بسم الله الرحمن الرحيم

**BIOCHEMISTRY**

DOPAMINE
SCIENTIFIC TEAM

Lecture 27

# Enzymes III Regulation

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا لنصرة دينك


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## Introduction:

- Enzymes shouldn't be always active  and should be regulated (organising enzyme activity)
- Cells undergo fine tuning; which is what makes a living organism living organism
- Regulation of enzymes follow this fine tuning (not an on/off switch)
- When an enzyme (or any other gene or protein) is very important, there would be different levels of regulation; so that if one mechanism doesn't work efficiently another mechanism would do the trick

# Mechanisms of regulation

- Non-specific regulation (temperature, pH, diffusion, and expression)
  - Localization (compartmentalization and complexing of enzymes)
  - Expression of isoenzymes
- Regulation of enzymatic activity
  - Inhibitors
  - Conformational changes
    - Modulators
    - Reversible covalent modification
    - Irreversible covalent modification
    - Allostery

There are different types of regulation:

1. Non-specific: affects hundreds of enzymes all at once
2. Very specific: targets a specific enzyme

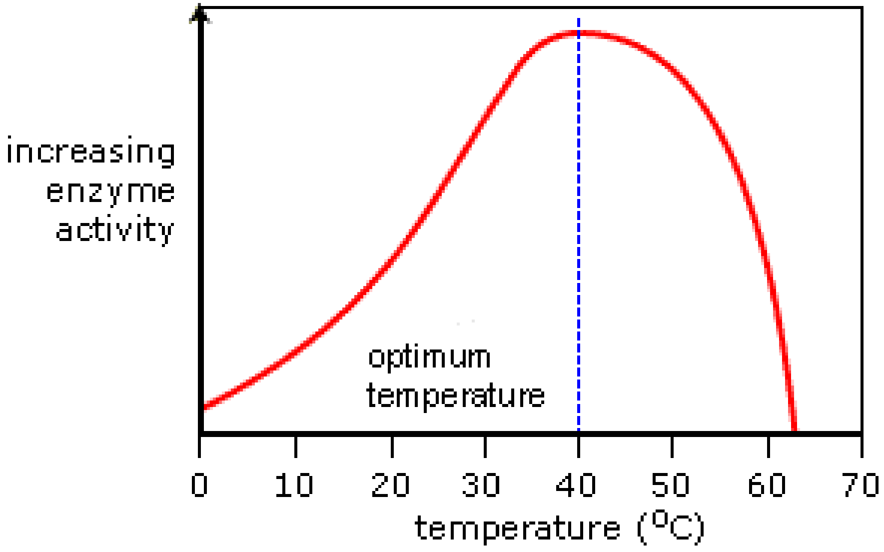
Specific: enzyme specific

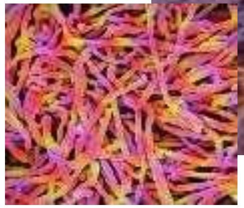
# Temperature
(Non-specific)

Our body temperature is about 37 while the optimal temperature in our cells for enzyme activity is 38 → From 37 → 38 Kinetic energy increases and rate of collision increases; more enzyme activity Still our control is 37 degrees.

If temperature reaches (42-44), protein denaturation occurs, which is why people could die with uncontrolled fever

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



- Temperature affects enzyme activity, other primitive organisms (like bacteria) love high temperature, they can survive at 65degrees
- In PCR (during DNA synthesis), the enzyme starts at a temperature of 95 in which enzyme is still functional
- Organism Specific (like people & bacteria)

# pH

(Non-specific)

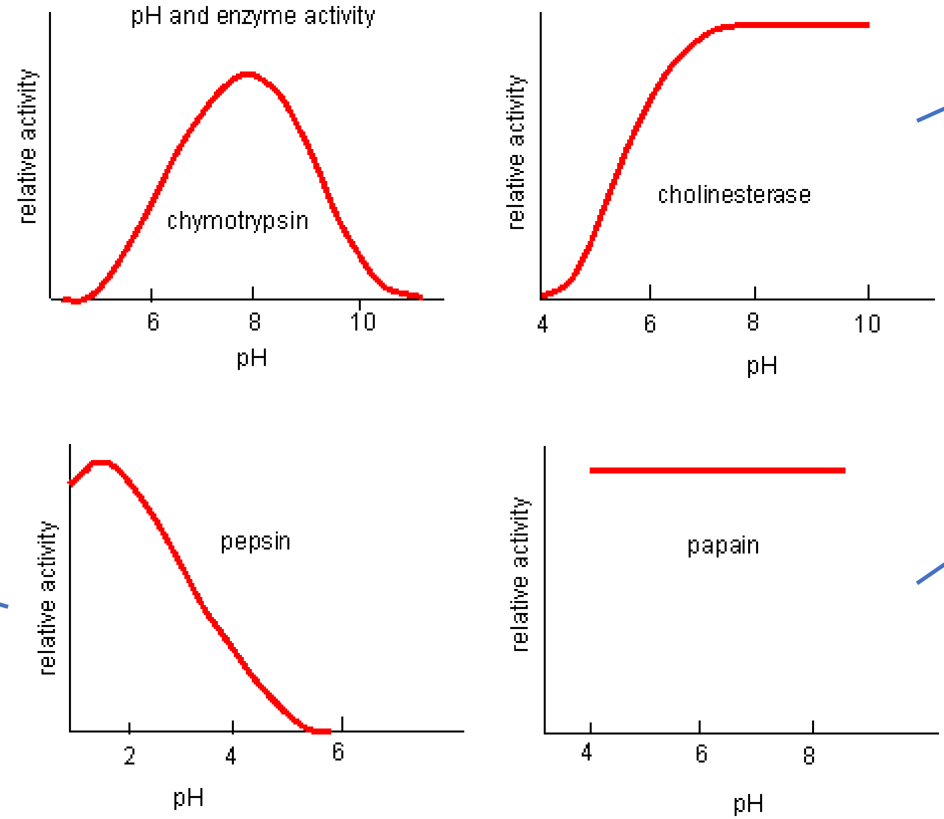
pH affects protonation state, electrostatic interactions, hydrogen bonds, etc.
Affects enzyme stability

If we change pH of cytosolic & mitochondrial enzymes, we would change enzyme efficiency
A lot of enzymes in cytosol will not work in pH=5 or 9 but lysosomal enzymes love acidic pH, their pH is (5.5-6)

- pH alters the protonation state of the substrate and/or the enzyme and, hence, their binding.
- The effect of pH is enzyme-dependent.

Chymotrypsin is a digestive enzyme, works in intestines in which the pH=8, optimal pH=8

Cholinesterase works in CNS, optimal pH is >6, doesn't function below 6

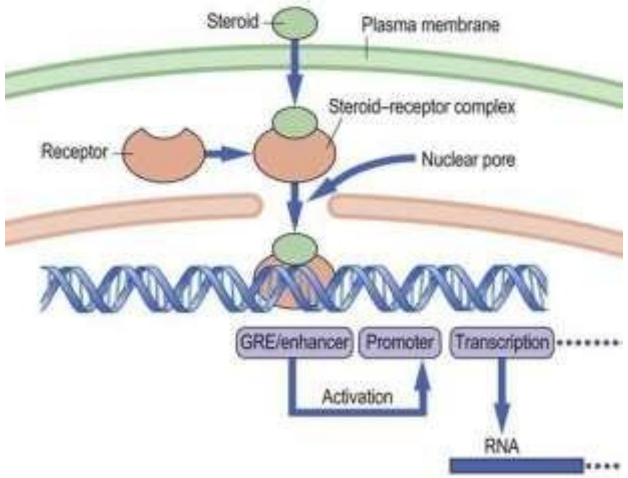



Pepsin in stomach works optimally at pH=2, doesn't function at pH=7

Papain is in papaya fruit, not affected by pH

pH can affect multiple enzymes, but can be enzyme specific as well

# Regulation of enzyme amount :amount of enzymes in cells
(can be enzyme specific)




- Three mechanisms: Regulated at different levels
  - Enzyme synthesis at the gene level Active gene, transcriptional translation
  - Enzyme degradation by proteases
  - Synthesis of isozymes

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

- Half life of enzyme differs by the type of enzymes
- Enzymes are degraded when cells don't need them anymore, amino acids are produced & used
- When amino acids are degraded they give energy

| Enzyme | Half-life (days) |
|---|---|
| Catalase | 1.4 days |
| Glucokinase | 1.2 days |
| Lactate dehydrogenase | |
| LDH1 (heart) | 1.6 |
| LDH5 (liver) | 16 |
| LDH5 (muscle) | 31 |

What is meant by **half life?** 🤔
After the half life→ fifty percent of enzymes gets degraded

# Compartmentalization

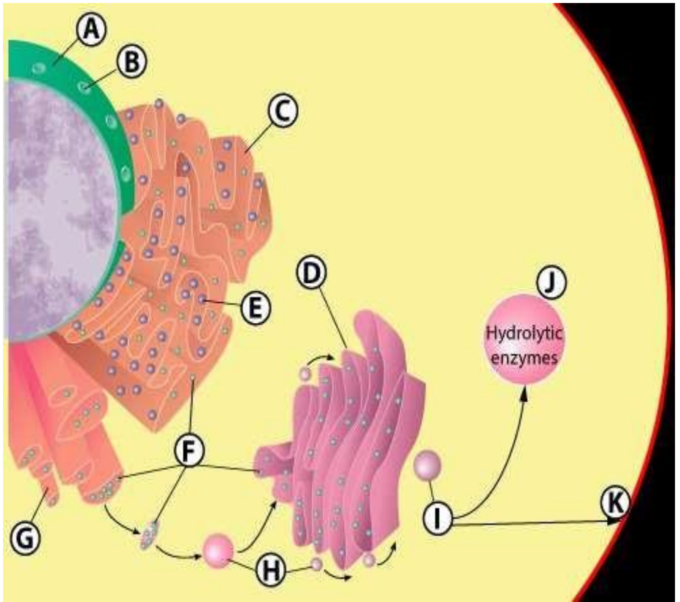
Rate of diffusion is an enzymatic reaction problem, enzymes & substrates meet randomly, so cells place enzymes & substrates in small compartments to limit the rate of diffusion

- Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide.
  - Example 1: lysosomal enzymes

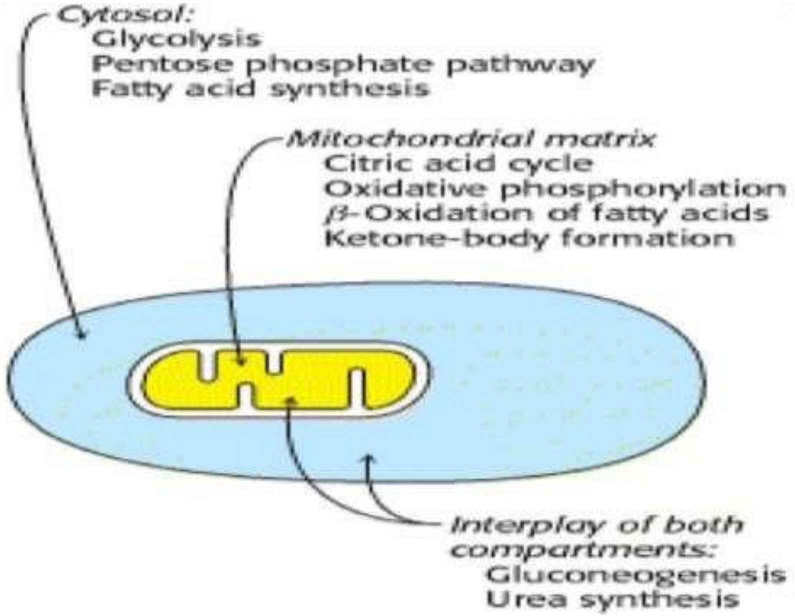
    lysosomal enzymes degrade everything not needed (hydrolysis: use water to break bonds)
    pH in lysosomes is low which denatures protein
  - Example 2: fatty acid metabolism

    Another purpose for compartmentalization is regulation of metabolic pathways,
    - Fatty acids synthesis → cytosol
    - Fatty acids degradation → mitochondrial

    If they were both in same area, things may get out of control
    - Synthesis occurs in cytosol, whereas break-down is mitochondrial.




Main reason for putting substrates & enzymes in smaller compartments is to limit diffusion rate to produce higher collision rate



# Enzyme complexing

Another mechanism that limits rate of diffusion

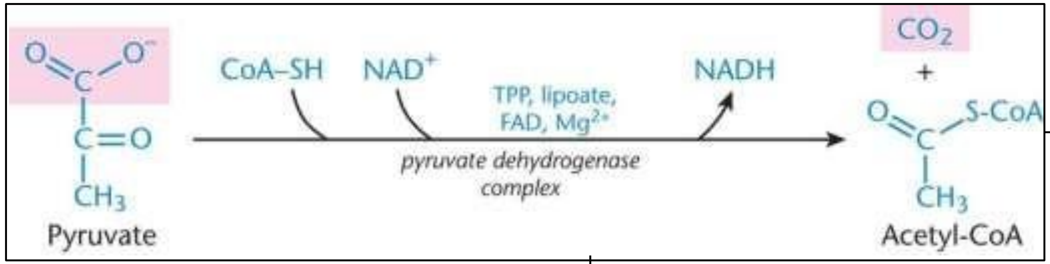
Instead of enzymes converting A→b, then b diffuses and second enzyme searches for b until it finds it in random collision converting b→c & third enzyme from c→d; three enzymes form one complex which doesn't allow diffusion

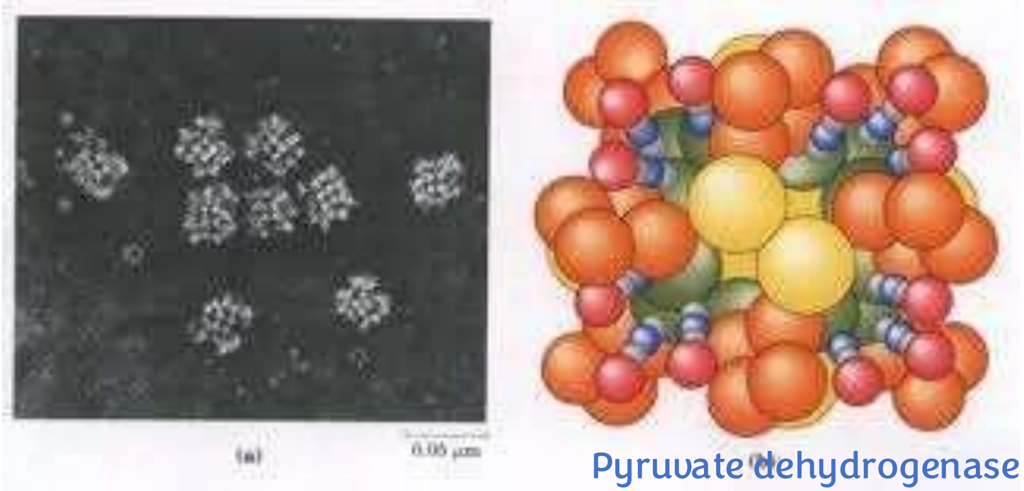
- Formation of a complex of multiple enzymes also reduces diffusion.

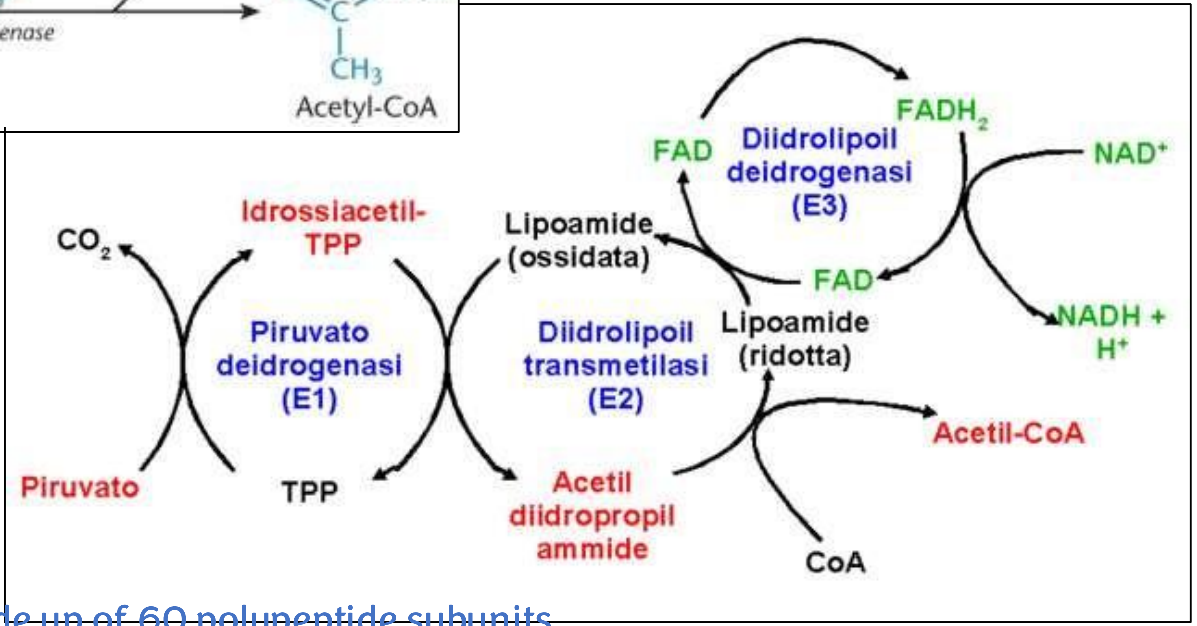
  Catalyses the conversion of pyruvate to acetyl-co enzyme A by three reactions (three enzymatic activities )
- Example: Pyruvate dehydrogenase (mitochondria) is composed of 3 enzymes: decarboxylation (1), oxidation (2), & transfer of the acyl group to CoA (3). Lyase enzyme

Three carbons------→ two carbons (cO2 released)

**Memorise names of enzymes during this lecture**






Pyruvate dehydrogenase is made up of 60 polypeptide subunits, decarboxylase (30), oxidation enzyme (20), transfer reaction(10)

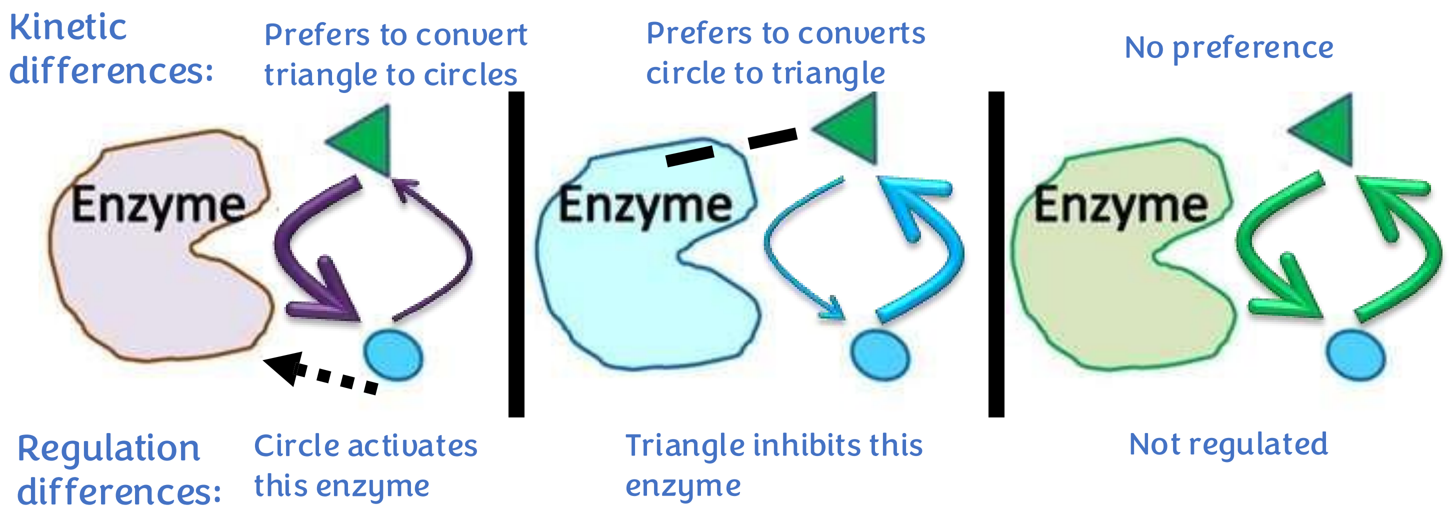
# Isoenzymes (isozymes)

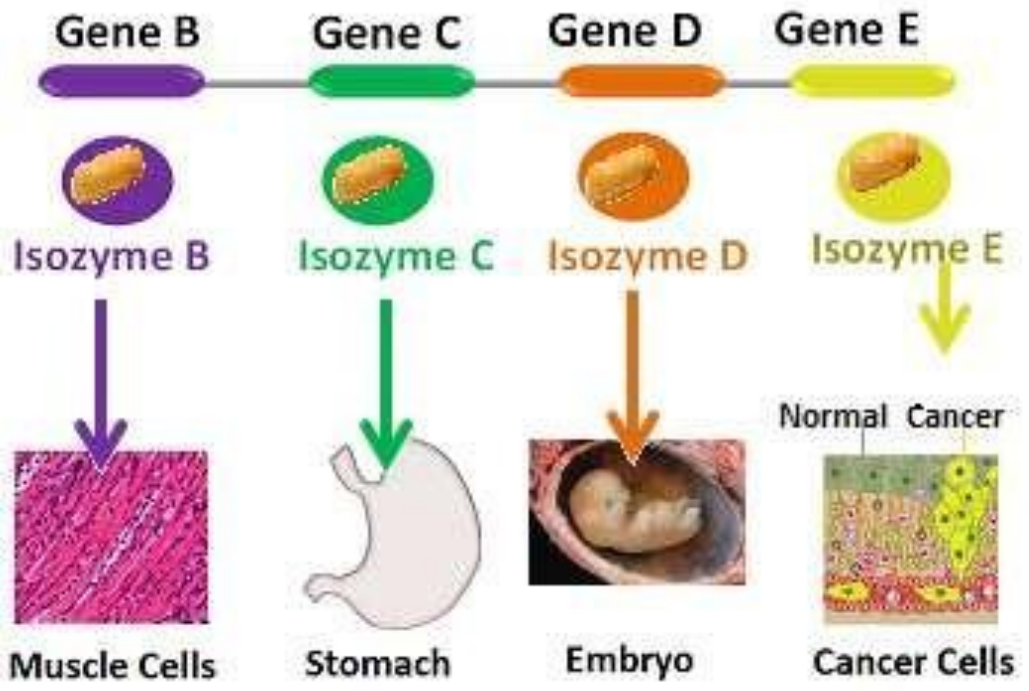
Different enzymes from same gene: isoforms
Different genes catalyse same reactions: isoenzymes

Differences are:
1. Produced from different genes
2. Produced by different cell type in different tissues
3. Different kinetic activities (Vmax, Km..)
4. Regulated differently

Catalyse same reaction

- Isoenzymes are enzymes that can act on the same substrate(s) producing the same product(s).
- They are produced by different genes that vary only slightly.
- Often, various isozymes are present in different tissues of the
- body. They can be regulated differently .
- They can have different catalytic activities.

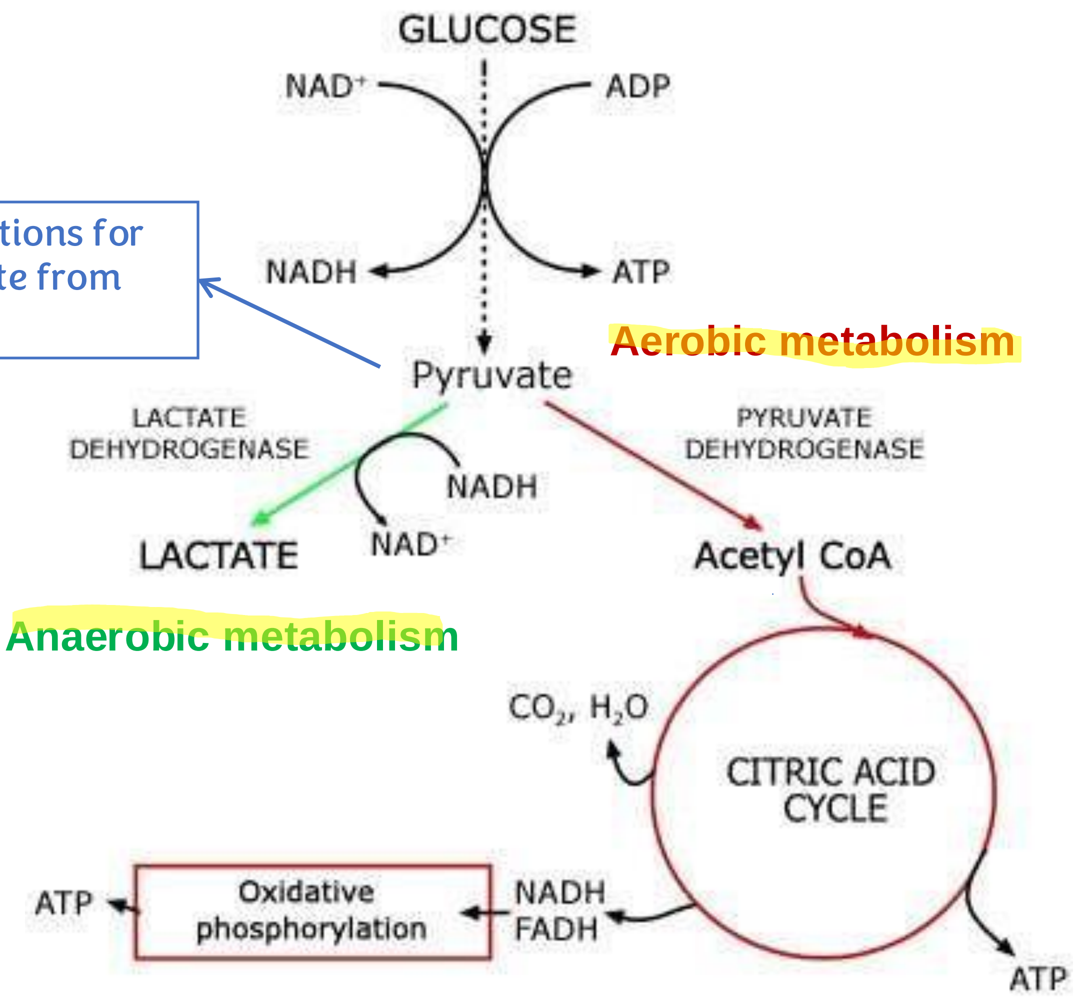


# Aerobic vs. anaerobic metabolism

Glycolysis (gly: sugar, lysis: break)
Breaking glucose → pyruvate

Two options for pyruvate from here




Cells decide based on oxygen availability

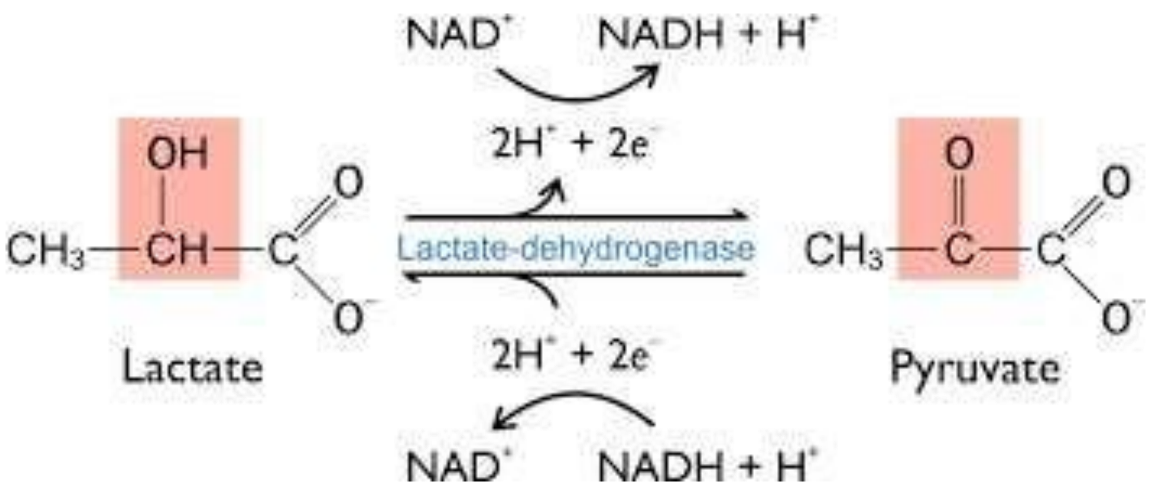
- There is oxygen → acetyl coA → Krebs cycle → oxidative phosphorylation (high ATP)
- No oxygen → lactate dehydrogenase (pyruvate to lactate or reverse reaction)

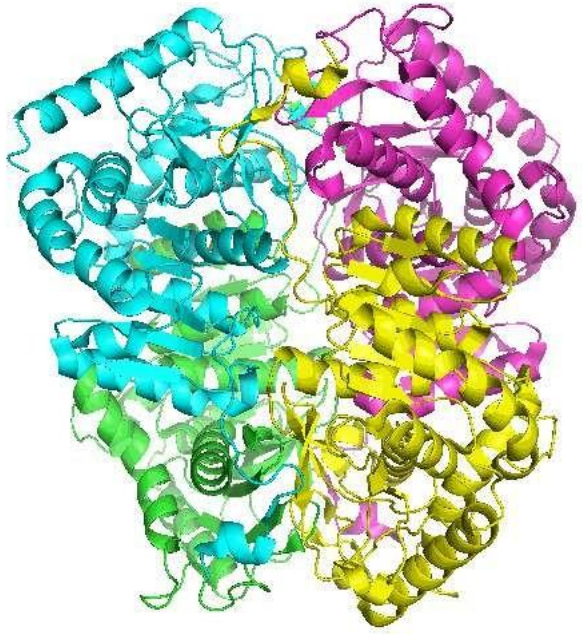
# Lactate dehydrogenases (LDH)

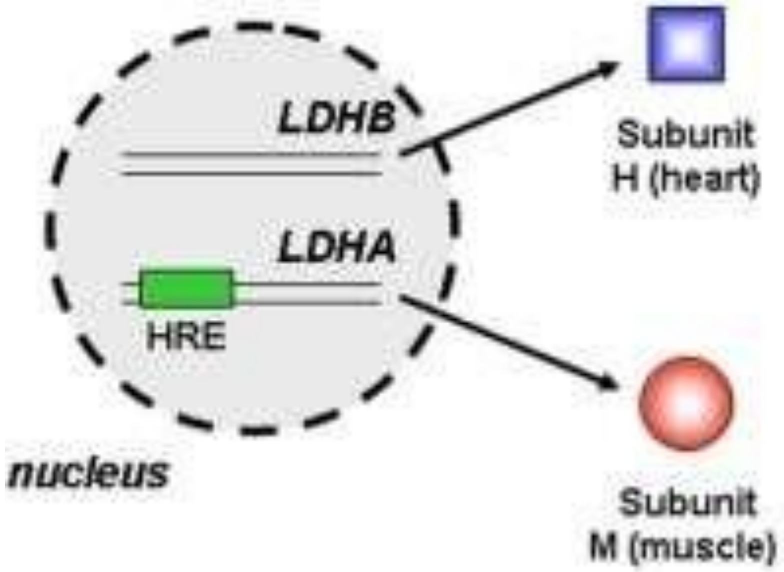
Lactate dehydrogenase: Lactate → pyruvate OR pyruvate→ lactate (with same reaction)

Four polypeptide subunits, can be made of different combinations of two polypeptide chains (H or M); two chains =different genes

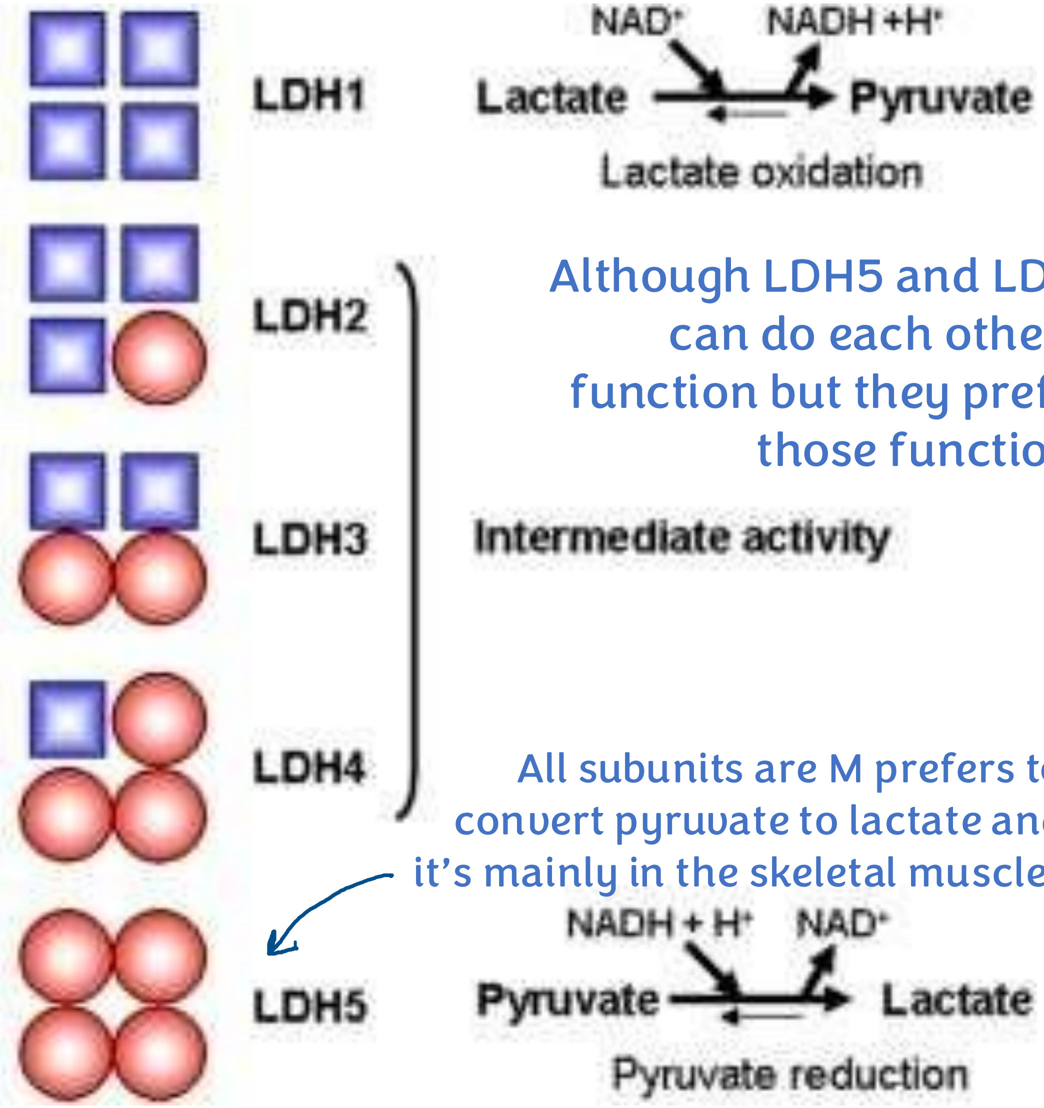
- LDH is a tetrameric enzyme composed of a combination of one or two protein subunits: H (heart) and M (skeletal muscle).
- These subunits combine in various ways leading to 5 distinct isozymes leading to 5 distinct isozymes (LDH1-5) with different combinations of the M and H subunits (H4, H3M. H2M2, HM3, and M4). Homotetramers:H4, M4
- The H4 isozyme is characteristic of that from heart tissue, and the M4 isozyme is typically found in skeletal muscle and liver.






All subunits are H prefers to convert lactate to pyruvate and it's mainly in the heart



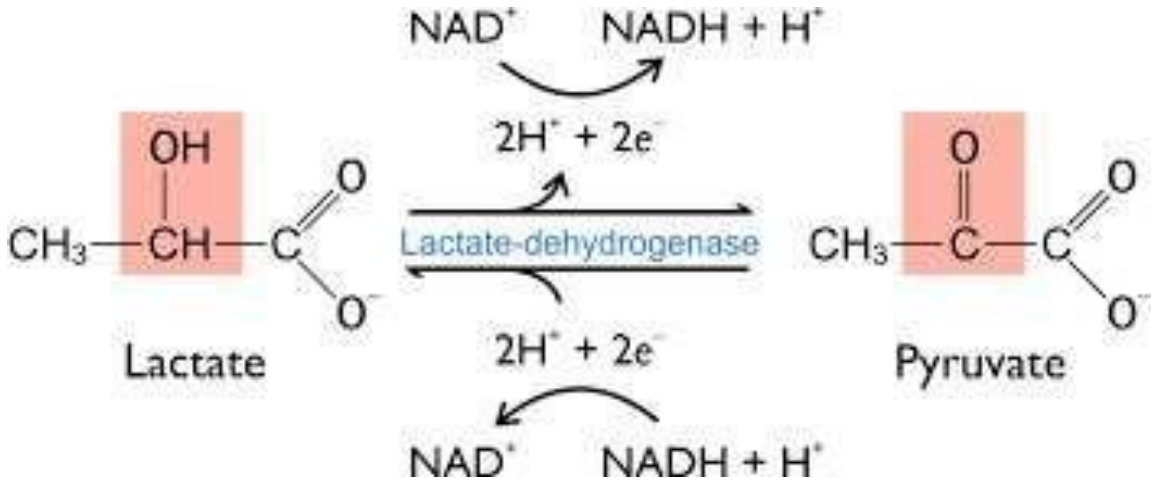

Although LDH5 and LDH1 can do each others' function but they prefer those functions

All subunits are M prefers to convert pyruvate to lactate and it's mainly in the skeletal muscles

- Although the five (izozymes) catalyze the same reaction, they differ in their primary structure, kinetic properties, tissue distribution, affinity to the substrate, regulation, and isoelectric point. And in genes
- The M subunit a higher affinity towards pyruvate, thus converting pyruvate to lactate and NADH to NAD+.
- The H subunit has a higher affinity towards lactate, resulting in a preferential conversion of lactate to pyruvate and NAD+ to NADH.

# Logic behind tissue distribution

Extra: athletes feels pain in their muscles cuz when lactate is traveling through the blood it reduces its ph and for people trying to loose weight they must focus on the aerobic exercises so the glucose breaks down directly not converting to lactate




Lactate which is produced by the muscles goes by the blood then to the heart that use it in the krebs cycle to produce ATP and pyruvate cuz it function aerobically only

Only memories LDH1 &LDH5

| Isoenzyme | Structure | Present in |
|---|---|---|
| LDH1 | ( $H_4$) | Myocardium |
| LDH2 | ($H_3M_1$) | RBC |
| LDH3 | ($H_2M_2$) | Lungs |
| LDH4 | ($H_1M_3$) | Kidney |
| LDH5 | ($M_4$) | Skeletal muscle, Liver |

As muscles are lazy metabolically and they can survive by producing lactate and small amount of ATP

- Muscles can function anaerobically, but heart tissues cannot.
- Whereas the M4 isozyme catalyzes the reduction of pyruvate into lactate, the H4 enzyme catalyzes the reverse reaction.

They are considered as isozymes

# Hexokinase vs glucokinase

They differ in kinetics and regulation They are also produced by different genes

Can phosphorylate other sugars

Phosphorylation of the glucose

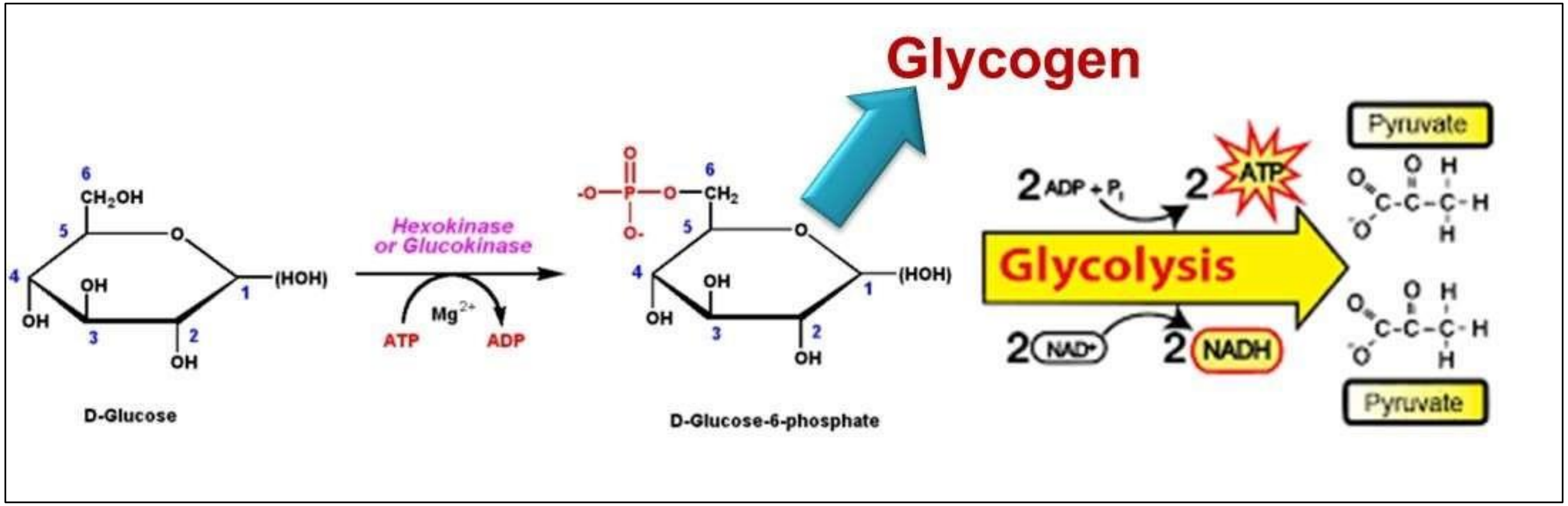
- Hexokinase and glucokinase (hexokinase IV) are allosteric isozymes that catalyze:

ATP + **Glucose → Glucose-6-Phosphate** + ADP

- Glucokinase is a liver (and pancreatic) enzyme, whereas hexokinase is a RBC (and skeletal muscle) enzyme.
  - The purpose of liver glucose is to balance glucose level in the blood.
  - The purpose of RBC glucose is to produce energy.

As liver is the main bank of glucose producing it from breaking down glycogen

As RBCs doesn't have mitochondria so their only source of energy is to convert glucose into lactate



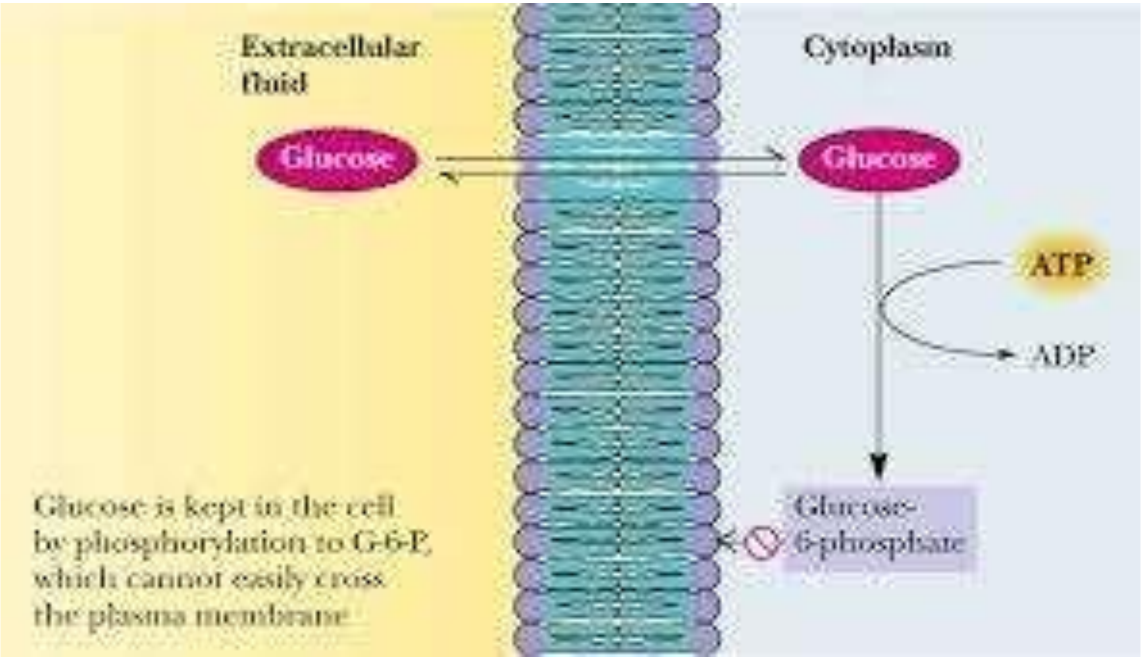
# Biological significance

As phosphate groups are negatively charged so they prevent the glucose from getting out

- Note: once glucose is phosphorylated, it cannot cross plasma membrane out of cells.
  - Liver: low efficiency enzyme to provide glucose to other organs.
  - RBC and skeletal muscles: high efficiency enzyme to trap glucose.

So it doesn't actually phophorelate directly because its main function is to provide other tissues with glucose

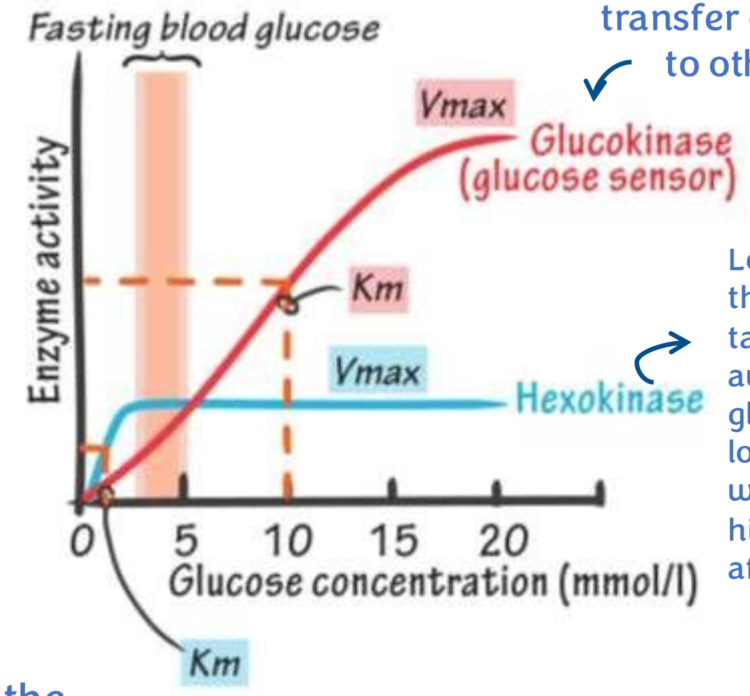
As it's the main source of energy of the RBC



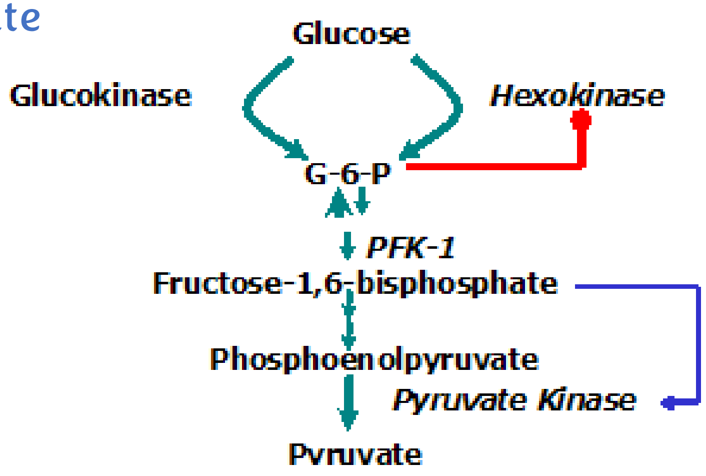
# Regulation of hexokinase and glucokinase

Higher Km so lower affinity that facilitate the transfer of glucose to other tissues

- Note Vmax and $K_M$ values (low - 0.1 mM for hexokinase and (high - 10 mM for glucokinase)
- Regulation
  - Hexokinase is inhibited by glucose-6-phosphate, but glucokinase is not. As liver can store high amounts of glucose as glycogen
  - Glucokinase is activated by insulin and inhibited by glucagon. Insulin increase the secretion of glucokinase when the sugar level is high and glucagon does the opposite
- Significance:
  - At fasting state, glucose is not stored. So it transfer to other tissues that need it
  - At well-fed state, RBCs and skeletal muscles do not consume all glucose in blood and liver can convert excess glucose to glycogen for storage.




Low Vmax so they don't take all the available glucose and low Kms which means higher affinity



# Regulation of enzymatic activity

# Inhibitors

# Enzyme inhibitors

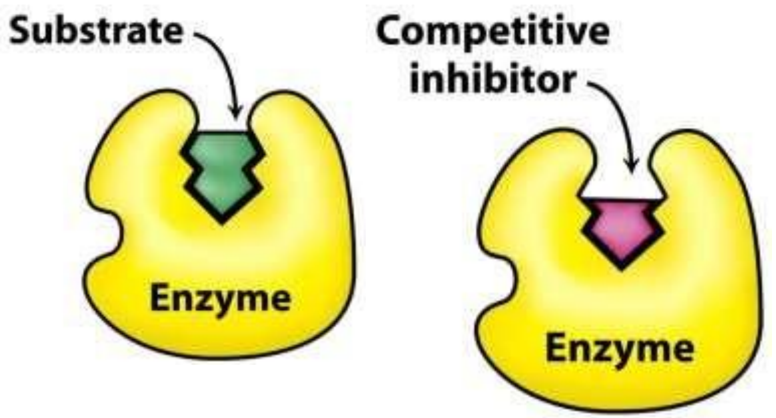
- Enzyme inhibition can be either reversible or irreversible.
  - Reversible inhibitors rapidly dissociate from enzymes (e.g. non-covalent binding).
    - Competitive, noncompetitive, or uncompetitive inhibition.
  - An irreversible inhibitor is tightly bound (e.g. covalently) to the enzyme.
    - Lower concentration of active enzyme.

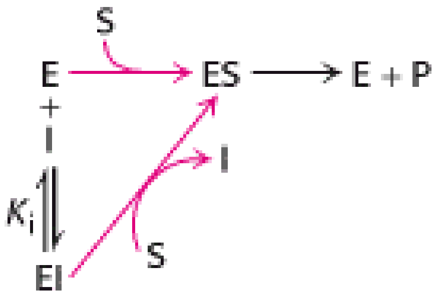
- All physiological inhibitors are reversible but synthetic ones can be irreversible

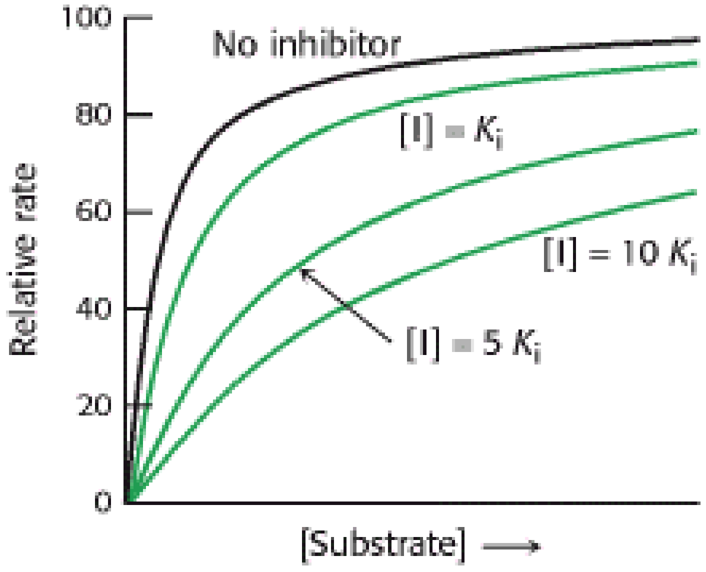
# Competitive inhibition

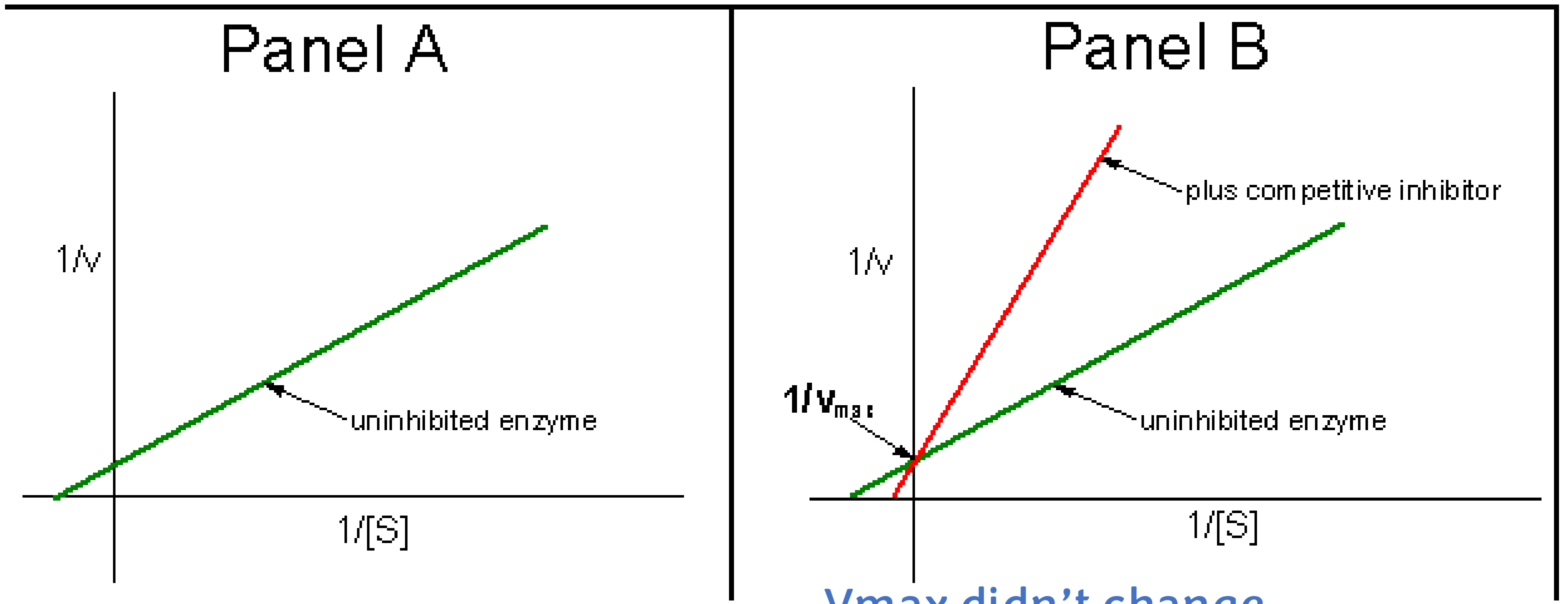
- Competitive inhibitors compete with the substrate for the active site.
  - Increasing substrate can overcome inhibition.
- Same Vmax, but higher $K_M$

Let's say we have specific inhibitor concentration and low substrate concentration and I started to increase substrate concentration will I ever reach Vmax? Yes I will but to reach half Vmax we need higher concentration of substrate because there's a competition



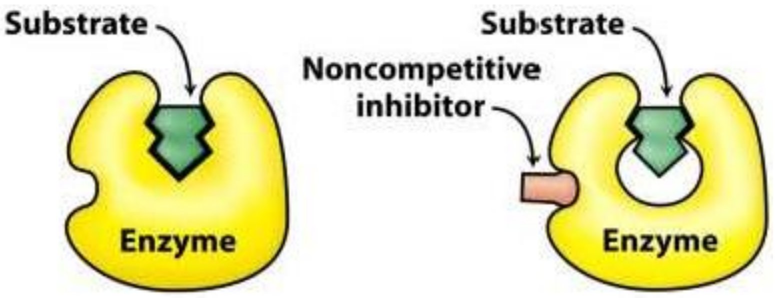



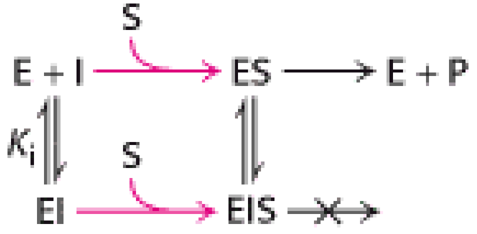

Vmax didn't change

# Noncompetitive inhibition

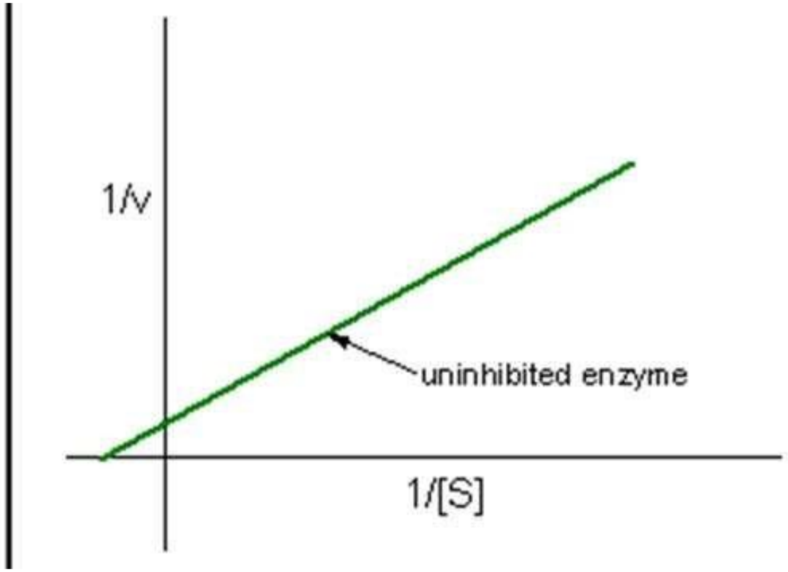
**Imp note** :The doctor said he loves hypothetical questions so focus on understanding the concepts

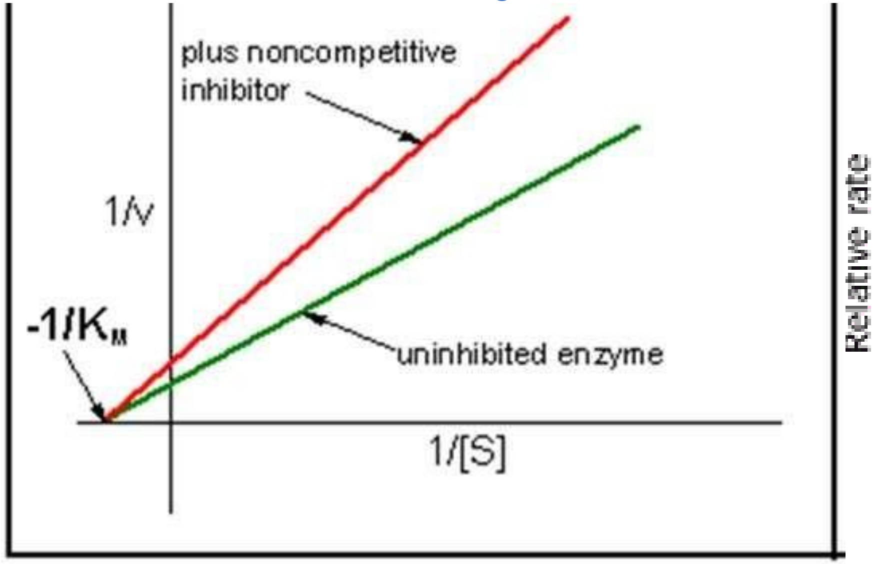
- Noncompetitive inhibitors bind E or ES complex at a site other than the catalytic site.
- Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product.
- Lower Vmax, but same $K_M$

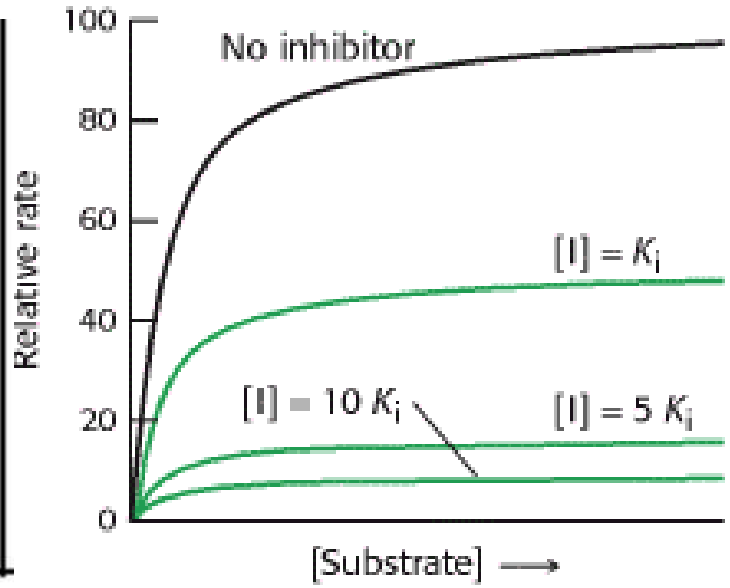



The active site changes but this doesn't affect the binding of the substrate but the enzyme cannot catalyze the reaction



# Mechanism-based inhibitors

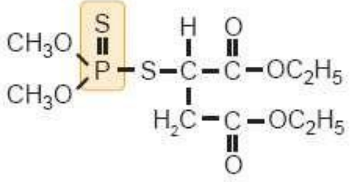
- Irreversible inhibitors
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction.
  - They include:
    - Covalent inhibitors
    - Transition state analogs
    - Heavy metals
- Irreversible inhibitors decrease the concentration of active enzyme.
  - As they bind to the enzymes and inhibit them irreversibly so they affect the Vmax

# Covalent inhibitors

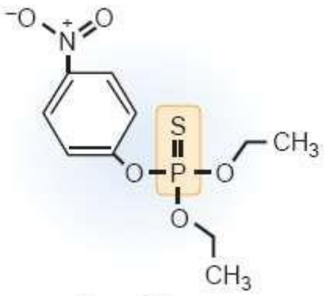
- They form covalent or extremely tight bonds with active site amino acids. They modify the active site chemically
  - Example: di-isopropyl fluorophosphate (DFP) is an organophosphate
    - The nerve gas sarin
    - The insecticides malathion & parathion.
    - DFP inhibits acetylcholinesterase preventing the degradation of the neurotransmitter acetylcholine.

    Causing paralysis because of the accumulation of acetylcholine in the nervous system

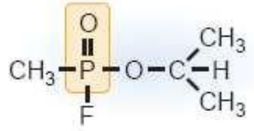
**DFP also inhibits other enzymes that use serine (ex. serine proteases), but not lethal.**



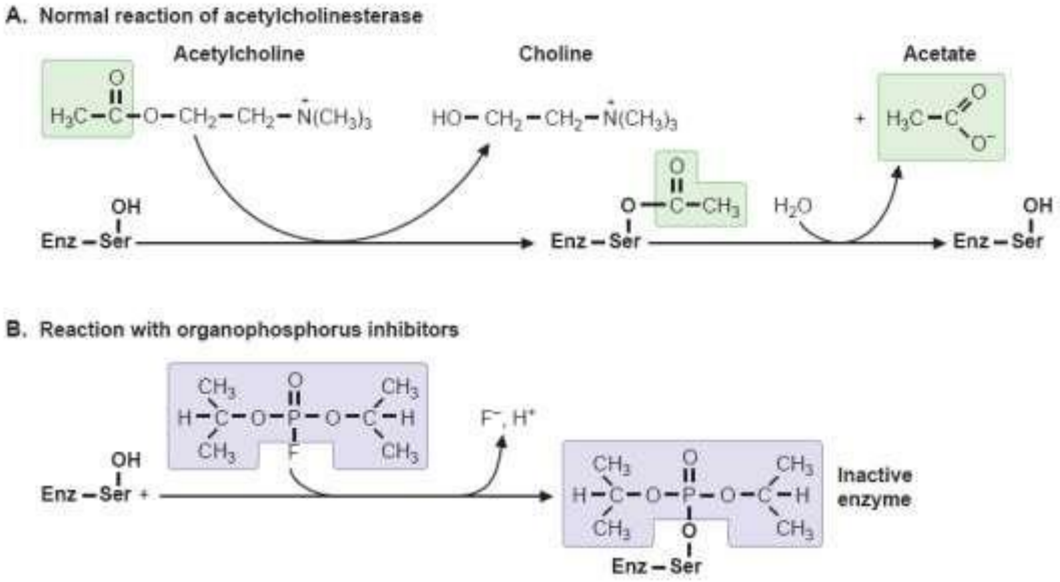
Malathion



Parathion

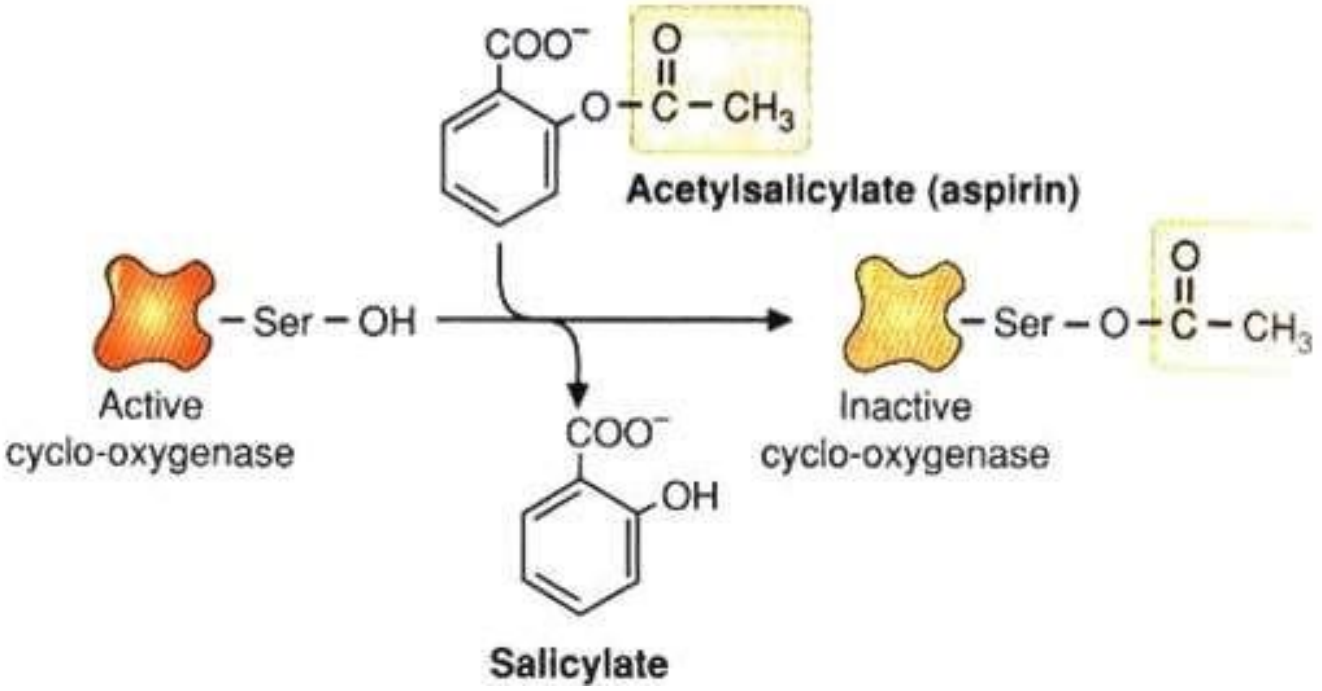


Sarin



# Aspirin

- Aspirin (acetylsalicylic acid) acetylates an active site serine of cyclooxygenase. Which inhibits the enzymes
- Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme.



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

Corrections from previous versions:

| Versions | Slide # and Place of Error | Before Correction | After Correction |
|---|---|---|---|
| V1 → V2 | 5<br>14<br>13 | Pepsin in stomach works optimally at pH=2, doesn't function at pH=2<br>**RBCs convert glucose into pyruvate or lactate**<br>Lactate goes to the by the blood | Pepsin in stomach works optimally at pH=2, doesn't function at pH=7<br>**RBCs convert glucose into lactate**<br>Lactate goes by the blood to the heart |
| V2 → V3 | 12 | Isoforms | Isozymes |

# Additional Resources Used:　　　　رسالة من الفريق العلمي:

وتحسبُ أنك جرمٌ صغير　　　وفيك انطوى العالم الأكبر

علي بن أبي طالب

اللهُم كُن لأهلنا بغزة عونًا ونصيرًا، وبدّل خوفهم أمنًا .اللهم احرسهم بعينك التي لا تنام .اللهم اجعل لأهل غزة النصرة والعزة.