25

BIOCHEMISTRY

faculty of medicine - JU2017

Sheet ○ Slides

DONE BY

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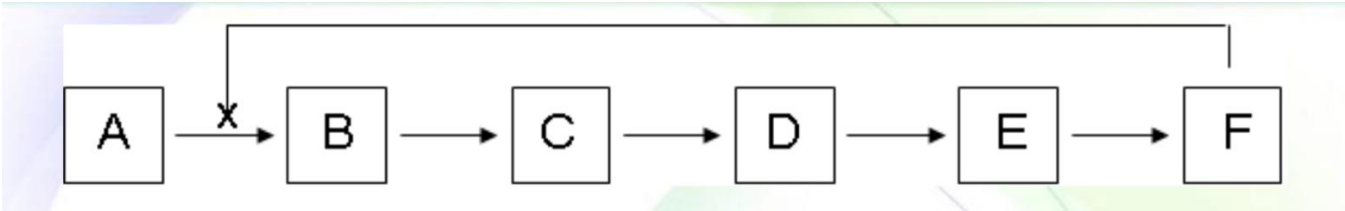
Review: -

Modes of regulation:-

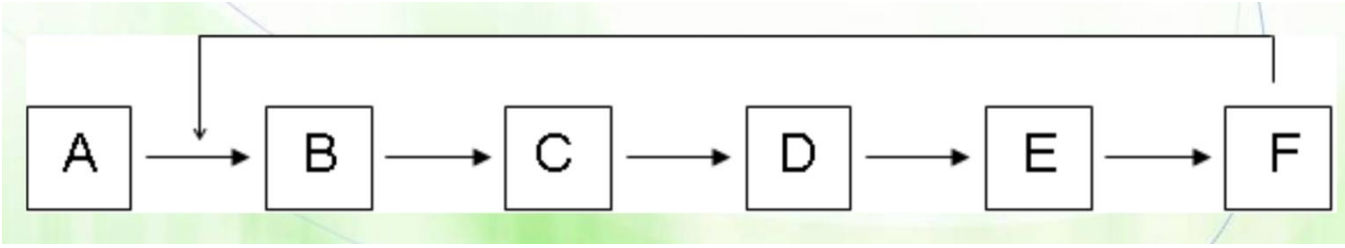
❖ **Feed-back regulation** is when an enzyme present early in a biochemical pathway is regulated by a late product of the pathway.

⇒ Feedback regulation could be either activation or inhibition.

* Feedback inhibition (negative feedback) regulation:
  - ✓ The figure below is an example of negative feedback regulation.
  - ✓ The end product F inhibits the first enzyme.



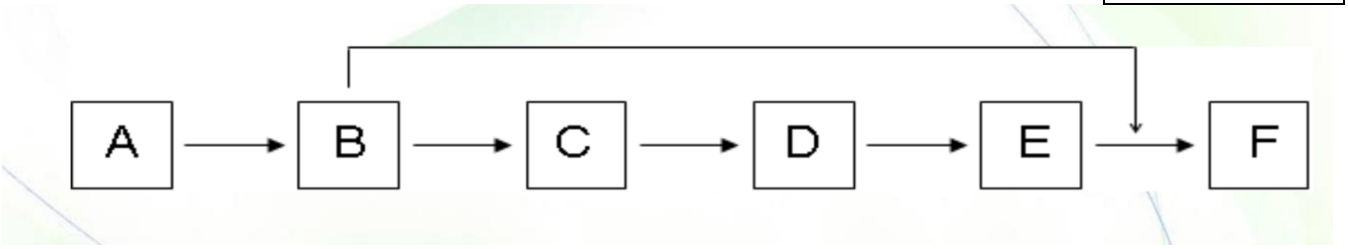
* Positive feedback regulation is when a product (as F in the below figure) stimulates the activity of an early enzyme.



❖ **Feed-forward regulation** is when a substrate produced early in a pathway activates an enzyme downstream of that same pathway.

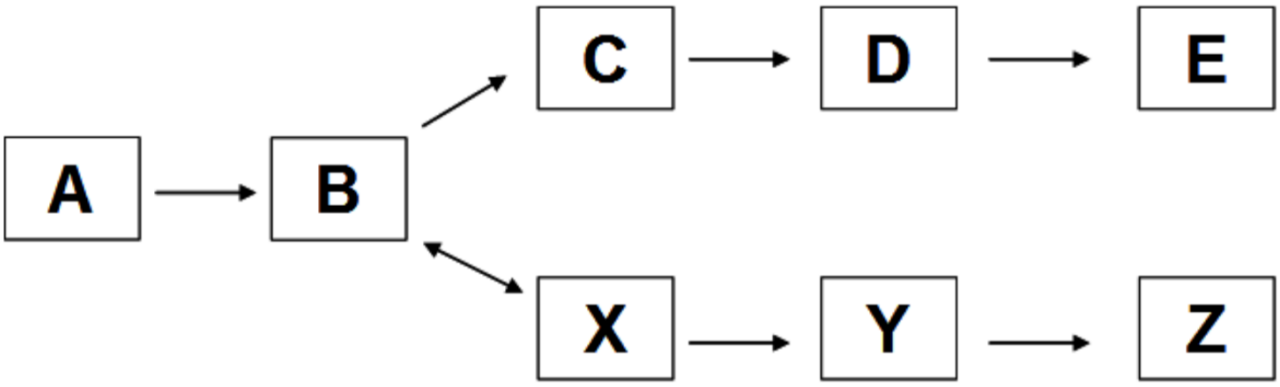
⇒ The main purpose is to speed up the reaction.

*It doesn't make sense for an early product to inhibit an enzyme.



❖ **A committed step** (a common word in biochemistry): is a step or a reaction in a pathway that finalizes the pathway committed (proceed to the end product with no point of return).

⇒ Committed steps are exergonic reactions.
⇒ Committed step is the first irreversible reaction that is unique to a pathway.



⌛ Q1:- According to the figure above, what are the committed steps for the formation of products E & Z ?

☑ For the conversion from A -> E, the committed step is B -> C; because it's irreversible.

☑ For the conversion from A -> Z, the committed step is X -> Y.

❖ **Rate-limiting steps** are the slowest steps in a pathway (rate of rxn depends on this step.)

★ WHY? Because (their characteristics) :-

- They require high amount of energy.
- Strict regulation of enzymes.
- High Km values of enzyme toward its substrate.

⇒ **Requirement for high amount of energy**
⇒ the cell is so stingy (بخيلة) it asks itself should I consume ATP? Which is very expensive? So, the reaction becomes slower than normal.

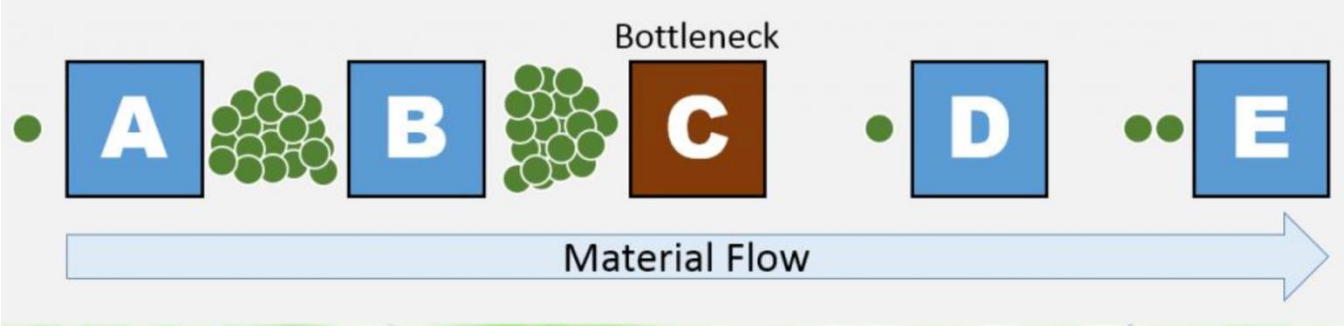
⇒ **Strict regulation of enzymes**

⇒ the reaction which is rate limiting is catalyzed by an enzyme that is highly regulated by many products or chemicals.

⇒ **High Km value of enzyme toward its substrate**.

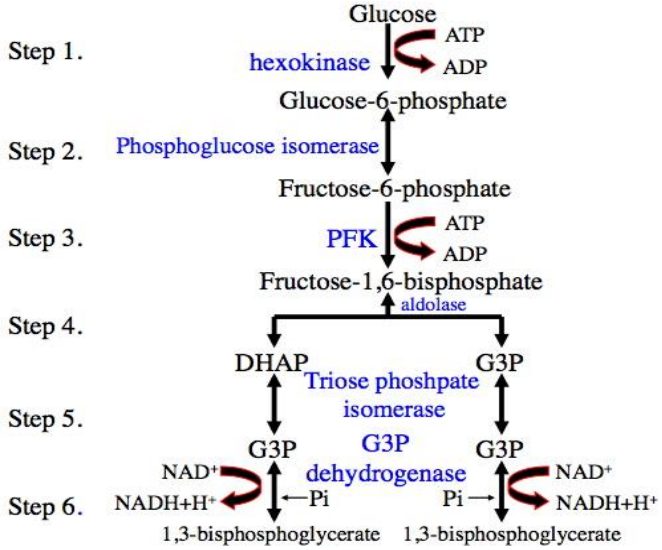
⇒ which means the enzyme has low affinity for the substrate.

★ These steps are usually, but not necessarily, the committed step.



★ According to the figure above, the rate limiting reaction is C -> D; because the reaction is slower than the previous reactions.

⌛ Q2:- Depending on the glycolysis reactions below, decide whether each of the first 3 steps is a committed step , rate limiting reaction, both or none.



*this mechanism is just for clarification and It's not mentioned in the slides, but Dr. Mamoun gave an example on it.

☑ Step 1;

* It's not a committed step; because the cell can use Glu-6-P in different pathways such as, (glycolysis) converting it to pyruvate, storing it as glycogen or converting it to Ribose-5-P to make DNA & RNA.

* It's a rate limiting step; because it requires ATP and the enzyme (hexokinase) is highly regulated by ATP and Glu-6-P.

☑ Step 2;

* It's not a committed step; because it's a reversible reaction.

* It's not a rate limiting reaction; because it doesn't require energy.

☑ Step 3;

* It's a committing step; because when Fru-1,6-bisP is produced it's difficult to go back again (Irreversible reaction).

* It's a rate limiting reaction; because it requires ATP and the enzyme (phosphofructokinase "PFK") is highly regulated by many factors such as, ATP/AMP/Fru-6-P/Fru-1,6-bisP/Fru-2,6-bisP.

Enzymes in disease diagnosis:-

⇒ One of the main functions of biochemistry is to help us in diagnosing diseases.

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⇒ We can diagnose diseases by blood tests by looking at the concentration of certain biomarkers such as enzymes, ions and molecules in serum (blood sample).

⇒ Normally, these biomarkers should be present in cells or in tissues, but not in the blood.

⇒ The measurement of enzyme amount in a serum is of diagnostic significance.

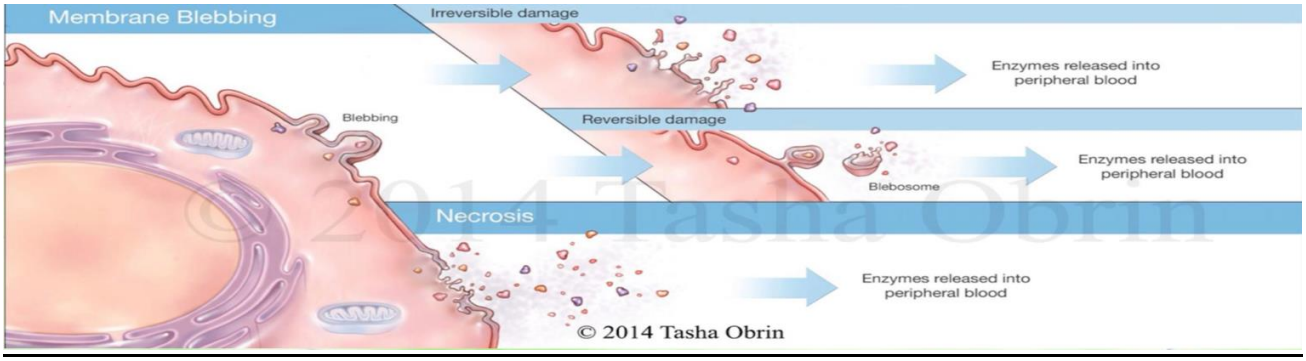
⇒ High levels of these biomarkers in blood indicates tissue damage that can be caused by, for example, being beat up, therefore, athletes after doing sports have high amount of skeletal muscle proteins in blood as a result of muscle damage (or cell rupture). Another example could be the viral infection in liver cells which get damaged and die. So, enzymes and proteins get released into the blood.

⇒ We are looking for certain enzymes like;

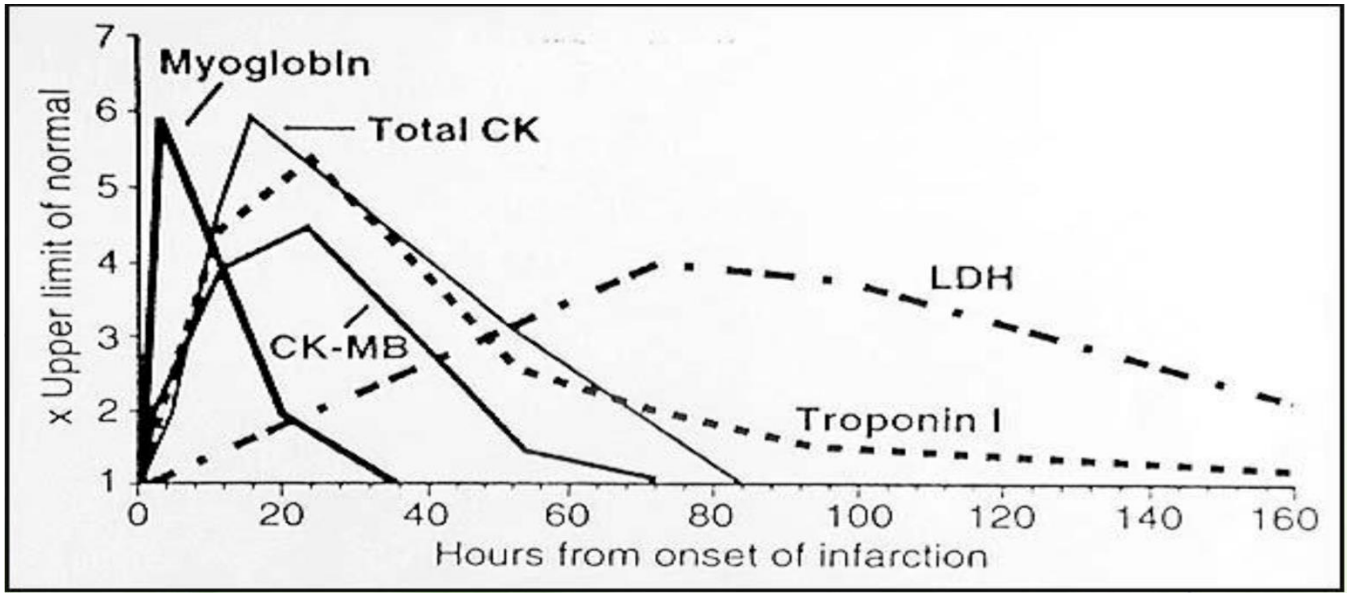
- Amino transferases (Transaminases):-
  - Alanine transaminase (ALT).
  - Aspartate aminotransferase (AST).
    - ✓ Good diagnostic markers of liver damage.
- Lactate dehydrogenase (LDH).
  - ✓ Good marker of tissue damage as well.
- Creatine kinase (CK) also called Creatine Phosphokinase (CPK).

❖ **ASL and ALT:-**

- Typical Liver enzymes.
- ALT is predominantly in hepatocytes (ALT is more specific for liver).
- If both AST & ALT are released (increased conc. in blood) it could be liver damage.
- If the conc. of ALT is normal and the conc. of AST is high in blood, it could be that the kidney is not functioning well because AST is also present in kidney.
- The ratio of ALT \ AST is diagnostic:
  - If the conc. of ALT is high, and the conc. of AST is high in blood and the ratio < 1, the diagnosis is liver disease / damage (Not a result of a viral origin).
  - If the ratio > 1 the diagnosis is liver damage (a result of viral origin " **Viral hepatitis**").



Protein Profile in myocardial infarction [Heart attack]:-



⇒ When someone feels a chest pain and he rushes to the emergency room to check up, the doctors look for certain biomarkers, which are increased as a result of cardiac muscle damage (these biomarkers are a lot proteins), such as;

*we should look for sensitivity and specificity for each biomarker; to decide whether it's good or not. Is the test sensitive enough to detect changes in biomarker conc.?
specificity: is this biomarker specific to that organ/disease or not?

Both should be verified to say this is a good biomarker.

○**Myoglobin**

✓ It's **not** a good indicator because it's also present in skeletal muscles which might be damaged due to physical activity. (the example the doctor gave is a person who felt chest pain and rushed running to the hospital, in that case myoglobin will not be a good indicator of myocardial infarction because the increase in myoglobin might be due to skeletal muscle damage while running).

- ✓ It's sensitive.
- ✓ It's not specific.

- **CK-MB** (a good biomarker).
- **LDH** (a good biomarker as well).
- **Troponin I** (an excellent biomarker/it is the gold standard for detection of infarction and it's not an enzyme).

⇒ In the figure above, two things should be noticed :- (مش للحفظ)

1. Detection rate (Biomarkerال متى ببين) .
2. Duration in blood.

LDH, it appears after a day or 2 and it lasts for a long time (a week or longer).

CK-MB, it peaks after 24h and lasts for a day.

⇒ If someone had chest pain a week prior to his doctor's visit, then the CK-MB test won't be a good idea as it lasts for 2 days, but LDH and Troponin I would be a good idea to check as they last for a longer time.

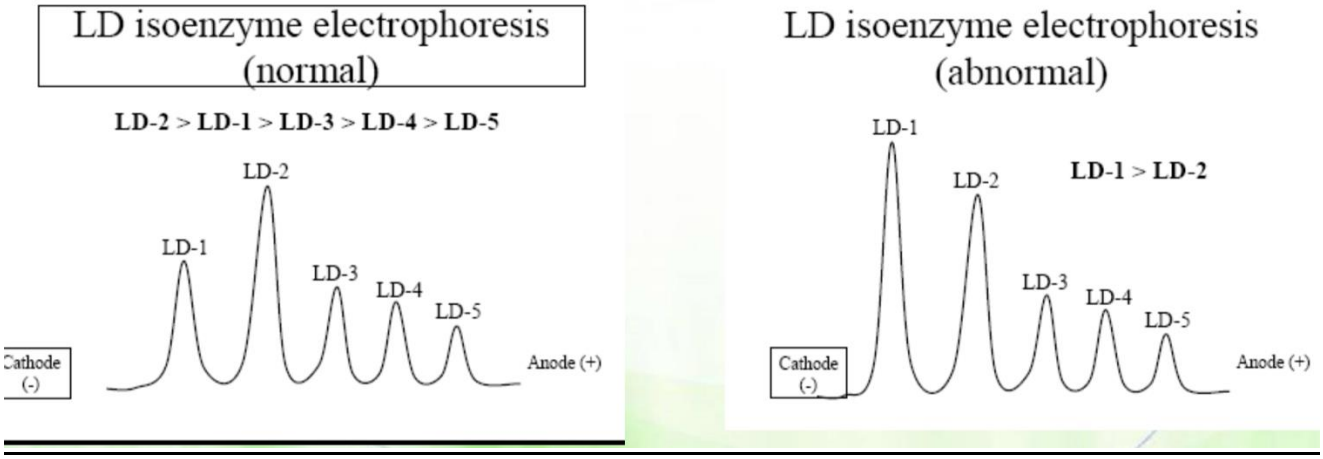
⇒ We also identify isoenzymes which have different tissue distribution, such as LDH-1 & LDH-2 .

LDH:-

⇒ A comparison of serum level of LDH-1/LDH-2 ratio is diagnostic for Myocardial Infraction (heart attacks).

- Normally, this ratio is less than 1, that means LDH-2 is higher than LDH-1.
- Following an acute myocardial infraction, LDH ratio will be higher than 1, because the increased release of LDH-1, which is highly present in heart muscles.

⇒ LDH is not commonly used clinically, unlike troponin I.



CPK:-

M= muscle

B= brain

⇒ Found primarily in heart and skeletal muscle as well as in the brain.

⇒ Three tissue-specific isozymes of CPK:

- CPK3 (CPK-MM) is the predominant isozyme in muscles. (skeletal (99%) and cardiac (80%) muscles).
- CPK2 (CPK-MB) accounts for about 35% of the CPK activity in cardiac muscle, but less than 5% in skeletal muscle.
- CPK (CPK-BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle. (Brain damage, CPK-BB increased).

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

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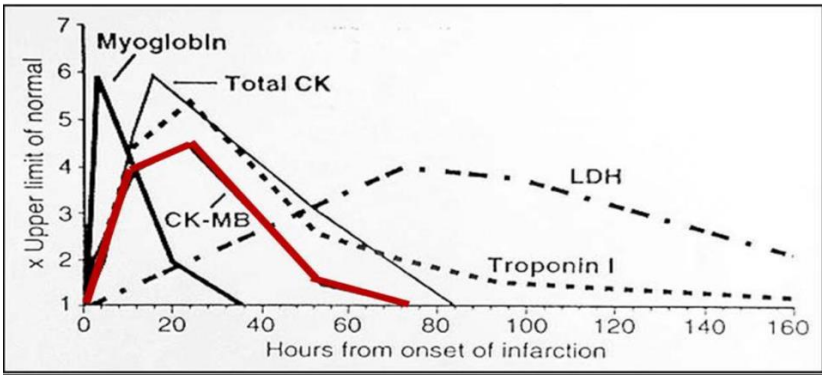
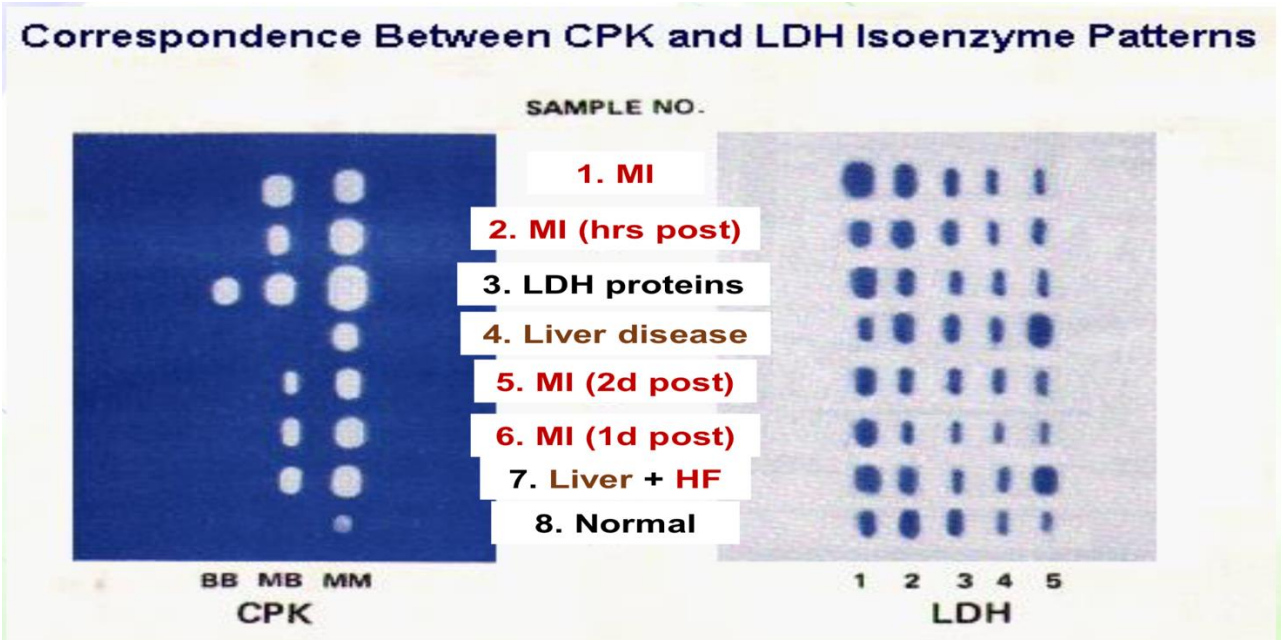
➢ CPK and Myocardial Infraction:-



- CPK-MB would be a bad idea if the patient suffered chest pain more than 2 days ago as CPK-MB will decrease within 2 days.
- CPK-MB would be a good idea if the patient felt chest pain more than 2 day ago and is not feeling well upon his doctor's visit; therefore, CPK-MB is a good biomarker when a second event happens (**re-infarction**). [In this case Troponin and LDH aren't the best idea because they stay for a long time].
    ⇒ The CPK-MB is also useful for diagnosis of re-infarction because levels begin to fall after a day and disappear in 1-3 days, so subsequent elevations are indicative of another event.

Example:-



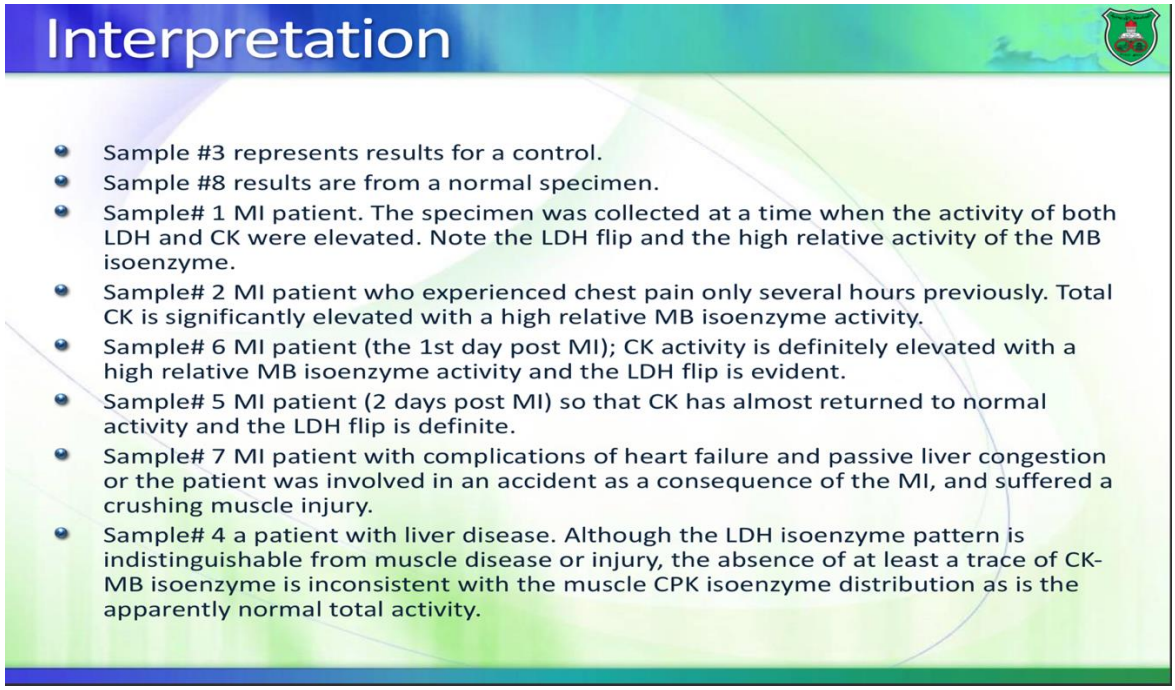
⇒ We separate isoenzymes according to charge or size.
⇒ CPK (BB, MB and MM) is identified.

1. MI, someone who is suffering myocardial infarction right now.
    * MM is high, and MB is high also, but BB is not; indicating that there is no brain damage but there is MI.
    * LDH-2 < LDH-1, confirming that there is MI.
2. MI (hrs post)
    * According to the figure in page 8, MB is still high
    * LDH-1 ≈ LDH-2
3. LDH proteins
    * Represent results for control.
4. Liver disease

- MM (it is present in the liver) is high, but MB and BB are not shown that means there is no MI.
- LDH-2 > LDH-1 (normal), confirming that there is NO MI.
- LDH-5 has increased, which is a biomarker for liver disease.

5. MI (2d post)
   - MM and MB levels are high, there is MI.
   - LDH-2 < LDH-1, confirming that there is MI.
6. MI (1d post)
   - MM and MB levels are high, there is MI
   - LDH-2 < LDH-1, confirming that there is MI.
7. Liver + Heart failure
   - MM is high, and MB is high also, there is MI.
   - LDH-2 < LDH-1, confirming that there is MI.
   - LDH-5 has increased, which is a biomarker for liver disease.
8. Normal
   - We can detect MM because it released by skeletal muscles as a result of tissue damage but not MB nor BB.
   - LDH-2 > LDH-1 (normal).

⇒ MM and MB are not good indicators. LDH, on the other hand, is a good marker for MI.



Troponin in MI:-

⇒ It's not an enzyme, it's an actin binding protein which helps in muscle contraction.

⇒ It has isoforms (NOT isozymes because they are not enzymes), one of which is specific for cardiac tissue.

⇒ 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 infraction if it occurred only days after the first.

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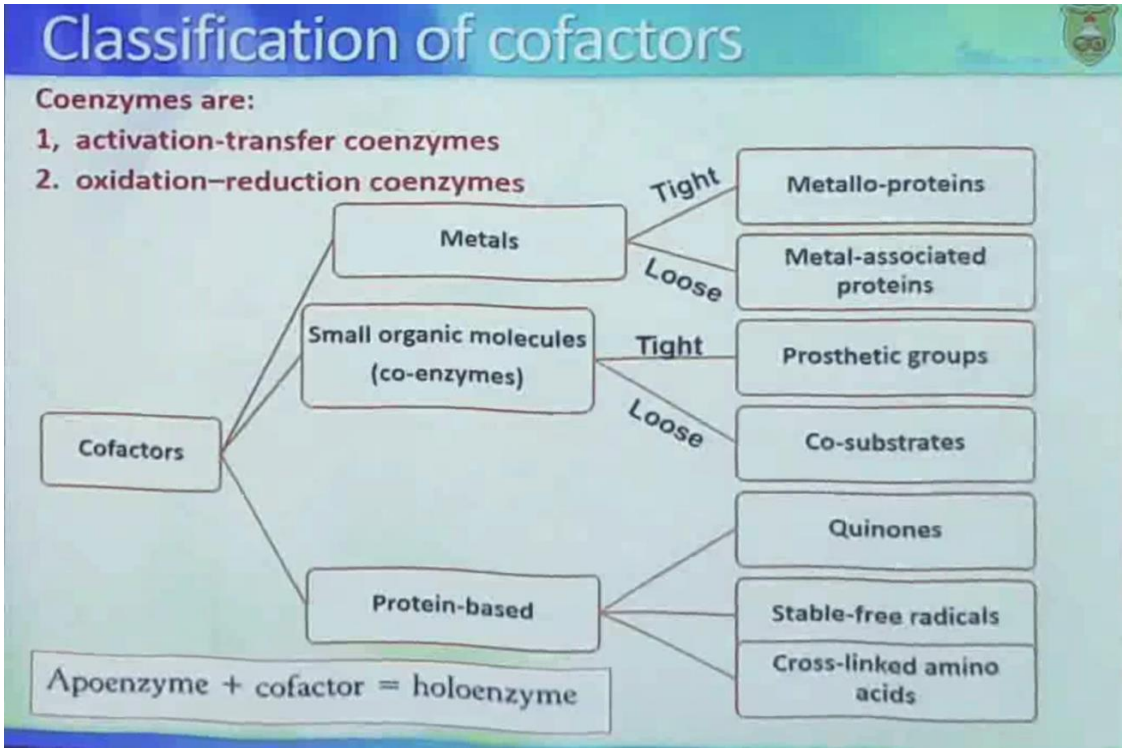
Slide 15:

Enzymes part IV: Cofactors:-

❖ **Catalytic strategies of enzymes:-**

- Enzymes carry out reactions utilizing different catalytic strategies.
  - Some enzymes, such as chymotrypsin, rely on amino acid residues within the active site.
    - Almost all polar amino acids participate in nucleophilic catalysis.
    - Ser, Cys, Lys & His can participate in covalent catalysis
    - Histidine: pKa, physiological pH & acid-base catalysis.
  - Other enzymes increase their repertoire by employing cofactors (non-protein compounds that participate in the catalytic process).
    - Cofactors can participate in the reaction (and are released as a product) or can facilitate the reaction (during the transition state).
    - They are called Conjugated enzymes (Holoenzymes).

❖ **Classification of cofactors:-**



- 3 types:
  - **Protein-based** (don't memorize).
  - **Metals** which are divided into (1) tightly and covalently associated with enzyme (Metallo-enzymes) and (2) loosely associated with enzyme (Metal-associated enzymes).
  - **Small organic molecules (co-enzymes)** [vitamins] which are also divided into (1) tightly associated to enzymes (Prosthetic groups) and (2) loosely associated to enzymes (Co-substrates →they participate in the rxn and are changed by the end of it).

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➢ **<u>Small organic molecules:-</u>**

- Lipid soluble vitamins, which are 4 (vitamin A, D, K, E).
  - Will be discussed in the next course.
- Water soluble vitamins:-
  - Most derived from vitamin B (B1, B2, B5, B6, B12, etc..)
  - Vitamin C.
  - Lipoic acid.
  - Folate.
  - ...

Water-Soluble Vitamins

Name	Coenzyme or Active Form	Primary biochemical function
Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
Riboflavin	Flavin mononucleotide (FMN)	Hydrogen-Atom (electron) transfer
	Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD)	Hydrogen-Atom (electron) transfer
	Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer
Pantothenic Acid	Coenzyme A (CoA)	Acyl-group transfer
Pyridoxine	Pyridoxal Phosphate	Amino-group transfer
Biotin	Biocytin	Carboxyl transfer
Folate	Tetrahydrofolate	One-Carbon group transfer
Vitamin B_{12}	Coenzyme B_{12}	1,2 shift hydrogen atoms
Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer
Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation

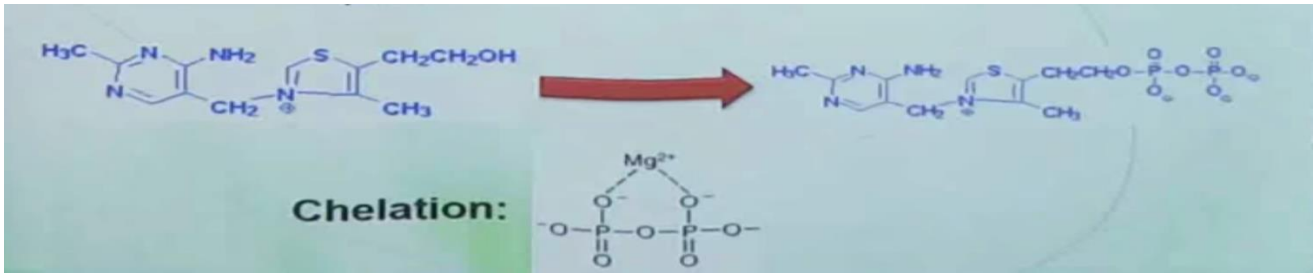
- **<u>Classification according to the reaction that they help:-</u>**
  - Activation-Transfer coenzyme:
    - Help in transferring of a group.
  - Oxidation-Reduction coenzyme:
    - Donating & accepting electrons.

⇒ Activation-Transfer coenzymes

* Participate directly in catalysis by forming a covalent bond.
* Characteristics:-
  - Two groups in the coenzyme.
    - ✓ A functional group that forms a covalent bond with substrate.
    - ✓ A binding group that binds tightly to the enzyme.
    - ✓ they could be the same site but usually they are separate.
  - Dependence on the enzyme for additional specificity of substrate & catalytic power. (they help in increasing the specificity of enzyme in binding to substrate).

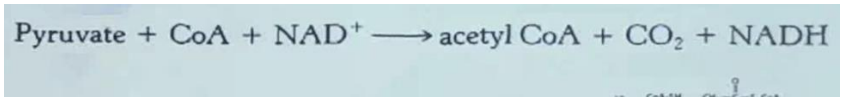
❖ Thiamin pyro-phosphate (TPP):-

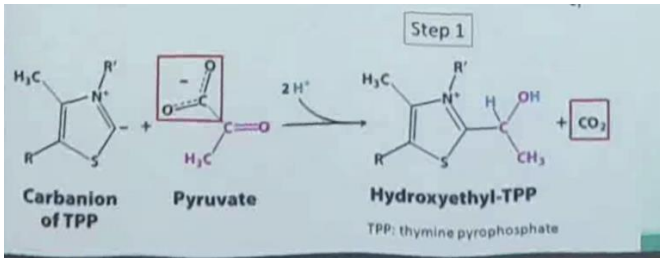
- Thiamin (vitamin B1) is converted (it must be modified to become active) to its active form, TPP, in the brain & liver, by the addition of 2 phosphate groups that help in the binding of cofactors with enzyme or with substrate.
- TPP is involved in decarboxylation reactions.
- The pyrophosphate provides negatively charged oxygen atoms and chelates Mg2+ that is tightly bound to the enzyme.
- The phosphate groups participate in chelation reactions.
- Chelation is binding of phosphate group with an ion like Mg2+ or Ca2+ resulting in the formation of a ring.(بمعنى انه بسحب الايون الموجود بالمحلول)

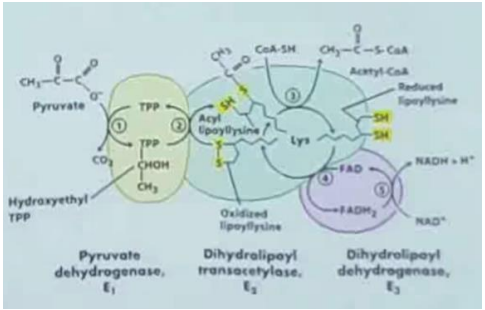


❖ Importance of Thiamine:-

- In a group of enzymes:-
  - Pyruvate dehydrogenase complex:
    - ✓ TPP is involved in decarboxylation of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex (Contains 16 polypeptide chains and it catalyzes 3 reactions).

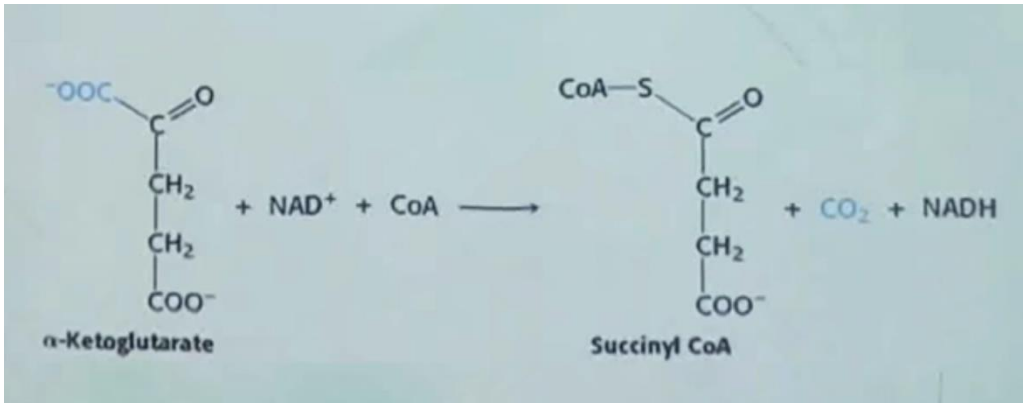






  - Alpha-Ketoglutarate dehydrogenase:
    - ✓ Decarboxylation of alpha ketoglutarate into succinyl CoA by alpha ketoglutarate dehydrogenase.

*Don't memorize reactions, but they are important in the next course of biochemistry.



❖ **Mechanism of action:-**

⇒ The functional group is the reactive carbon atom that forms a covalent bond with a substrate's keto group, while cleaving the adjacent carbon-carbon bond.