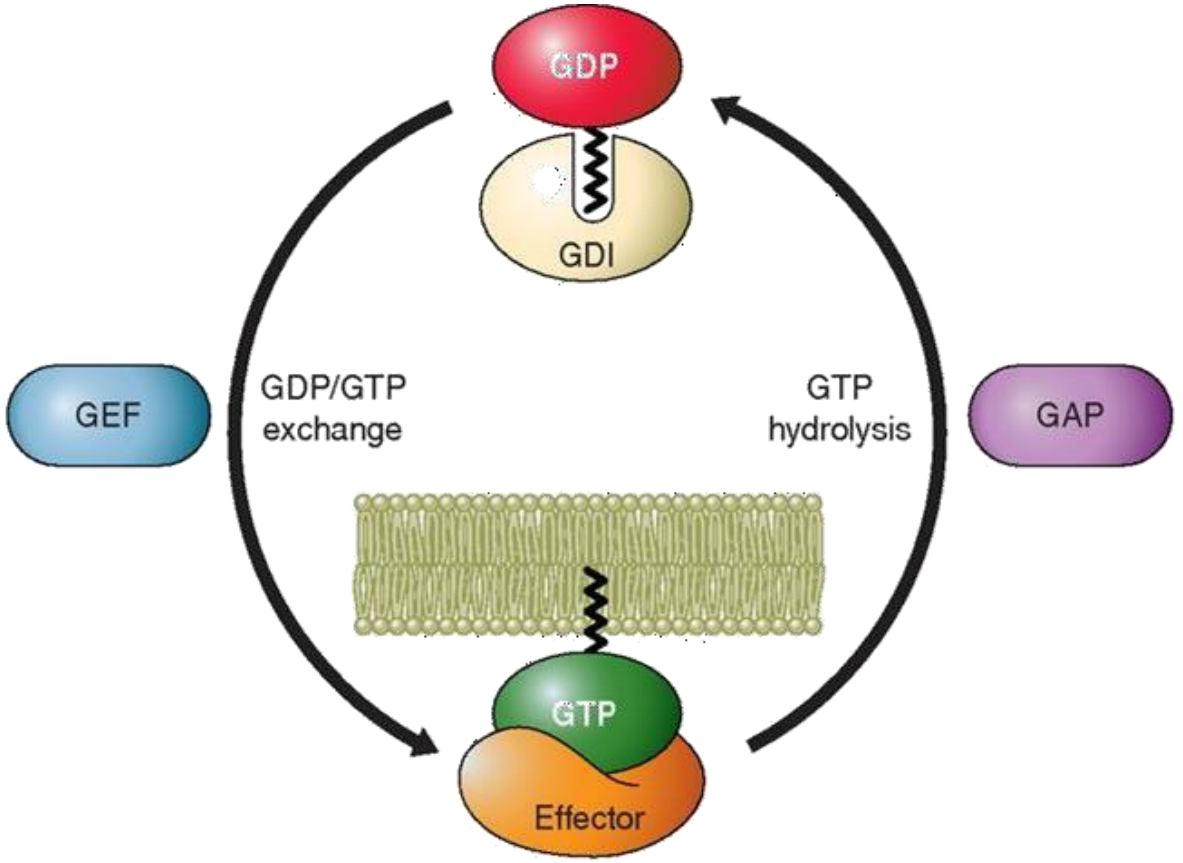
- The reason we focus on the alpha subunit is because it acts like an enzyme. It has the ability to hydrolyze GTP to GDP, which eventually returns it to its inactive form and allows it to re-associate with the beta-gamma dimer.

- It's also important to note that not all G proteins are trimeric. Some are monomeric and function similarly to the alpha subunit, but they are not membrane-bound and are instead cytosolic.



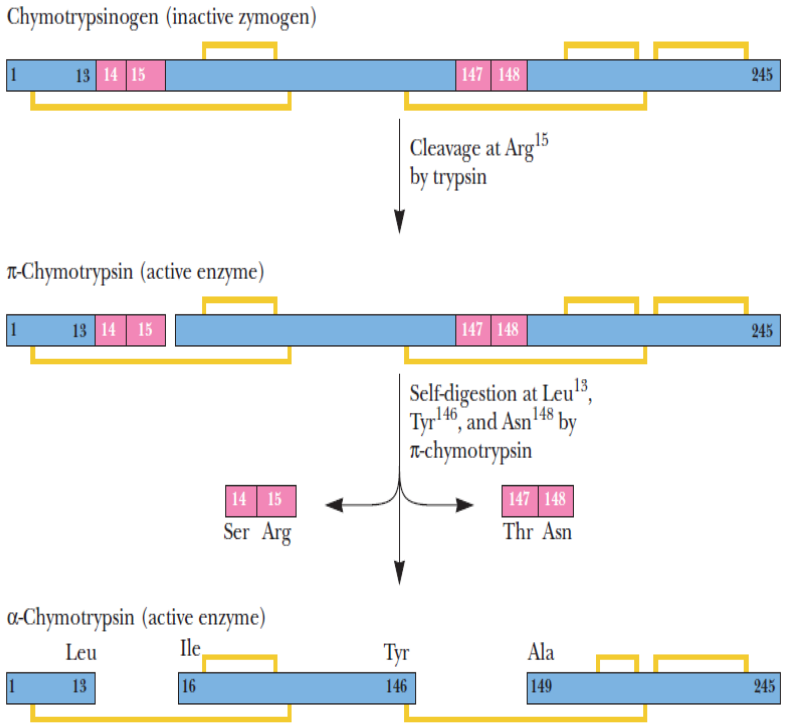
Monomeric "G" proteins

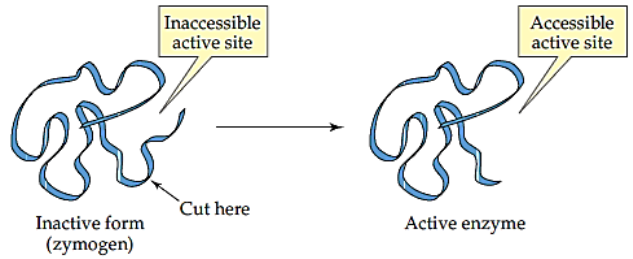
- Same as Gα
- Hydrolysis vs. exchange
- Activation or inhibition
- Example: RAS

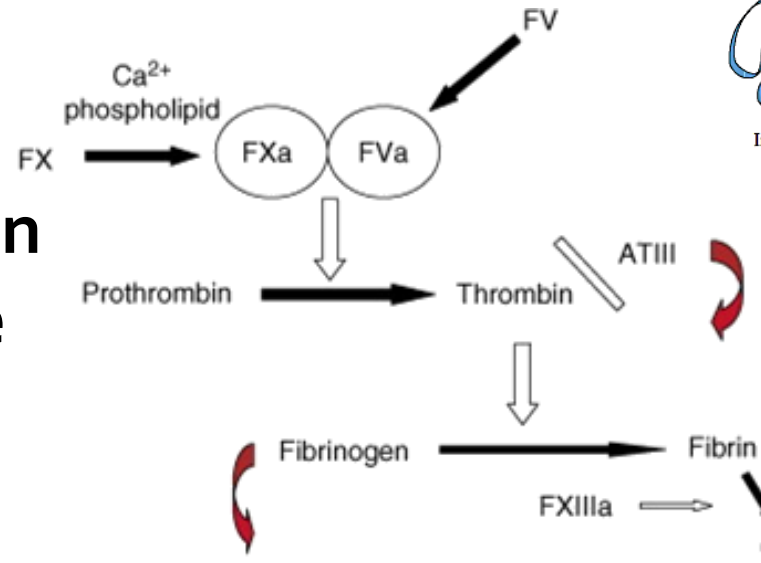


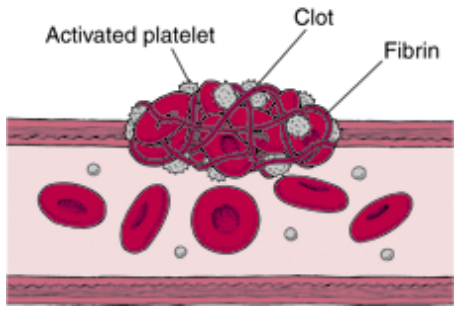
Proteolytic Cleavage Zymogens (Pro- or -gen)

- **Irreversible cut usually at the N- terminus**
- **Trypsin, chymotrypsin, pepsin (trypsinogen, pepsinogen chymotrypsinogen)**
  - ✓ **Chymotrypsinogen: single polypeptide chain (245 residues), 5 (S—S) bonds**
- **Blood clotting**
  - ✓ **The soluble protein fibrinogen is converted to the insoluble protein fibrin**









In the case of proteolytic cleavage, enzymes are initially synthesized with an extra segment that blocks their active site, making them inactive. We call these inactive precursors “zymogens.” The naming convention often involves adding the prefix “pro-” or the suffix “-ogen,” such as in the case of trypsinogen.

When the enzyme is needed, a proteolytic cleavage occurs, usually at the N-terminus, which removes that blocking segment and activates the enzyme. This process is particularly useful when the enzyme’s active site is located in a different region from where it’s synthesized, or when the enzyme needs to be ready for immediate action. This way, the body can keep the enzyme in an inactive form until it’s actually needed, making activation much faster than synthesizing the enzyme from scratch.

A common example of this is digestive enzymes, which are produced in the pancreas in their inactive forms. Once they reach their site of action, they become active and perform their digestive functions.

Non-specific regulators

Affect enzymes regardless of their nature

REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

A. Regulated Enzyme Synthesis

- Regulated by increasing or decreasing the rate of gene transcription (induction & repression)
  - Usually slow in humans (hours to days)
- Sometimes through stabilization of the messenger RNA

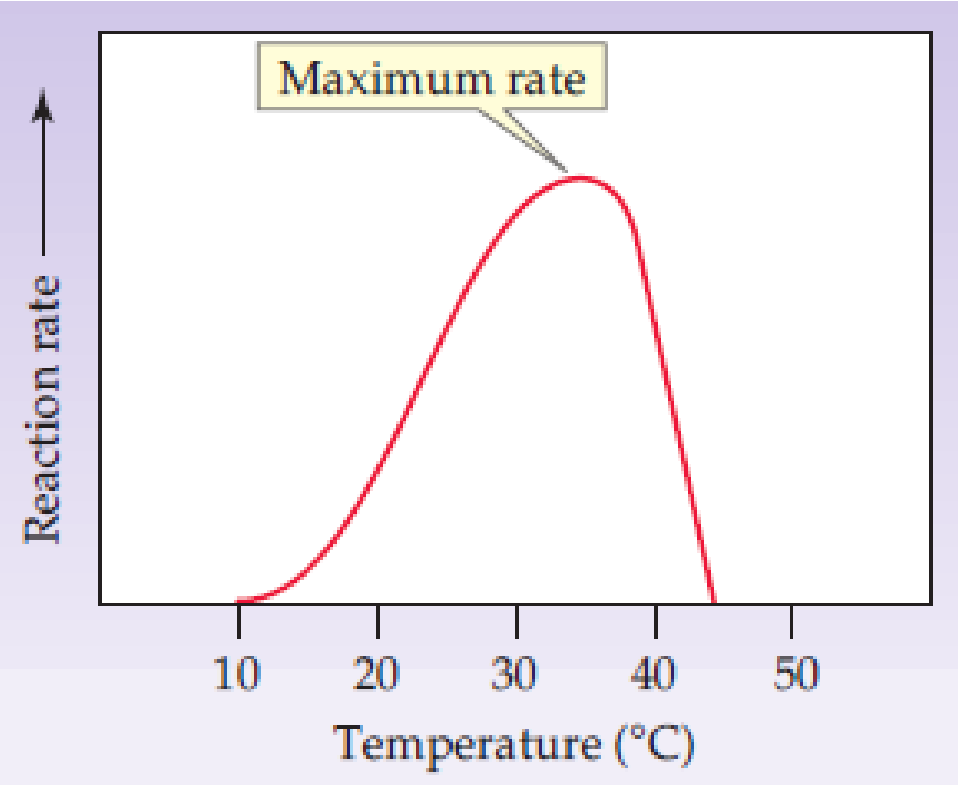
B. Regulated Protein Degradation

- Can be degraded with a characteristic half-life within lysosomes
  - During fasting or infective stress: gluconeogenesis increase & synthesis of antibodies (protein degradation increases)
  - Increased synthesis of ubiquitin

Ubiquitin acts as a tag marking other proteins for degradation.

Effect of Temperature

- **Increase in T° increases the rate until reaches a max (≈ 50°): the optimal temperature of each enzyme is its' denaturation**
- **Autoclave steam heating**
- **Hypothermia, metabolic reactions, cardiac surgery**

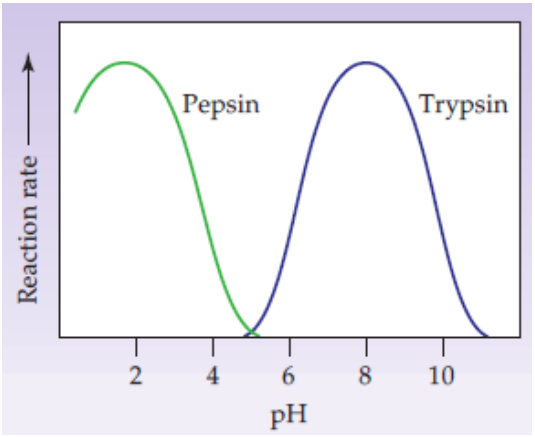


The optimal temperature is not really optimal, because the enzyme starts to denature at that point.

Effect of pH

- Usually a well defined optimum point
- Most enzymes have their max. activity between (5-9)
- Extremes of pH denatures protein
- pH can alter binding of substrate to enzyme (K_M) by altering the protonation state of the substrate, or altering the conformation of the enzyme

Pepsin is quite different as its optimum pH around 2.



The effect of pH is enzyme-dependent

Extremozymes

Enzymes extracted from organisms living in extreme conditions(thermophiles & psychriphiles), so they can survive and work in extreme conditions

Taq polymerase and PCR

Biobleaching of paper pulp using heat-stable xylanases

Thermophiles (heat lovers)

Psychrophiles (cold lovers)

lipases and proteases

Taq polymerase synthesizes new DNA strands during PCR.
It comes from **thermophile**; it works at high heat in PCR without being destroyed.

Heat-stable xylanases are used in paper industry. They bleach pulp in an eco-friendly way (instead of harsh chemicals).

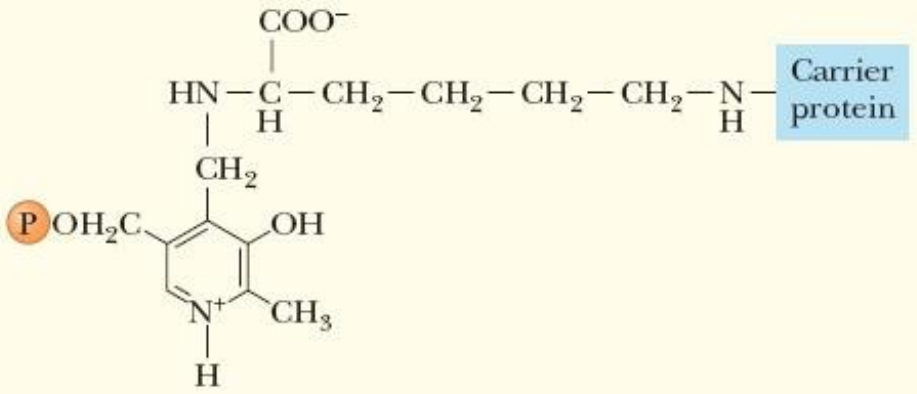
These enzymes from **psychrophiles** work well in cold water useful for energy conservation

(Antibody + Enzymes) Abzymes – cutting edge science

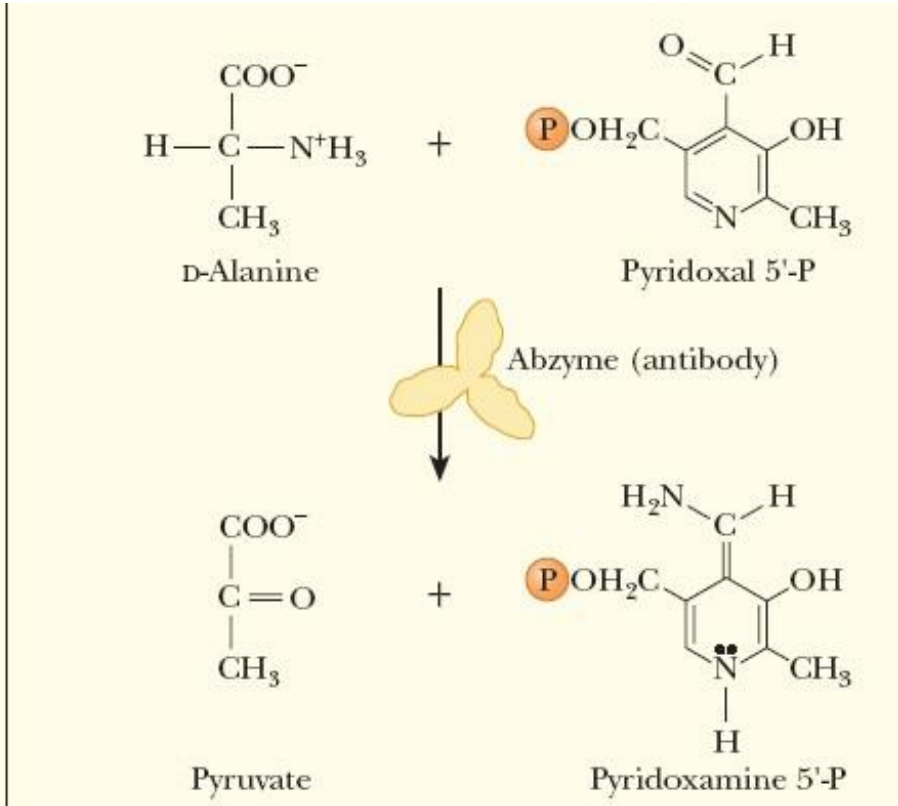
- **An antibody that is produced against a transition-state analog (active)**
- **An abzyme is created in animals**

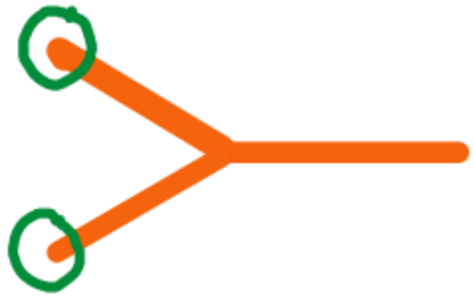
Abzymes are produced in animals by stimulating the immune system to recognize a transition-state analog of a chemical reaction.

In other words, it is an antibody that acts like an enzyme because it can accelerate a chemical reaction by mimicking the enzyme's active site(the tips of the Y shape) .



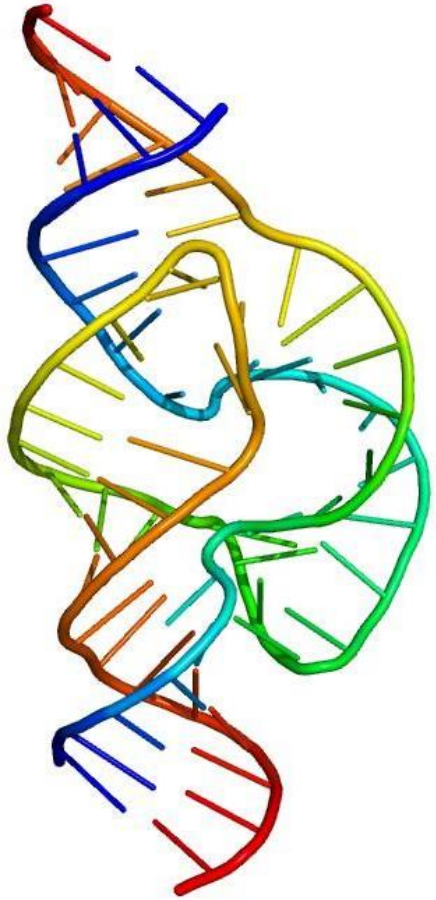
N^{α}-(5'-Phosphopyridoxyl)-L-lysine moiety (antigen)





An exception to protein enzymes
Ribozymes

- RNA molecules Special to rxns related to the RNAs themselves.
- Examples: telomerase & RNase P
- Catalyze splicing reactions and are involved in protein synthesis
- The catalytic efficiency of catalytic RNAs is less than that of protein enzymes, but can greatly be enhanced by the presence of protein subunits



Very weak in their catalytic activity **unless** they're coupled to proteins.

Metabolic pathways:series of biochemically catalyzed rxns where every rxn is leading to the other one till you achieve your final product and every step is catalyzed by different enzyme except for spiral pathways where the same set of enzyme catalyzes the pathway.

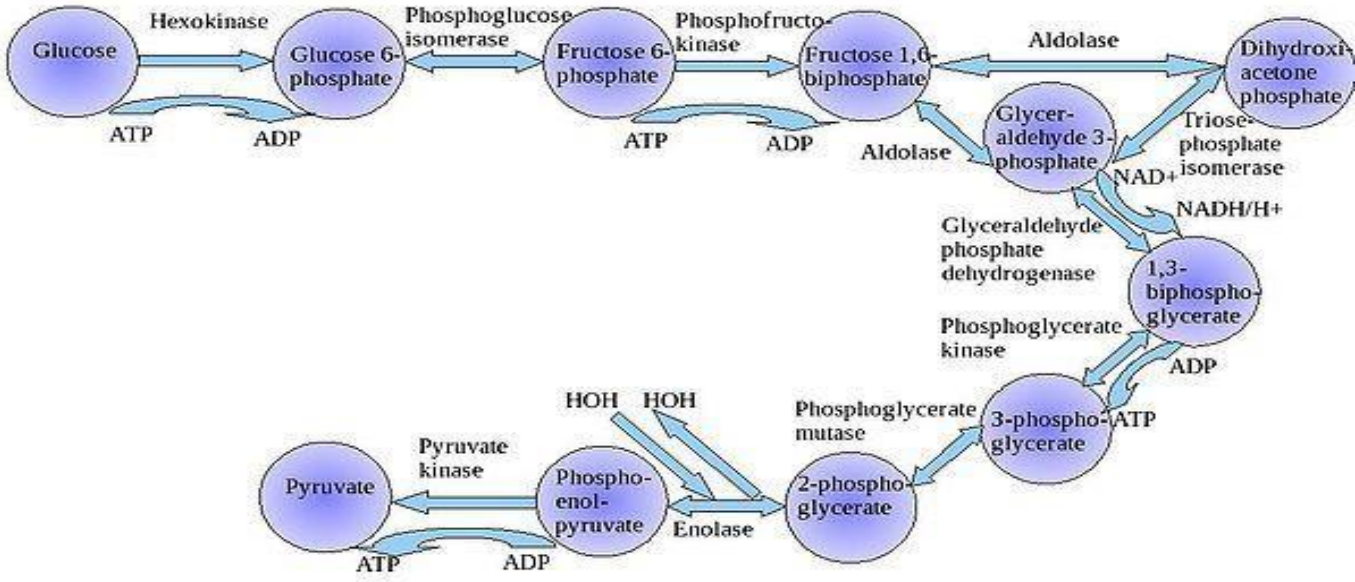
Metabolic pathways are generally classified into linear, cyclic and spiral.

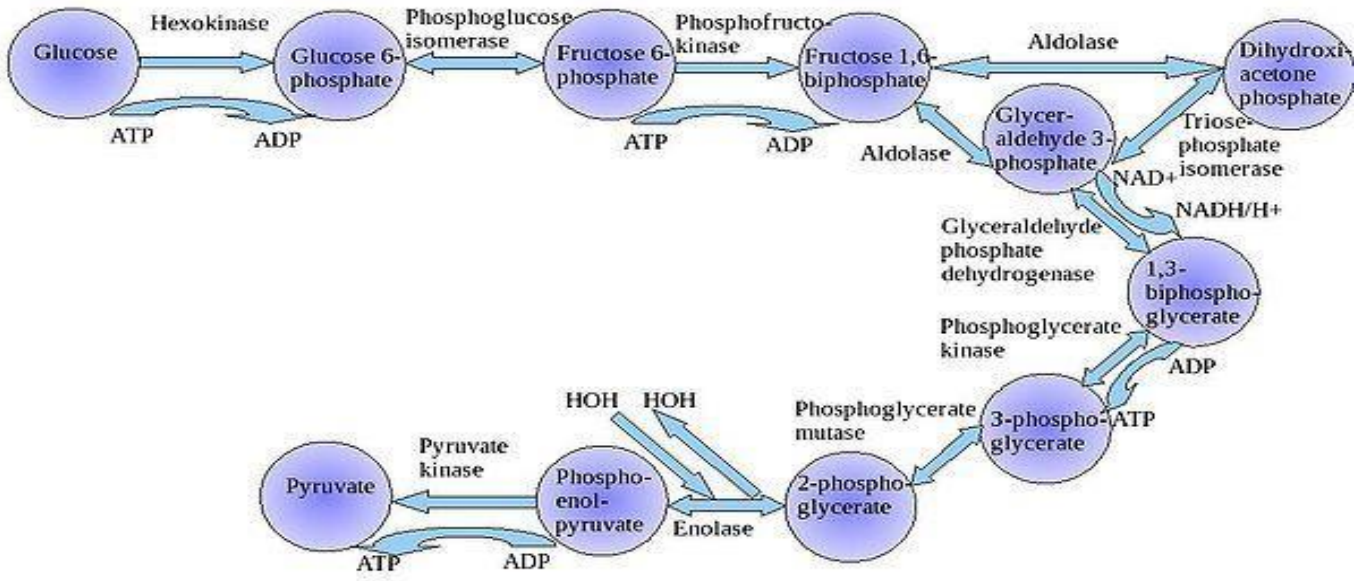
REGULATION OF METABOLIC PATHWAYS

Pay attention to the fact that there is no simple rxn in our body, for example the simplest split of glucose to pyruvat involves 10 steps.

Principles of Pathway Regulation

- *1. COUNTERREGULATION OF OPPOSING PATHWAYS*
- Synthesis vs. degradation (a different regulatory enzyme)
- *2. TISSUE ISOZYMES OF REGULATORY PROTEINS*
- *3. REGULATION AT THE RATE-LIMITING STEP*
- Pathways are principally regulated at their rate-limiting step
- The slowest step & is usually not readily reversible
  - Changes in this step can influence flux through the rest of the pathway
- Usually the first committed step in a pathway
- Requirement for high amount of energy
- High K_M values of enzyme towards its substrate



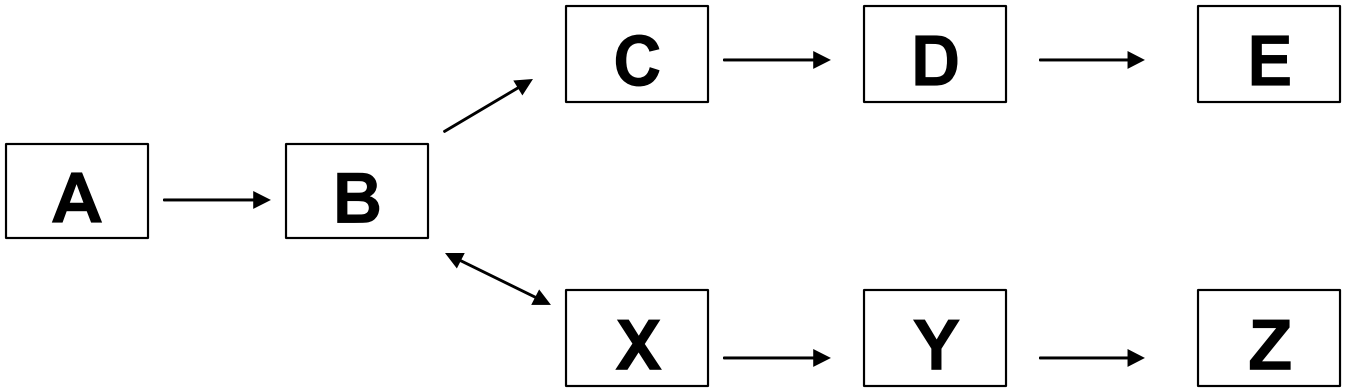


In the glucose splitting reaction to two pyruvate molecules, the rate-limiting step is the third one, which is the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, catalyzed by the enzyme phosphofructokinase because it is the slowest and has the lowest Km.

Principles of Pathway Regulation

- ***4. The committed step***

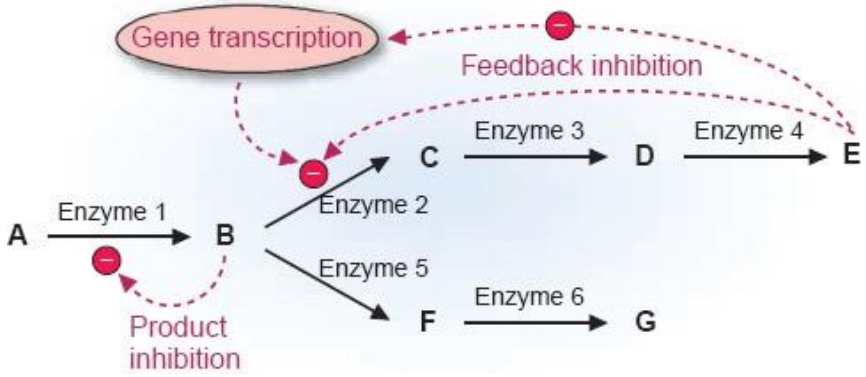
- **A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return**
- **Committed steps are exergonic reaction**
- **For example, the committed step for making product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

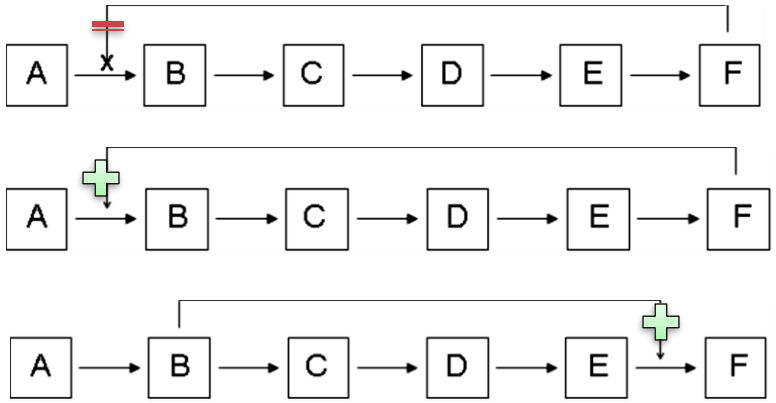


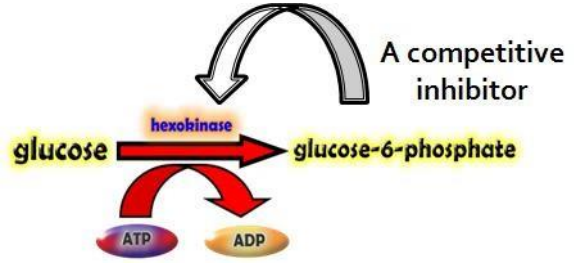
The committed step for making product Z is X>Y not B > X because it's reversible

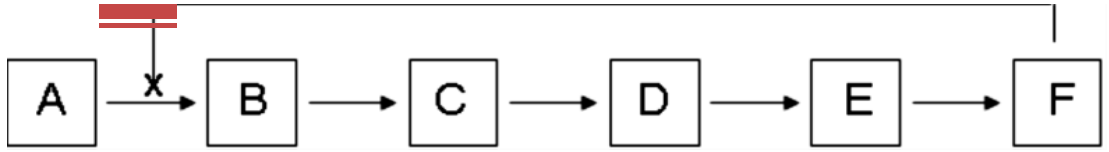
Principles of Pathway Regulation

- *5. FEEDBACK REGULATION*
- This type of regulation is much slower to respond to changing conditions than allosteric regulation
  - Negative feedback regulation (feedback inhibition)
  - Positive feedback regulation
  - Feed-forward regulation
    - Disposal of toxic compounds

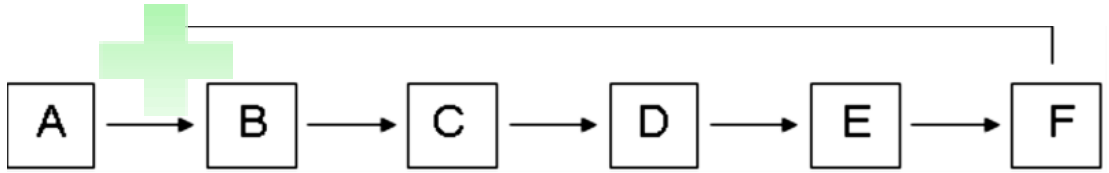




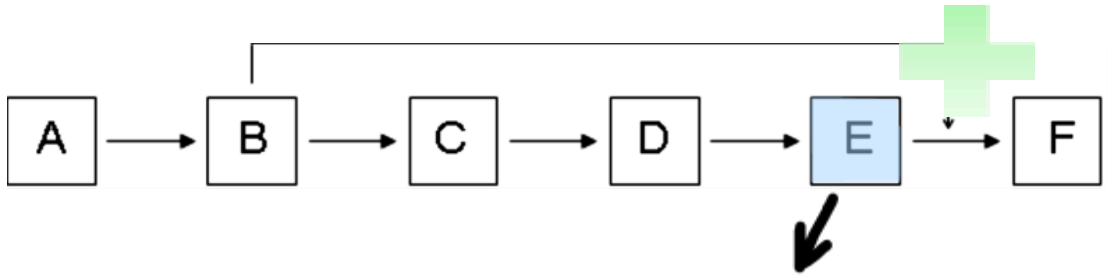




Negative feedback regulation



Positive feedback regulation



Feed-forward regulation

In feed-forward regulation the body prepares in advance for an upcoming change instead of waiting for it to happen.

The concentration of the enzyme responsible for detoxifying is increasing even before the compound is present, so the enzyme is ready to act as soon rapidly as the toxic substance appears.

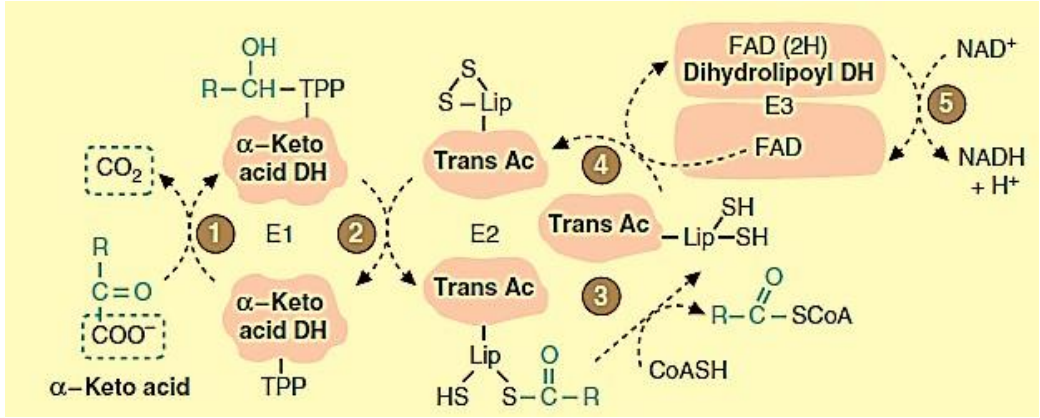
Principles of Pathway Regulation

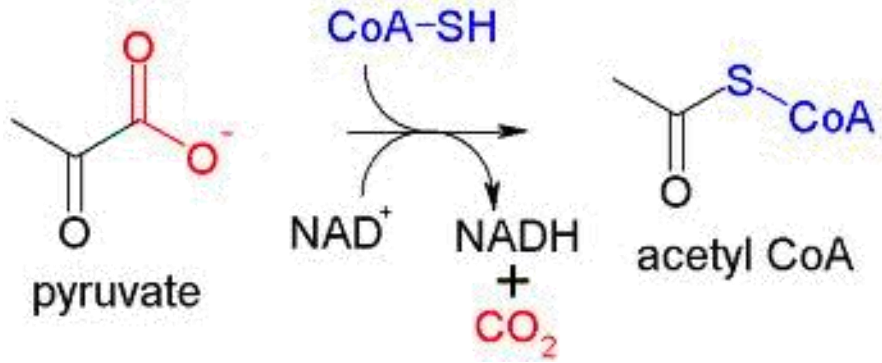
- ***6. Enzyme compartmentalization***
- **Both enzymes and their substrates are present in relatively small amount in a cell**
- **A mechanism by which rate of reactions become faster is their compartmentalization; reducing area of diffusion**
- **In this way, enzymes are sequestered inside compartments where access to their substrates is limited**
- **Lysosomes; proteins get transported to lysozymes**
- **Mitochondria; energy metabolic pathways**
- **Metabolism of fatty acids; synthesis (cytosol) vs. degradation (mitochondria)**

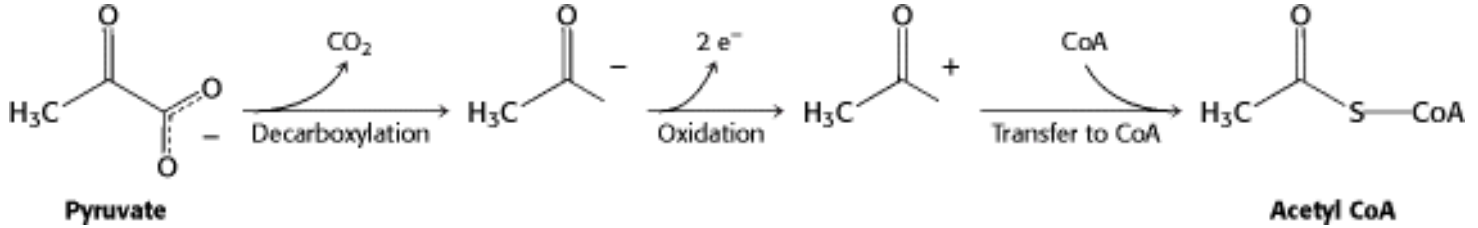
Principles of Pathway Regulation

- *7. Enzyme complexing (A multienzyme complex)*

- Complexing various enzymes that share one process
- Product of enzyme A pass directly to enzyme B
- Pyruvate dehydrogenase (mitochondria) 3 enzymes: decarboxylation, oxidation, & transfer of the resultant acyl group to CoA







Enzymes in Medical Diagnosis

Enzymes that are specific to a particular organ are normally present in low amounts in the blood. However, if there is a problem or damage in that organ, the concentrations of these enzymes in the blood increase, allowing us to detect and diagnose the organ dysfunction.

Diagnostic Enzymes & Liver Disease

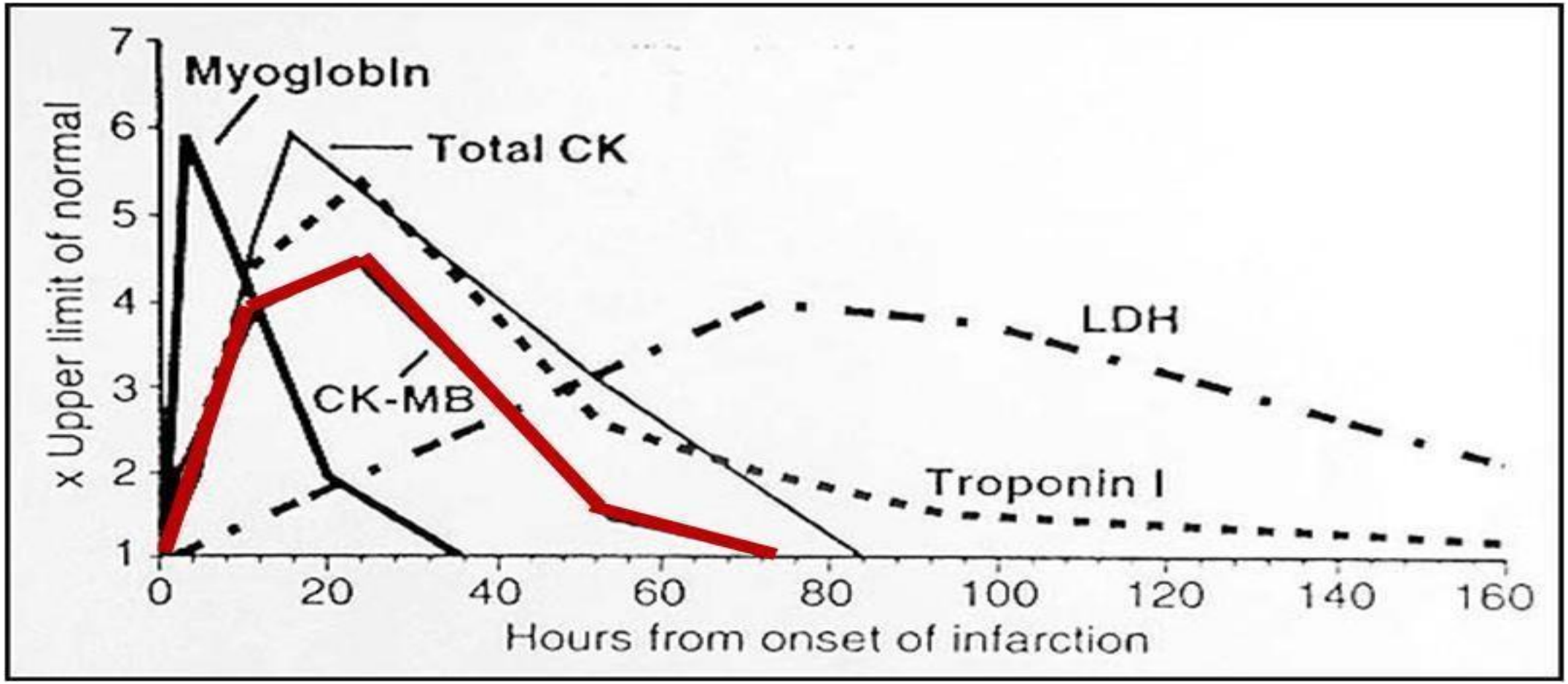
- Concept
- Examples: ALT, AST, LDH, CK (CPK)
- Liver disease: ALT & AST
  - ALT is the most specific
  - Ratio can also be diagnostic (ALT/AST)
  - In liver disease or damage (not of viral origin):
    - ratio is less than 1
  - With viral hepatitis:
    - ratio will be greater than 1

ALT present in good concentration in the liver but low concentrations in other organs so it's specific for the liver

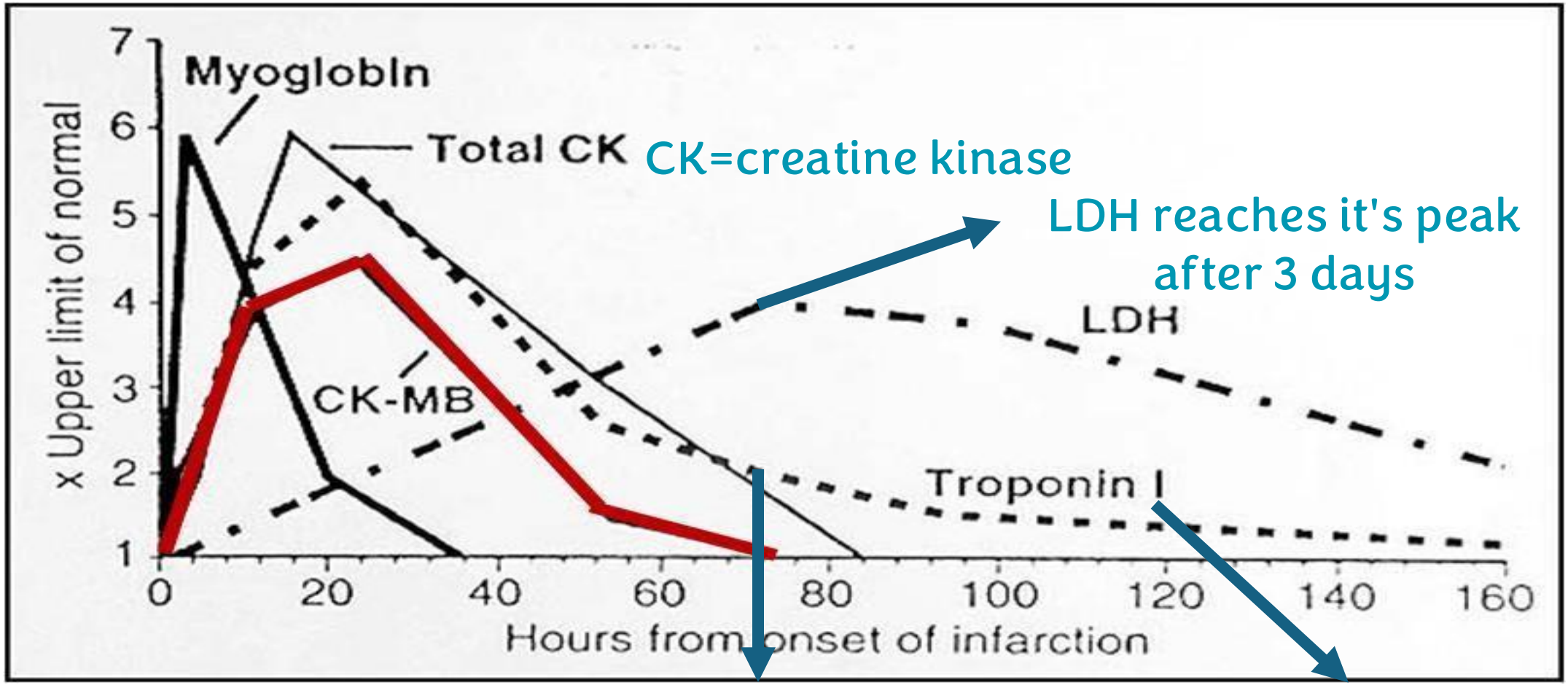
AST present in high concentrations in the liver and other organs so it's more sensetive for the liver but not specific

Protein profile in myocardial infarction

The Y-axis does not measure enzyme concentrations; rather, it shows how many times higher they are than the normal concentration.



Protein profile in myocardial infarction



CK=creatine kinase

LDH reaches it's peak after 3 days

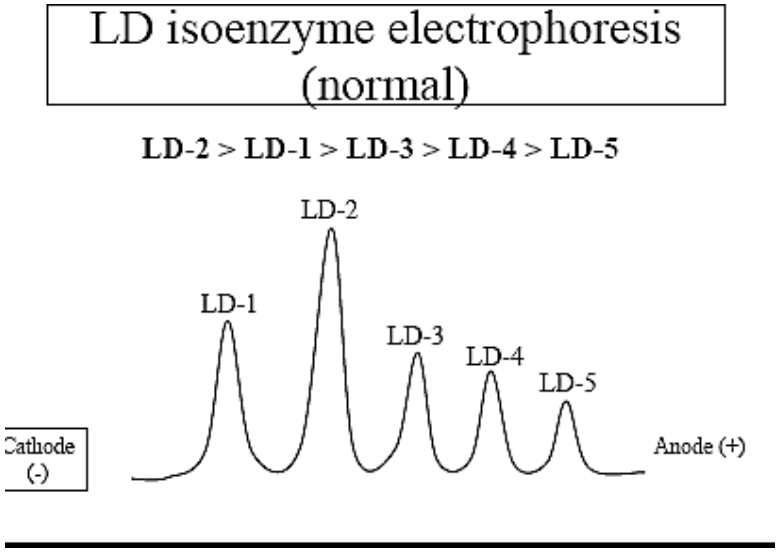
After 3 days CK almost return to it's normal value

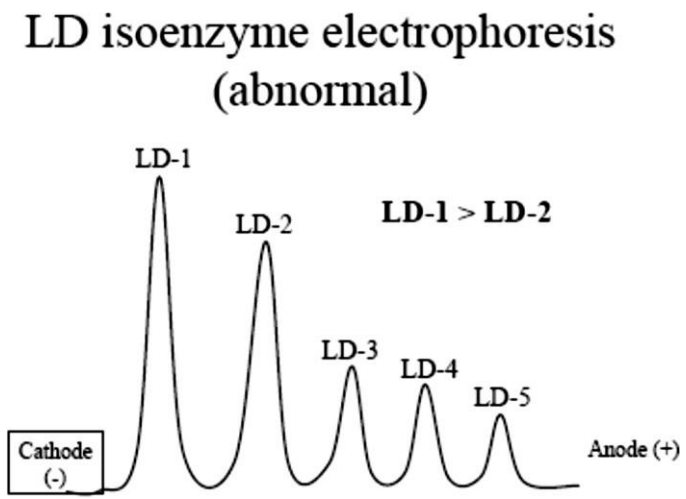
We won't discuss it because we are talking about enzymes and it's a protein

LDH

- 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

LDH1 in normal situations it's less than LDH2





CPK 3 copies:BB,BM and MM

- Heart, skeletal muscles, & brain
- Like LDH, there are tissue-specific isozymes of CPK:
  - CPK3 (CPK-MM): the predominant isozyme in muscle
  - CPK2 (CPK-MB): accounts for ≈35% of 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

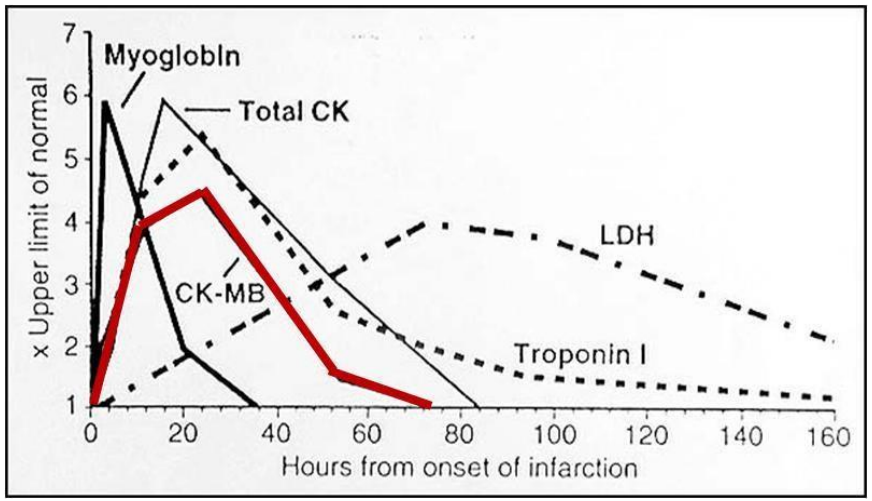
Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

The BB isoform of CK is specific to the brain, the MM isoform is found in both skeletal muscles and the heart, and the MB isoform is specific to the heart. Therefore, if MM levels are elevated, it is not possible to tell whether the problem is in the heart or the muscles, but an increase in MB indicates a heart-related issue so it's specific for the heart.

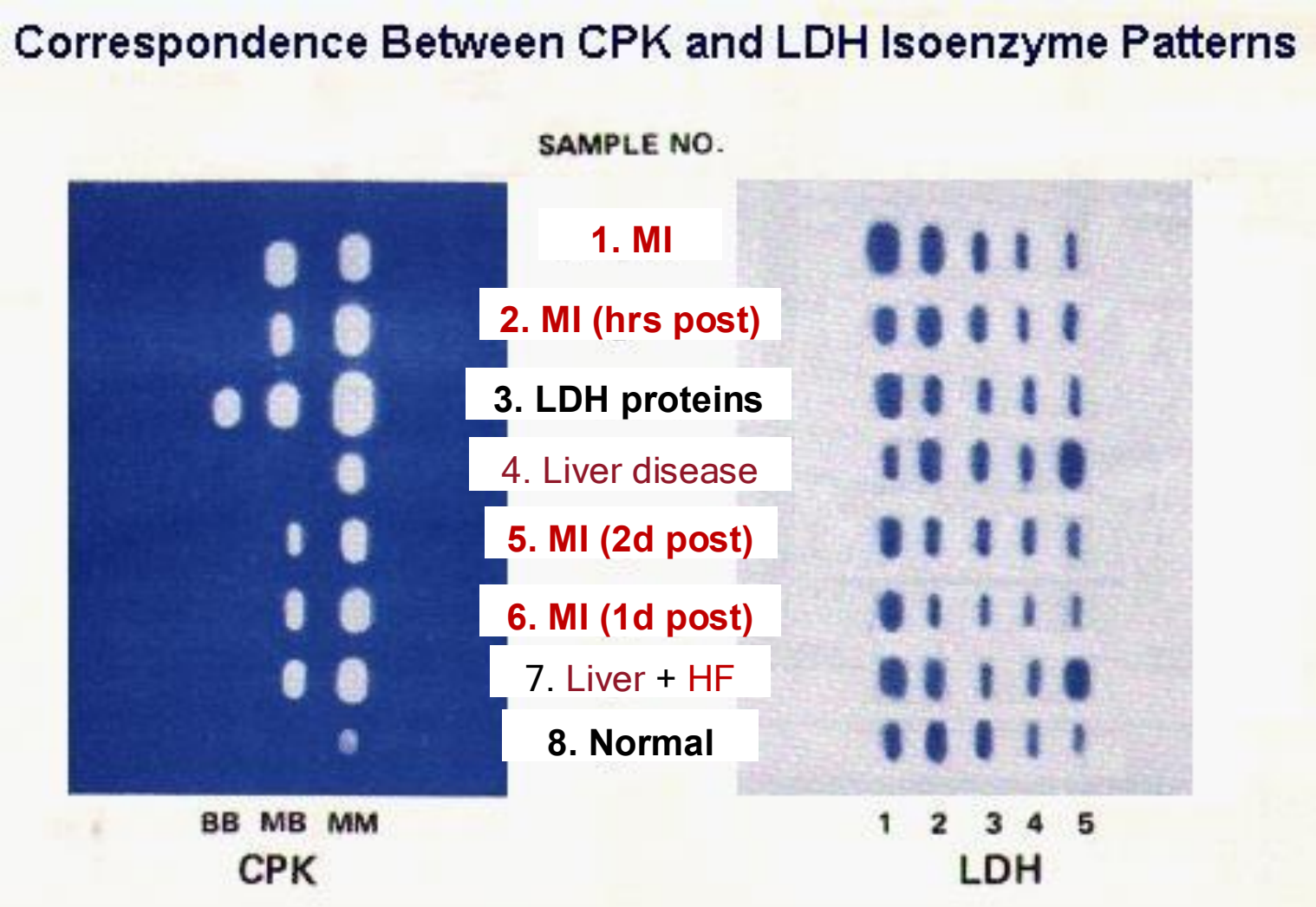
CPK and myocardial infarction

- Most of released CPK after MI is CPK-MB
- Increased ratio of CPK-MB/total CPK may diagnose 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

If we found CK concentration after 4 days in it's peak this means thatt here is another infarction (it's a common condition)



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

- Like all cardiac markers, troponins have a unique diagnostic window
- 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

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

رسالة من الفريق العلمي:

﴿ فَسَيَكْفِيكَهُمُ اللَّهُ وَهُوَ السَّمِيعُ الْعَلِيمُ ﴾

"So Allah will be sufficient for you against them, and He is the All-Hearing, the All-Knowing."

يا له من وعدٍ عظيم!
من القادر، المعطي، الكريم..

اللهم إني لا أملك من أمري شيئًا، فدبّـر لي أمري كما تحب وترضى، وحقق لي غايتي كما تعلم أنـــــــــــها خير لي

أشهدك يا الله أني قد سلمت إليك أمري، ورضيت بقضائك وشكرتك على حكمك، فلك الحَمد في الأولى والآخـــــــرة ولك الشكر سرًّا وجهرًا، ما علمتُ وما لم أعلم...