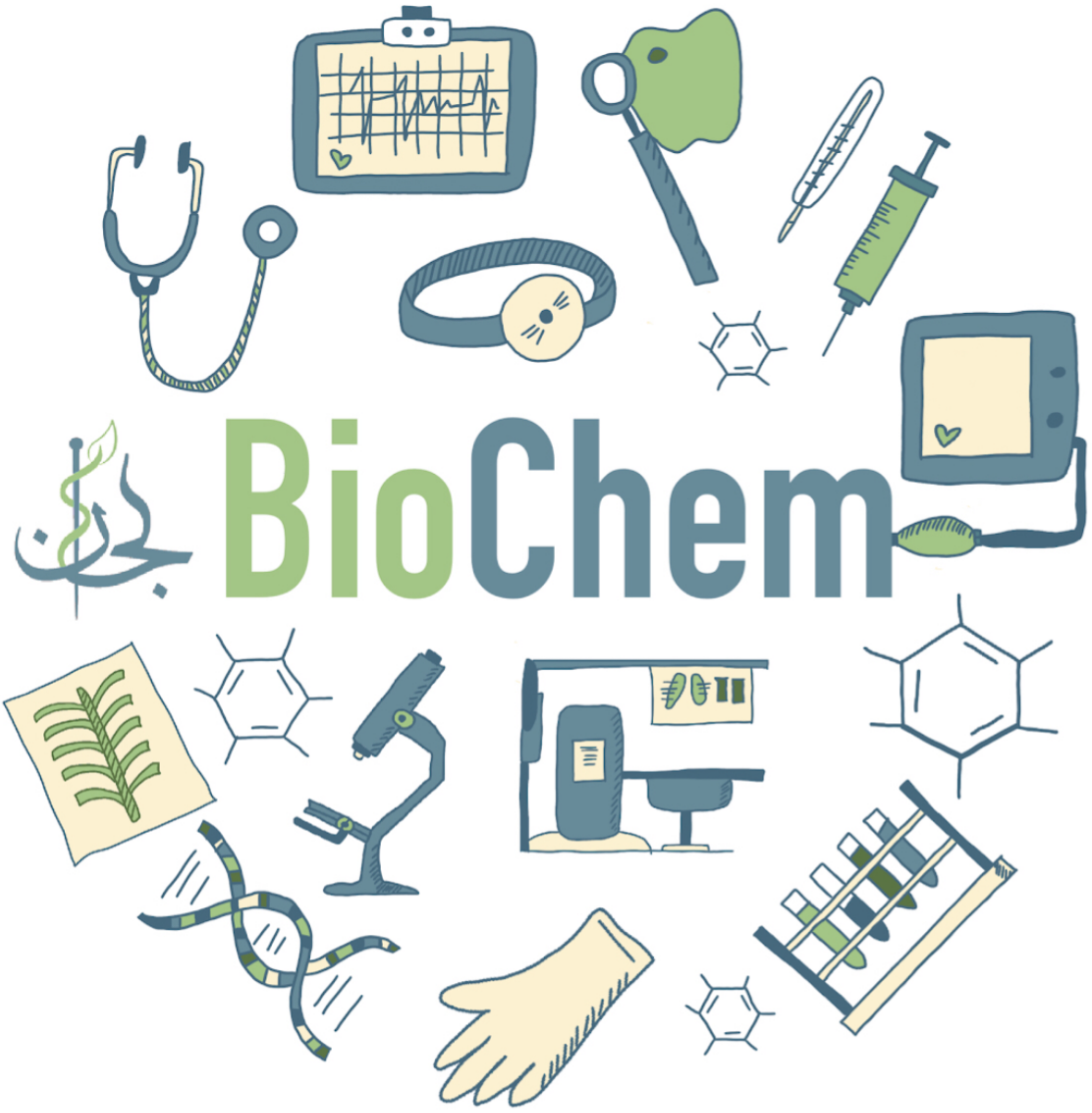


# BioChem


**Writer:** Jude Maslamani&Raghad Jariri

**Science:** Jude Maslamani&Raghad Jariri

**Grammar:** Jude Maslamani&Raghad Jariri

**Doctor:** Nafiz AbuTarboush

☐ **Regulation of enzymes:**

**-Do enzymes need to be regulated?Why?**

Yes, mainly to maintain homeostasis.

**-Do they need to be highly regulated?Why?**

Yes, they are highly processive so we need them to work on the spot ,
Like race cars=>they are highly regulated.

**- In the human body thousands of diverse enzymes are regulated to fulfill their individual functions**

☐ **How do we regulate the enzymes:(**in which cases**)**

1. **Regulate the enzyme itself :** as a single enzyme.
2. **Control the pathways of the reactions:** like the glycolysis (10 steps reaction , is controlled by many enzymes) so we need to regulate them all.

**-Do all enzymes affected by all majors of enzyme regulation?**

No, it might be affected by one way or more at the same time.

**-Do we have an enzyme which is affected by ALWAYS regulation?**

No

**-Regulation matches function.**

- **Modes of regulation( methods that enzymes could be affected by):**

1. **Isozymes**
2. **Inhibition**
3. **Conformation**
4. **Amount**
5. **None-specifically**

**-Let's explain them one by one:**

- **Isozymes:** Same substrate & product, different gene, different localization, different parameters (Km, Vmax, kcat).

More explanation:

**What does it mean as the name implies?**

Different structure (different amino acids sequence) , same function.

**Why?** To control the enzyme (the way it works) , change the parameters of enzymes function such as: Km , Kcat , Vmax. without changing the role of the enzyme.

**Same** enzymes work **differently** in **different** tissues => because the need of each tissue differs.

**-All the body cells need glucose (**source of energy**)**

**What happens when it enters the body cell?**

Be phosphorylated,**why?** To not get out of the cell(be trapped inside the cell) because the phosphate group is a bulky group with negative charge.

**-The tissue needs differ.**

**-The enzyme that adds phosphate to the glucose=>>glucokinase (**branch of hexokinase **=**which are composed of mini mini isozymes= their number depends on the need of each tissue**) .**

**-Hexokinase found in RBCs & in the liver , catalyzes the first step in glucose metabolism.**

☐ **Let's talk about 2 examples of hexokinases:**

**1-Hexokinase I** (RBCs)

**2-Hexokinase IV (**glucokinase**)** (Liver & Pancreas)

**The comparison =>** will illustrate how different isozymes participate in the regulation inside the body.

**Quick reminder:**

Km of the enzymes determines the substrate's concentration that is needed to achieve 50% of the maximum velocity

-When the substrate's concentration is way **less** than Km **=>** the enzymes are **weakly functional** and vice versa.

- **The differences between the RBCs & Liver cells?**

1. RBCs don't have organelles (no mitochondria which is the main source of energy production ) **=>** so RBCs depend mainly on glycolysis to produce energy (they **need** glucose all the time ) **=> so** the hexokinase I has **less** Km ( more efficient) and **high binding affinity** . (Check the note below to understand more)

While the liver cells have mitochondria consequently they will produce a good amount of energy so **no need** for glucose all the time.

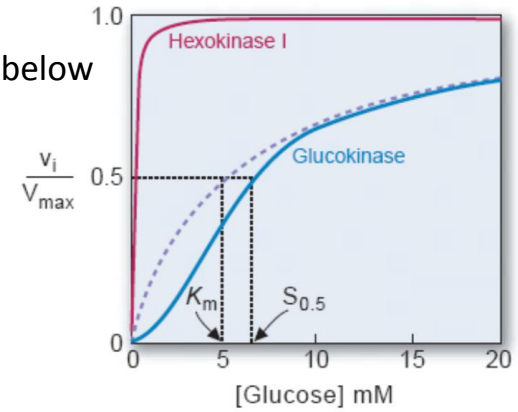
2. Liver cells store glucose as **glycogen** , while the RBCs do not store it ( they only consume it by converting it to energy by glycolysis).

**-In general : Km expresses the affinity**
(High Km => low affinity) ->so it needs much higher concentration of the substrate to be more effective and to bind well so the reaction will occur in much better efficiency.

---

☐ **Fasting level of glucose = 5mM**

-The Km in the **hexokinase I(RBCs)** will be above or below 5mM?why?
ANS: below, regardless of whether you are fasting or not , RBCs have to take most of the glucose that you have (cause it needs to function all the time so the glucose has to be provided for it all the time )




**-Hexokinase I (RBCs): KM (glucose) ≈ 0.1 mM** which is 50 times less than the fasting glucose level.

-RBCs: when blood glucose falls below its normal fasting level (≈ 5 mM), RBCs could still phosphorylate glucose at rates near Vmax
**In simpler way:**
When the person is fasting => glucose level is over saturating this enzyme which means that it works at its **maximum** capacity (**we reach Vmax)**

**-Hexokinase IV (glucokinase, liver, pancreas) ≈ 10 mM** which is 100 times greater than hexokinaseI & greater than the fasting level.

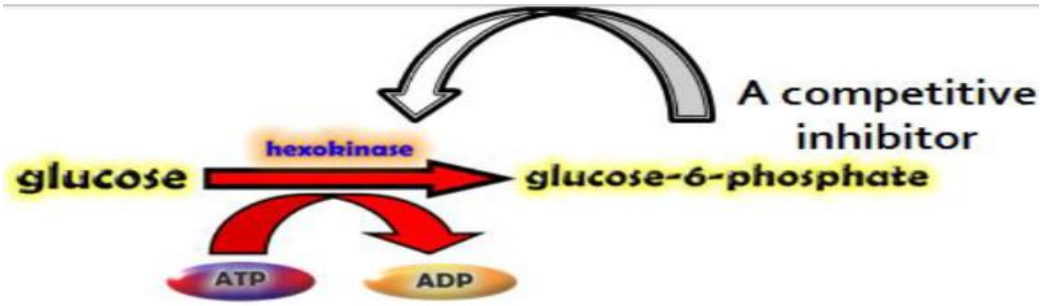
**In fasting condition is the enzyme processive?** No , it works weakly.

It becomes efficient when the glucose level is **much greater** than the Km.

The liver will work as =>storage

Pancreas will work as => sensor (to detect the glucose amount in the blood => to detect the amount of secreted insulin or glucagon )

---




**-Glucose-6-phosphate works as what for hexokinase?**

A competitive inhibitor.

**What do we call this?** Negative feedback inhibition.

☐ **Which enzyme has to be affected by the inhibition?**

**RBCs=>** when they take energy as they need => they won't need more glucose , so the enzyme will need **inhibition** , that's why the **glucose-6-phosphate** comes back to the active site of the enzyme which designed as it takes glucose so it will take glucose-6-phosphate.

**The liver and pancreas=>** won't need this inhibition. **WHY?**

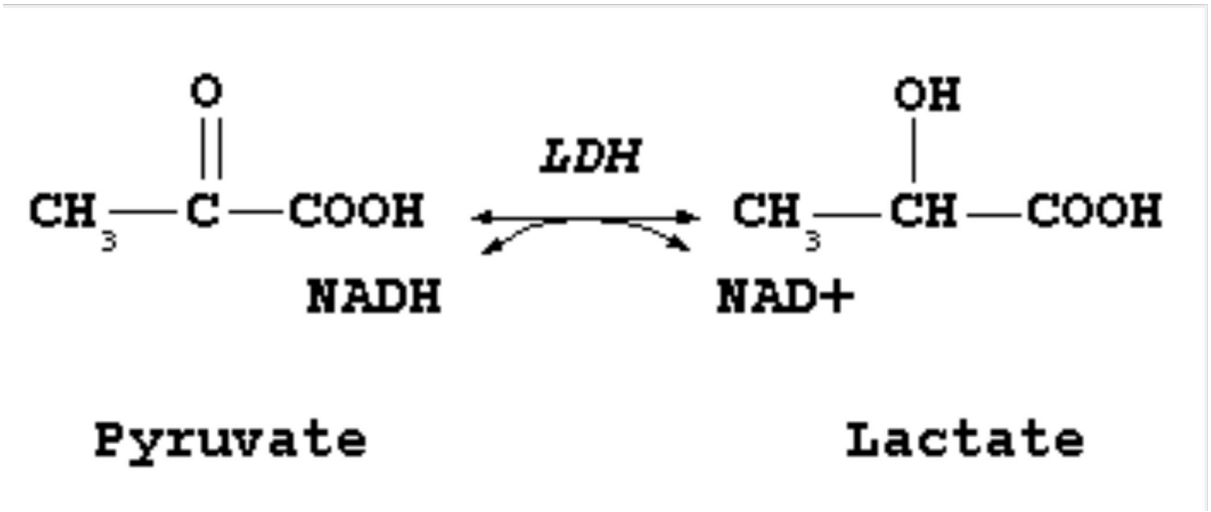
because the pancreas **ALWAYS** needs to detect the glucose level in the blood by taking it.

& The increase of glucose in the liver will be controlled by storing it , so the active site has been designed to absorb glucose.

**Lactate Dehydrogenase (LDH)**

An **enzyme** that catalyse the conversion of **pyruvate** into **lactate** and the lactate into pyruvate "both directions, same enzyme".

The conversion of pyruvate into lactate is the last step in anaerobic glycolysis.




-What is the difference between the heart muscles and skeletal muscles with respect to energy in general?

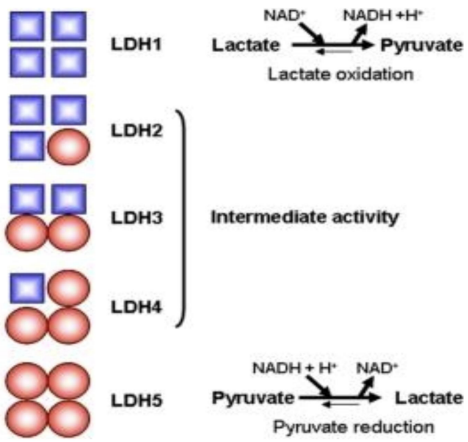
The heart doesn't respire anaerobically, lactate doesn't accumulate in the heart yet it gets converted into pyruvate and that's why LDH is required in the heart muscles.

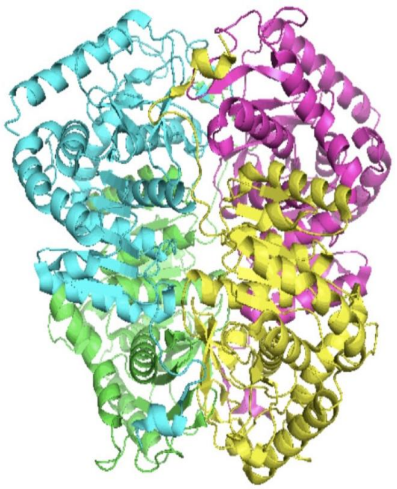
The structure of the enzyme;

It's a tetramer formed of 4 subunits and the different combination in between these subunits forms 5 different isozymes: LDH1, LDH2, LDH3, LDH4, LDH5.

-LDH1: present in heart.

-LDH5 : present in skeletal muscles and liver.



**-How do LDH1 & LDH5 differ?**

**LDH1** is composed of **4** subunits of (H subunit) calling it **H4**.

**LDH5** is composed of **4** subunits of (M subunit) calling it **M4**.

- * the combination between these two subunits (H&M) forms the 5 isozymes *

The **H4** enzyme in the heart is required to convert lactate into pyruvate and not the opposite, but the enzyme in the skeletal muscle is required to convert pyruvate into lactate.

**-Why is the production of lactate present in the skeletal muscles?**

Exercise requires more oxygen to respire aerobically and produce the energy needed. However, sometimes cells don't get enough oxygen to produce energy so anaerobic respiration takes place to produce lactate from pyruvate, lactate accumulates only to get converted into pyruvate in the backward reaction.

- this accumulation of lactate leads to Fatigue (إعياء) of muscles.
- " pyruvate is allowed to be converted into lactate in skeletal muscles yet not allowed in heart muscles cause we don't want Fatigues in heart muscles ".

**With respect to Km:**

-The Km of H4 is higher in respect to lactate compared to the Km of M4 and the opposite to pyruvate, Km if H4 is lower in respect to pyruvate compared to Km of M4.

- The Km of H4 " heart enzymes" is lower **in respect to pyruvate** compared to M4.

-**pyruvate** will bind to the higher affinity, to the enzymes of the heart, with concentration, once the pyruvate binds to the enzyme it causes a consent regulation called Substrate Inhibition, pyruvate binds with high affinity and gets too attached to the active site by that the reaction won't go further so no production of lactate, on the other side it encourages more lactate to be converted into pyruvate.

**In conclusion,** both enzymes differ in their Km's in respect to both lactate and pyruvate.Also, they differ in mode of inhibition as H4 is inhibited but not M4.

**Aldehyde Dehydrogenase (ALDH)**

-It's an oxidoreductase that causes the oxidation of it's substrates.

-oxidation of acetaldehyde to acetate.

Alcohol consumption:

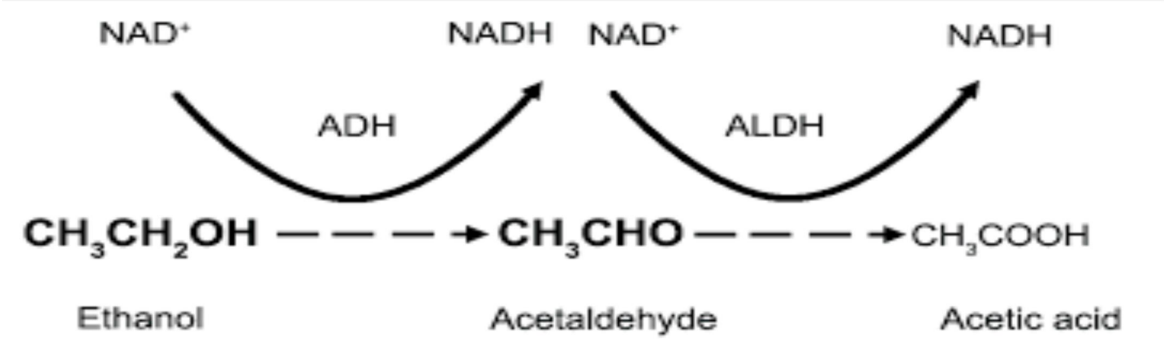
Ethanol —> gets into our bodies, reaching liver cells where it undergoes metabolism —> enters the mitochondria —gets converted to —> acetal dehydrate .

**IN GENERAL** :

**Alcohol** —oxidation—> **Aldehyde** —oxidation—> **carboxylic acid.**

**The enzyme's reaction:**

**Ethanol** —oxidation—> **acetaldehyde** —oxidation—> **acetate.**




- This enzyme has four tetrameric isozymes (I-IV).

**ALDH I** is present in **mitochondria**, has a low Km for Acetaldehyde (high affinity ) so :

- it binds to it directly converting it to **Acetate** - to uproot the Toxicity that **Acetaldehyde** in charge of in Alcohol- as **Acetate** is safer compared to Acetaldehyde.

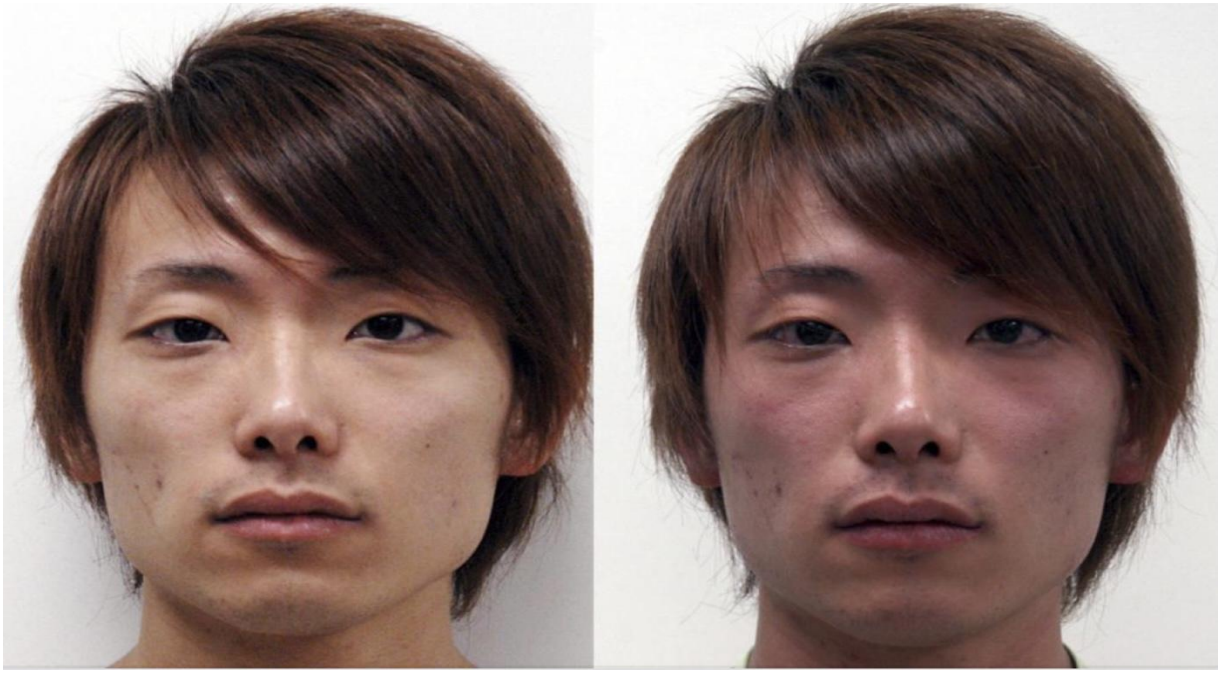
- Some of the **Acetaldehyde** escapes to the **cytosol** where another copy of ALDH is placed that has higher Km called **ALDH II :**

- When **Acetaldehyde** occurs in the cytosol it binds to it converting it to Acetate.

In certain populations like Chinese & Japanese, the **mitochondrial** copy of ALDH (**ALDH I**) has a problem compared with coucasian & negroid population, the enzyme isn't working effectively as Acetaldehyde going in high concentration, very high concentration of it would escape to cytosol.(**ALDH II**) will work on them but it won't be enough for the amount of Acetaldehyde. So

it escapes further from the **cytosol** into the **bloodstream** reaching different body tissues including:

- the brain and by so causing Toxicity.
-vascular tissue causing redness & flushing .
- the heart causing Tachycardia.



- Again the Toxicity of Alcohol comes from Acetaldehyde depending on the population examined shows how values vary and how it can be affecting the toxicity control of some materials.

---

**Inhibition:**

- Enzymes can be inhibited to regulate their functions.

*Inhibitors of enzymatic function can be classified into two majors :*

**1- reversible inhibitors.**

**2- irreversible inhibitors**;they aren't physiological, they are either poisons, toxins or drugs & medicine we create to control the situation.

-The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme

- **Mechanism-based inhibitors** (irreversible)

Named mechanism-based because they interfere with the mechanism of the enzymes.

- **It's classified into 3 subunits**:

**A- covalent inhibitors**; which bind the enzyme initially in a covalent manner.
**B- Transition state analogs**; which mimics the transition state in their structure.
**C- Heavy metals**.

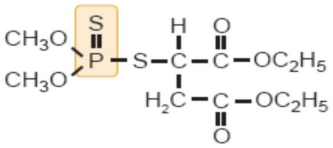
## A- Covalent inhibitors:

They bind to the enzyme from the start, initially in a covalent manner then this material won't desiccate from the enzyme by that, killing the enzyme (losing the function of the enzyme for good).

- Covalent or extremely tight bonds with active site amino acids.
- Amino acids are targeted by drugs & toxins.

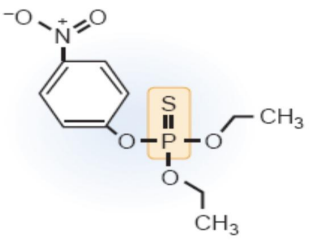
---

1- **The lethal compound [DFP]** is an organophosphorus compound that served as a prototype for:



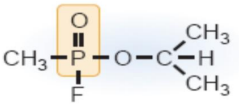
Malathion

A- The nerve gas sarin: first used in Tokyo 1996 then lately in Syria. Those who were exposed to this gas started to suffocate shortly after the exposure.



Parathion

B- The insecticides malathion & parathion.
DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal.



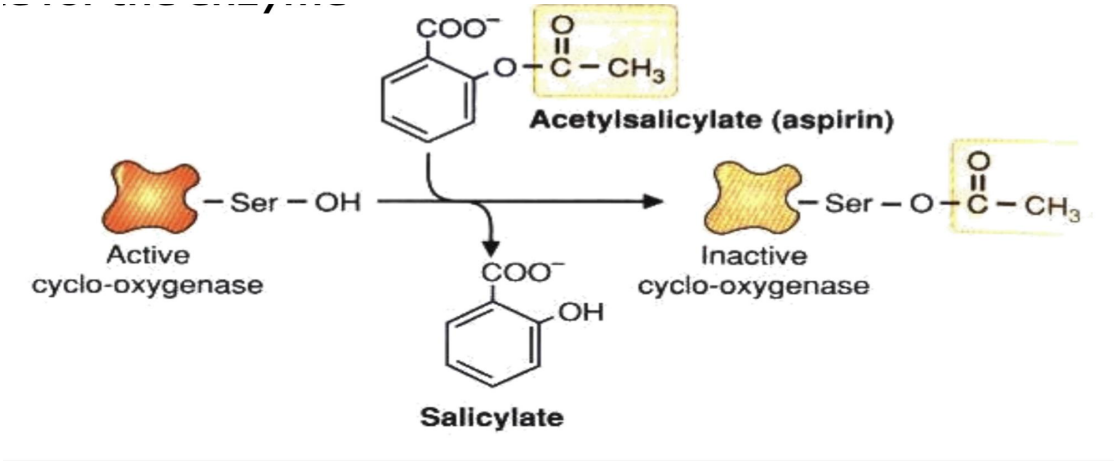
Sarin

2- **Aspirin** acetylsalicylic acid

Cyclooxygenase is an enzyme which synthesis prostaglandins & thromboxane by converting Arachidonic acid.
Aspirin inhibit cyclooxygenase it acts as an acetylating agent where an acytal group is covalently attached to a sarin found in the active site of cyclooxygenase causing the inactivation of the enzyme in a permanent manner,

meaning no production of prostaglandins nor thromboxane and accordingly there won't be an inflammatory response…

-Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme.

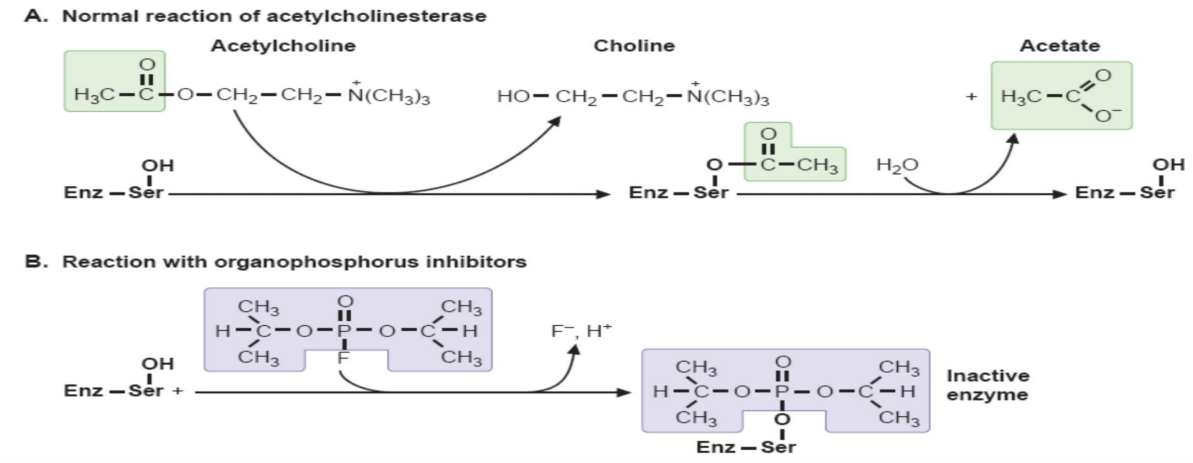



**Why are 1 & 2 considered fatal?**

-Because they affect an enzyme called acetylcholinesterase .

- acetylcholinesterase: an enzyme that degrades acetylcholine which is a neurotransmitter that breaks out of the nerve endings, binds to the receptors on the surface of the muscle causing its contraction and once they get degraded the muscle relaxes.

-making it defective would make acetylcholine proceed affecting the muscle putting it in a state of repetitive contractions without reaching the state of relaxation which is a condition named **tetanization of muscles** , not a big deal if it occurs in limbs yet a problem if in the diaphragm as tetanization causes loss of energy sources, that loss of ATP causes respiratory arrest resulting in death.



## B- Transition state analogs

-extremely potent inhibitors (bind more tightly).

-Drugs cannot be designed to precisely mimic the transition state! (highly unstable structure).

- we can't synthesize a transition state because it’s unstable,but we can make a structure that looks like the substrate.

- it’s designed to look like the transition state, however it has a certain modification for the reaction not to go further. Once they bind to the enzyme they kill it.

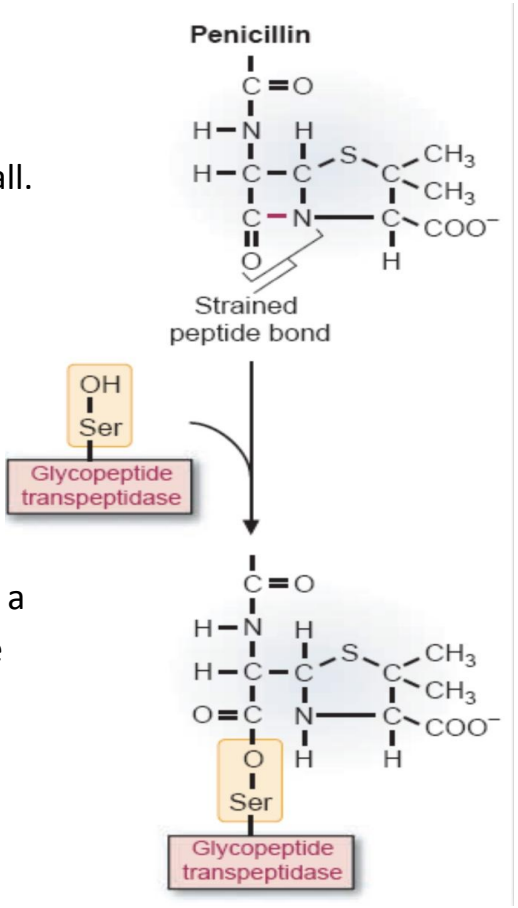
- the designed transition state has higher affinity to the active site than the substrate as it’s created to fit perfectly in the active site.

- called suicide inhibitors: because the enzyme thinks this transition state is better than the substrate, binds to it autonomy free but it’s what kills it.

---

1- **Penicillin** : most common antibiotic in the world.

- A transition-state analog to glycopeptidyl transferase or transpeptidase.
- Required by bacteria for synthesis of the cell wall.
- The reaction is favored by the strong resemblance between the peptide bond in the β-lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction.
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed suicide inhibitors
- The enzyme breaks the amide bond thinking it’s a peptide bond but it’s not! So it covalently binds to the active site killing the enzyme.

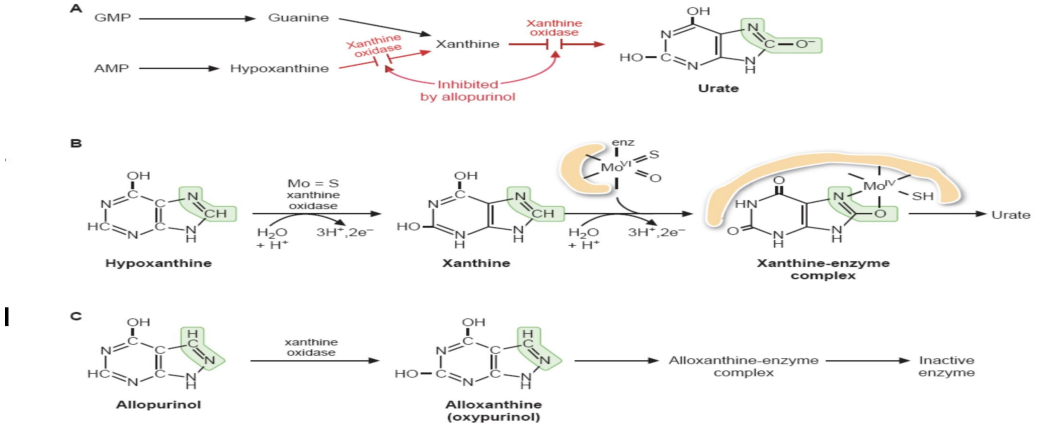


## 2-Allopurinol

- A drug used to treat gout( النقرص ), Decreases urate production (xanthine oxidase).
- **The enzyme commits suicide** by converting the drug to a transition-state analog.
- **The enzyme contains a molybdenum– sulfide (Mo-S) complex** that binds the substrates and transfers the electrons required for the oxidation reactions.
- **Xanthine oxidase oxidizes the drug allopurinol to oxypurinol**, a compound that binds very tightly to a molybdenum–sulfide complex in the active site.

-How is Allopurinol different from the substrate?(C)

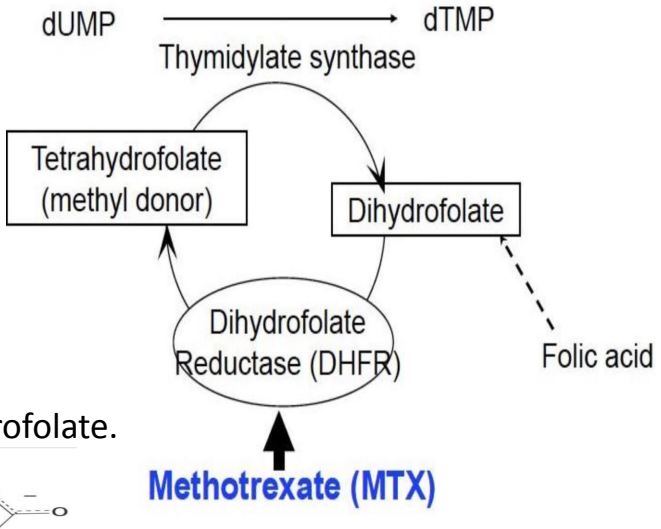
The N=CH is switched in between the two structures.

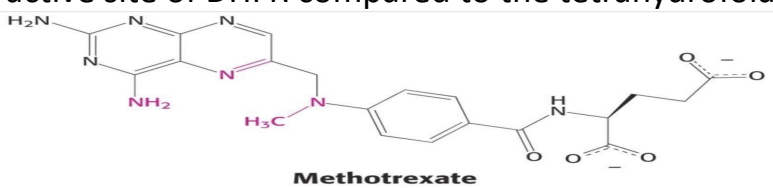



---

## 3-Methotrexate

- Synthetic inhibitor.
- Anti-cancerous.
- Analog of tetrahydrofolate.
- Binds to enzyme a 1000-fold.
- more tightly.
- Inhibits nucleotide base synthesis.
- It binds with much higher affinity to the active site of DHFR compared to the tetrahydrofolate.





Methotrexate


Tetrahydrofolate

## C- Heavy metals

- Tight binding of a metal to a functional group in an enzyme.
- **Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)**.
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is.
- associated with high-dose toxicity.
- Mercury: binds to so many enzymes, often at reactive sulfhydryl groups in the active site.
- Whenever having high concentration of mercury —> disturbance in enzymes, specifically **central nervous system enzymes** causing **Toxicity**.
- It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity.
- Lead provides an example of a metal that inhibits through replacing the normal functional metal in an enzyme, such as calcium, iron, or zinc.
- Its developmental & neurologic toxicity may be caused by its ability to replace Ca+2 in several regulatory proteins that are important in the central nervous system and other tissues.
- Lead based paint was banned because the paint peels and toddlers are interested to eat those peelings exposing themselves to lead which will affect their brain, nervous system, affecting their growth and developing mental issues.

**BEST WISHES**