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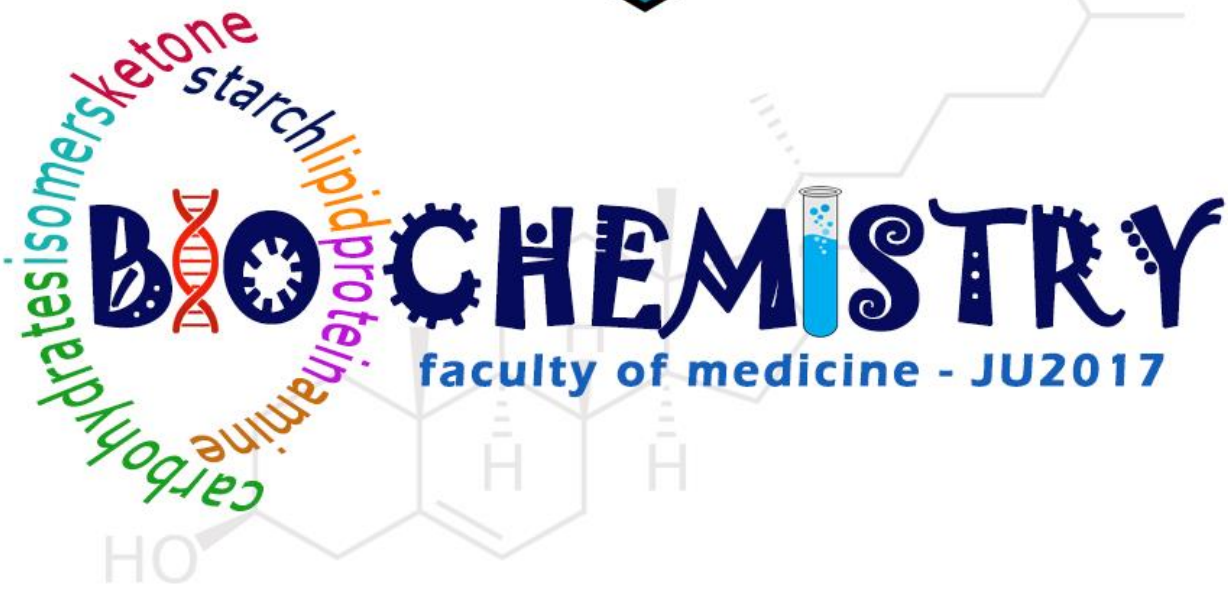

◉ Sheet ○ Slides

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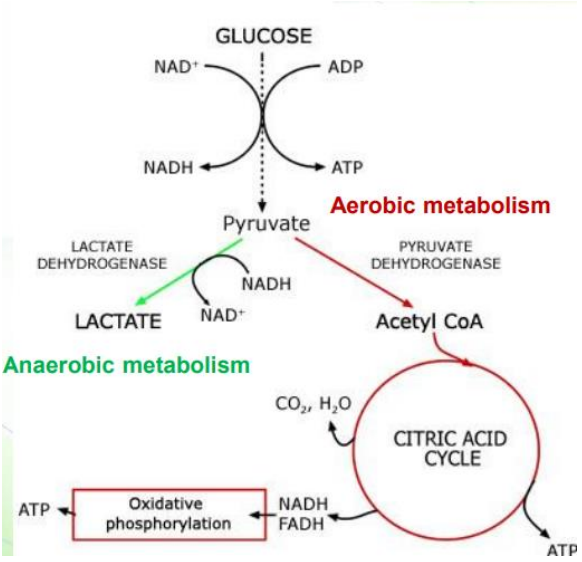
There are several ways in which enzymes can be regulated:

1. **Expression of isoenzymes**
2. **Regulation of enzymatic activity**
    a) Inhibitors
    b) Conformational changes
        I. Allostery
        II. Modulators
        III. Reversible covalent modification
        IV. Irreversible covalent modification
3. **Regulation of enzyme amount**
4. **Location** (Compartmentalization and complexing of enzymes)
5. **Non-specific regulation**

In metabolism (oxidation of glucose), the first step is glucose phosphorylation - recall Glycolysis from Bio 101 Course - until we get Pyruvate.

Now it's decision time for the cells, Pyruvate either goes through Aerobic or Anaerobic metabolism.

- **Aerobic Metabolism:** Citric Acid Cycle, Oxidative phosphorylation (Respiratory Chain Reaction) and it gives a lot of ATP.
- **Anaerobic Metabolism:** Pyruvate is reduced to lactate by **lactate dehydrogenase.**
  This happens in tissues like: RBCs and Skeletal Muscles.



Then Lactate gets transferred to cardiac muscles, where **lactate dehydrogenase** is also found, *BUT* it's a bit different than the one found in RBCs and Skeletal Muscles because what it does is that it catalyses the **reverse reaction** (takes Lactate and transforms it into Pyruvate). The resulting Pyruvate enters Aerobic Metabolism; because cardiac muscles can ONLY do Aerobic Metabolism.

Lactate dehydrogenase in Cardiac Muscles and Lactate dehydrogenase in RBCs and Skeletal Muscles are very good examples of isoenzymes. But what are **isoenzymes** ?

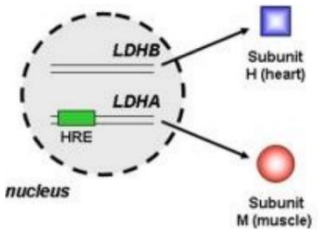
Isoenzymes: enzymes that catalyse the same reaction, the same substrate(s) and the same product(s). But their regulation and catalytic activity may differ. Also, they are produced in different tissues and from different genes that vary only slightly. (Table 1)

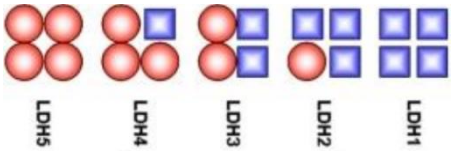
Similarities	Differences
Catalyze the same reaction	Regulation
The same substrate(s)	Catalytic activity
The same product(s)	Produced in different tissues
	Produced from different genes

Table (1)

Lactate dehydrogenase (LDH)

LDH is a tetrameric enzyme -made of 4 polypeptides (subunits)- which come from 2 genes, one of those genes produces the M (skeletal **m**uscle) polypeptide while the other produces the H (**h**eart) polypeptide.





These subunits combine in various ways leading to 5 different isoenzymes (LDH 1-5) with different combinations of the M and H subunits.

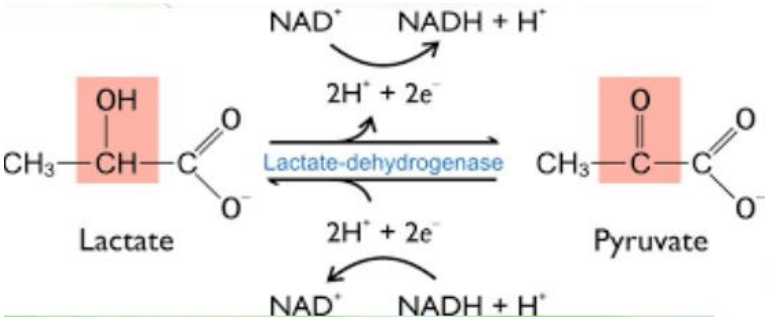
The **all H isoenzyme (LDH1)** is a characteristic of the heart tissues. It functions aerobically and catalyses the reverse reaction. While **the all M isoenzyme (LDH5)** is typically found in skeletal muscle and liver. It functions anaerobically and catalyses the production of Pyruvate into Lactate.

***Note:** LDH**2**, LDH**3** & LDH**4** have intermediate activity. (Table 2)

Isoenzymes	Structure	Present in	Elevated in
LDH1	(H_4)	Myocardium, RBC	Myocardial infraction
LDH2	(H_3M_1)	Myocardium, RBC	
LDH3	(H_2M_2)	Kidney, Skeletal muscle	
LDH4	(H_1M_3)	Kidney, Skeletal muscle	
LDH5	(M_4)	Skeletal muscle, Liver	Skeletal muscle, liver diseases

***Note:** LDH1, LDH2, LDH3, LDH4 and LDH5 are all isoenzymes.

As mentioned before, the LDH isoenzyme in cardiac muscles catalyses the conversion of Lactate to Pyruvate, and then Pyruvate enters the Citric Acid Cycle. While the LDH isoenzyme in skeletal muscles and RBCs catalyses the conversion of Pyruvate to Lactate. In other words, they work INVERSELY.



Regulation and catalytic activity of LDH.

- RBCs can function anaerobically, but heart tissues cannot. The reason for that is, RBCs don't have mitochondria and nucleus, so when metabolism of glucose happens there is no option left for Pyruvate other than being converted into Lactate. While in the heart and in skeletal muscles it has an option to go aerobically or anaerobically.

Regulation

LDH1 (H_4) has a relatively high K_m (low affinity) for Pyruvate and is inhibited by Pyruvate. The H_4 isoenzyme favors the oxidation of Lactate.

LDH5 (M_4) has low K_m (high affinity) for Pyruvate and is not inhibited by Pyruvate. The M_4 isoenzyme is always active even at high levels; ensuring that Pyruvate is always funnelled to anaerobic metabolism.

Again, RBCs and skeletal muscles convert Pyruvate to Lactate, then Lactate gets transferred to the heart where it's converted back to Pyruvate

Q- What will happen if there was high concentration of Pyruvate in the cardiac muscle?

A- LDH in the cardiac muscle will get inhibited, because the heart muscle is not selfish, it will not convert all the Lactate to Pyruvate to enter aerobic metabolism.
The extra Pyruvate will exit the cardiac muscle to the blood all the way to the liver, where it gets converted to Glucose and store it in the form of **Glycogen**.

Hexokinase & Glucokinase

Hexokinase is responsible for the phosphorylation of glucose to glucose-6-phosphate. Phosphorylation means that a phosphate group is added to glucose. But, phosphate has a negative charge and that will prevent glucose-6-phosphate from crossing the plasma membrane out of cells, it will get TRAPPED!

There are many types of hexokinases in tissues. We will focus on hexokinase I which is found in muscles & hexokinase IV (Glucokinase) which is found in the liver.

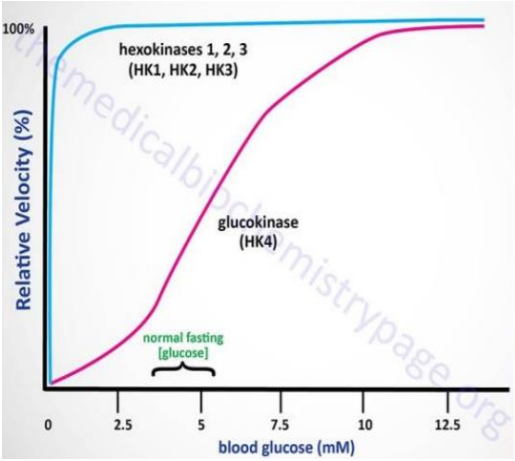
	Hexokinase I	Glucokinase (Hexokinase IV)
Found in	Muscles , RBCs	Liver
Regulation	Inhibited by glucose-6-phosphate	Not inhibited
Km Values	Low , 0.1 mM	High , 10 mM
Affinity & Efficiency	High	Low
Significance	RBCs do not consume all glucose in blood	Liver can convert excess glucose in glycogen for storage

- The purpose of liver glucose is to balance glucose levels in blood, ensuring that all tissues have enough glucose. Glucokinase is a low efficiency enzyme; to provide glucose to other organs.
- The purpose of muscles and RBCs glucose is to produce energy. Hexokinase I is a high efficiency enzyme; to trap glucose.

As we can see, the K_m values for hexokinase is low (0.1 mM), and for Glucokinase its high (10 mM).

Hexokinase has higher affinity to glucose, its binds to it very quick and phosphorylates it instantaneously.

However, Glucokinase has lower affinity to glucose; the liver only phosphorylates glucose when it makes sure that glucose is not needed by any other tissue.



Enzyme Inhibitors

1. **Reversible inhibition:** All physiological inhibitors are reversible, they are bound to enzymes through weak non-covalent binding
    a) Competitive inhibition
    b) Non-competitive inhibition
2. **Irreversible inhibition:** They are mainly synthetic e.g. Drugs. They bind *mainly* covalently, or with very strong high affinity non-covalent interactions

a) Competitive Inhibition

Competitive inhibitors compete with the substrate in binding to the active site. So, increasing substrate concentration can overcome inhibition.

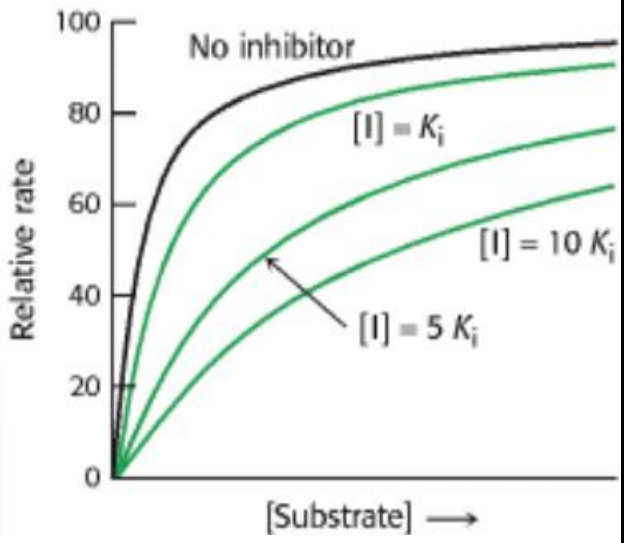
- K_m will be increased, but V_{max} will remain the same.
- It looks like the affinity of the substrate to the enzyme has decreased, but in fact some active sites have been blocked by the inhibitor which causes less binding to it

Q- Can we reach V_{max} in the presence of a competitive inhibitor?

A- If the inhibitor to substrate ratio was 1:1 (50% substrate, 50% inhibitor) then **NO**. If we have 100 molecules of substrate and 1 molecule of inhibitor, **YES**. Eventually it'll reach V_{max}.

The following graph shows the effect of increasing concentration of inhibitors.

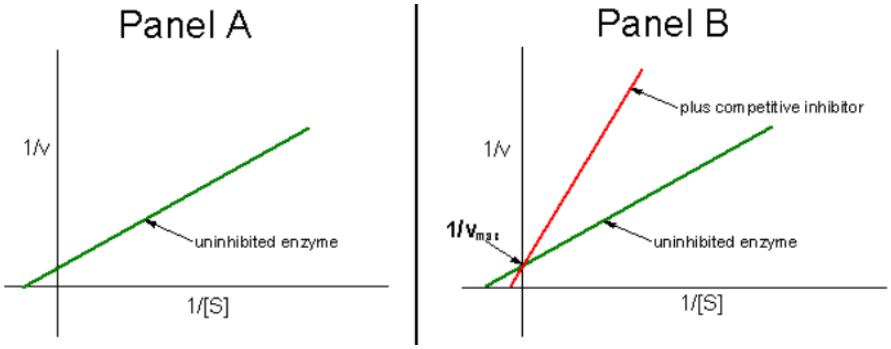
At very high concentration of the substrate, it'll eventually reach V_{max} as if the inhibitor is absent. When we have high amount of substrate relative to the inhibitor, the substrate will win the competition and it will bind to the active site of the enzyme and it will reach V_{max}.



Q- Can we calculate V_{max} from that graph?

A- No, because we can never reach V_{max}, It's hypothetical. But how can we calculate V_{max}? using the **Lineweaver plot** (it's linear).

By extending the line to the left (it's hypothetical because we can never have negative concentration of a substrate), eventually we will hit a point on the y-axis which is $1/V_{max}$.



- In the presence of an inhibitor (the red line) V_{max} didn't change, so the line will cross the same point on the y-axis, but Km will change, it will increase. Notice that the x-axis represents 1/[S] (just flip the concentration of the substrate).

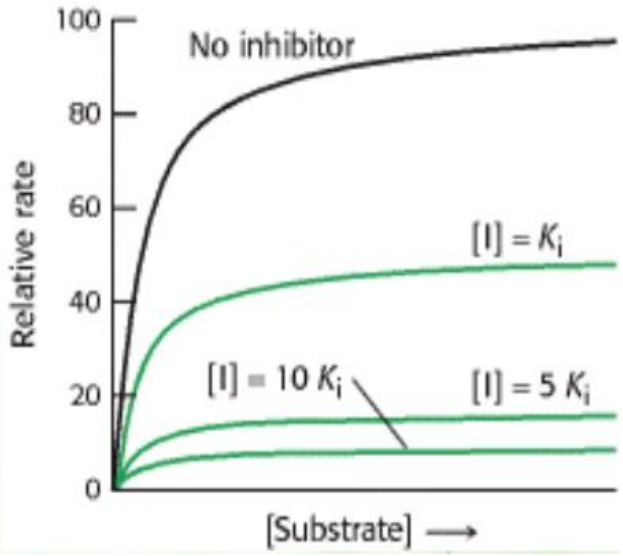
b) Non-competitive Inhibition

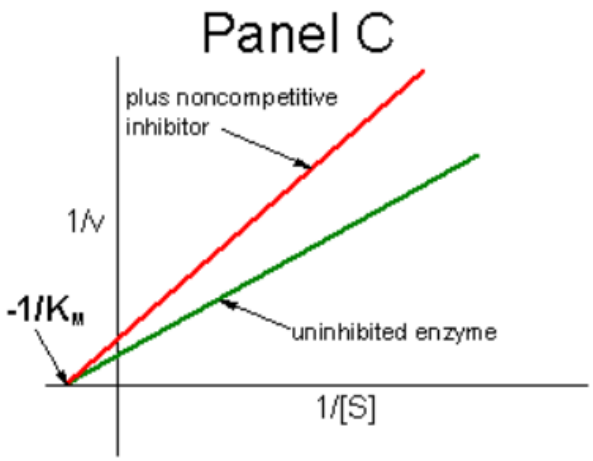
Non-competitive inhibitors bind to E or ES complex at a different site than the catalytic site, and they don't affect the substrate binding to the active site, there is no competition between the inhibitor and the substrate. But it will affect the enzyme activity.

- Substrates can bind to the Enzyme-Inhibitor complex, but ESI cannot form a product.

V_{max} will be decreased, K_m will stay the same;

Because no matter how many substrate molecules we add, the enzyme is inhibited (the same fraction of enzyme is inhibited), even though the interaction between the inhibitor and the enzyme is reversible; when the inhibitor molecule dissociates from one enzyme molecule it will bind to another, so **the same fraction of enzyme molecules remains inhibited**.





Notice that the values of K_m are all the same, but V_{max} is getting lower as we increase the inhibitor concentration.

Also, on the Lineweaver plot we will get same K_m but a lower V_{max}.

The green line represents the uninhibited ES complex. The Red line represents the ESI complex.

We can have different types of inhibitors, we already discussed two of them. The doctor said that in the exam he might put different plots of different inhibitors and ask questions about them. There might also be some inhibitors that increase the affinity to the substrate, in that case K_m will decrease, and V_{max} will decrease as well, so different inhibitors have different functions and different effects on the enzyme.

Again, it is not affected by the substrate concentration because the inhibitor is not bound to the active site of the enzyme, so V_{max} will be decrease, and K_m will stay

Q- How can an inhibitor increase the enzyme affinity to the substrate ?

A- The inhibitor will bind to the enzyme, then it'll increase the interaction between the substrate and the enzyme, the affinity is higher, K_m is lower, but the active site is NOT functioning, so the substrate is always bound to the enzyme, even though the affinity is higher, but the enzyme is not functioning.

Irreversible Inhibitors

Irreversible inhibitors are mechanism-based, that means they bind to the active site and the enzyme remains inactive all the time; because the interaction between them is very strong, very tight binding and mainly its via covalent bonds.

- Irreversible inhibitors decrease the concentration of active enzymes.

Irreversible inhibitors include:

1. Covalent inhibitors
2. Transition state analogs
3. Heavy metals

1. Covalent inhibitors

They mimic the substrate, binding to the active site covalently and modifying it, thus the active site cannot be modified again (return to its normal structure).
Example: organophosphates like (DFP) diisopropyl fluorophosphate, the nerve gas sarin (used in wars ☹), The insecticides malathion & parathion

- **The nerve gas sarin:** normally the brain releases neurotransmitters which induce a signal in nerves that cause an excitation of nerve cells, that excitation has to be terminated or else the nerve cell will stay excited the whole time. How can we terminate that excitation? through degradation mechanisms (recall physiology course).
  For example, the neurotransmitter acetylcholine, it is inhibited by the enzyme acetylcholinesterase. DFP inhibits acetylcholinesterase by binding to the active site of Sarin covalently preventing the degradation of the neurotransmitter acetylcholine, so acetylcholine will remain available in the synapse for a longer time
- **DFP** also inhibits other enzymes that use serine (ex. serine proteases), but it's not lethal.
- **Aspirin:** what it does is it can also modify serine of cyclooxygenase, it can acetylate it, deactivating the active site and that will inhibit the prostaglandin precursor that is a physiologic substrate for the enzyme.

2. Substrate and transition-state analogs

Recall back to when we studied the transition-state of enzymes, in the reaction between the enzyme and the substrate the enzyme binds to the substrate non-covalently, a series of reactions happen like what we can call a black box, then we get the product. In that series of reactions, a covalent interaction between the transition-state and the enzyme might happen (on the active site), so we can have a very strong interaction between the enzyme and the transition-state molecule.
scientists made a transition-state analog molecules, they're inhibitors that look like transition-state, which bind with a high affinity to the substrate, but they stop the reaction

Why? because the transition-state analog is missing a group, an example is the enzyme transaminase that binds to an amino-acid and a keto-acid, now the keto-acid looks like a transition-state molecule, but it's missing a group, the enzyme takes the amino-group of the amino-acid and gives it to the keto-acid, BUT since the keto-acid is missing a group (there is no place where it can put this amino-group) now the reaction is stuck, the enzyme can't give the amino-group neither to the amino-acid nor to the keto-acid. The enzyme is now killed, it is DEAD ☹. That's why these inhibitors are called suicide inhibitors.

Note: the inhibitor here mimics the keto acid

Transaminases are enzymes that cat alyze a transamination reaction between an amino acid and an α-keto acid. They are important in the synthesis of amino acids, which form proteins.

It's hard to make transition-state analogs; because they are unstable.

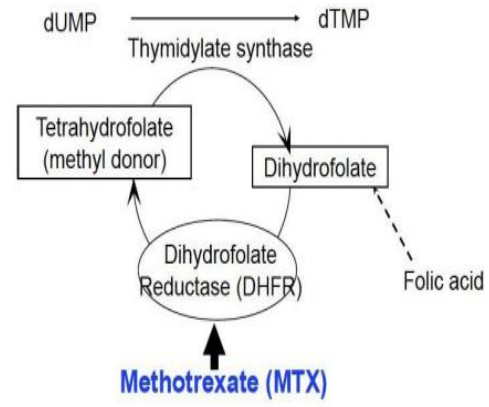
Methotrexate

it's is a cancer chemotherapeutic agent, which is given to cancer patients.

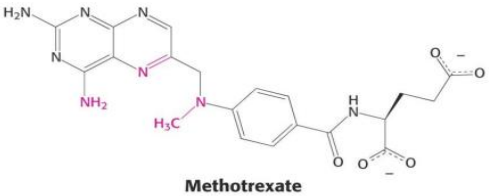
How does it work ?

- Cancer cells keep on growing and dividing, so they need to produce more of their DNA. **Basically, what methotrexate does is that it inhibits DNA synthesis**. As a result, cancer cells cannot grow anymore.
  the problem with methotrexate is it's not specific for cancer cells, it can target other cells which divide and synthesize DNA, causing hair loss and other side-effects of chemotherapy.

Methotrexate inhibits the synthesis of dTMP. To synthesize dTMP, a co-enzyme is required, and in this case, it is Folic acid *(vitamins are an example of co-enzymes)*. Folic acid is converted to Dihydrofolate. Dihydrofolate will be renewed by an enzyme known as Dihydrofolate-Reductase (DHFR). Now, if we inhibit the renewal of Dihydrofolate, Thymidylate synthase *(the enzyme responsible for the synthesis of dTMP)* will be inactive because it needs Tetrahydrofolate, and the synthesis of dTMP will be stopped.



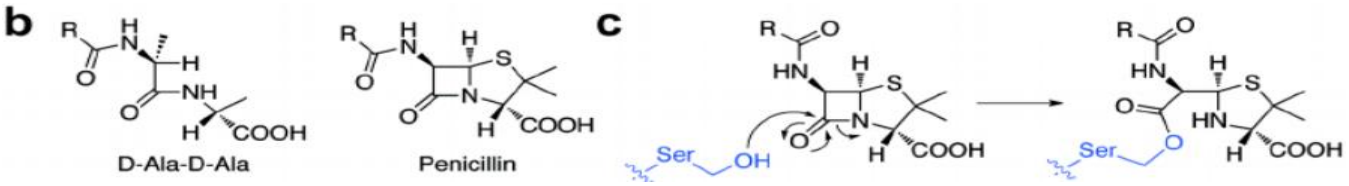
That's why pregnant women are advised to take Folic acid for the first 3 months of pregnancy; because the embryo needs a lot of DNA since there is a lot of cell growth



- Methotrexate mimics co-enzyme Tetrahydrofolate.
  It binds to dihydrofolate-reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis.
- It binds and stops the series of reactions (as we mentioned in page 8).

Penicillin

- It is a transition-state analog to glycopeptidyl transpeptidase, an enzyme essential for the formation of peptidoglycans, which are required for synthesis of the bacteria cell wall.
- The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex.
- The active site serine attacks the highly strained β -lactam ring, resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme.
- Penicillin looks like **dialanine,** but it is missing a group. It participates in the reaction and stops it, then the enzyme that synthesizes the peptidoglycan is inhibited.



3. Heavy Metals

- E.g. Mercury (Hg), Lead (Pb), Aluminium (Al) and Iron (Fe). result in tight binding to a functional group in an enzyme. Nonspecific inhibition at high doses.
- **Mercury (Hg)** binds to reactive sulfhydryl groups that are present in the active sites of enzymes, there are a lot of enzymes which contain Cystine or sulfhydryl groups at their active site, and these enzymes are killed by mercury.
- **Lead (Pb)** replaces the normal functional metals in an enzyme such as calcium, iron, or zinc by irreversible mechanisms.
  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.

Abzymes *(Ab = Antibody, zymes = Enzymes)*

- Abzymes are immunoglobins, they can spot immune cells, and they can bind to antigens (foreign bodies) very specifically.
  Immunoglobins are composed of amino-acids, they are polypeptides.
- They can act as enzymes and catalyse reactions
- An antibody is produced against a transition-state analog & has catalytic activity like that of a naturally occurring enzyme. An abzyme is created by injecting a host animal with a transition-state analogue. The host animal makes antibodies to the foreign molecule, & these antibodies have specific binding points that mimic an enzyme surrounding a transition state.