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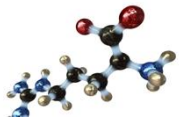


BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 14

Enzymes Pt.4

Written by : Jana Albreizat, Rand Alkhateeb, Dareen Tahrawi, Lamar Khorma

Reviewed by : Rand Alkhateeb, Dareen Tahrawi

Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Enzymes III

Regulation

Prof. Mamoun Ahram

Mechanisms of regulation

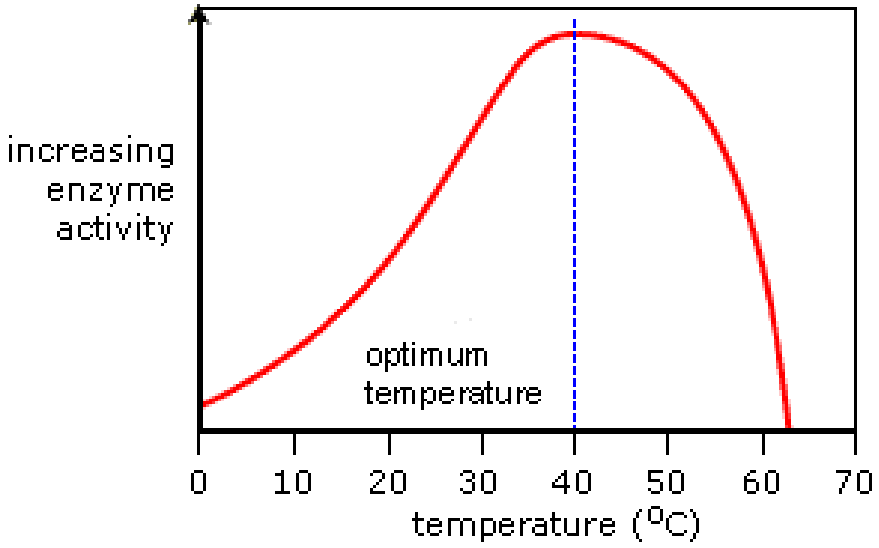
Some regulatory mechanisms are non-specific, meaning they can affect multiple enzymes. Other mechanisms involve specific regulators that can act on particular enzymes.

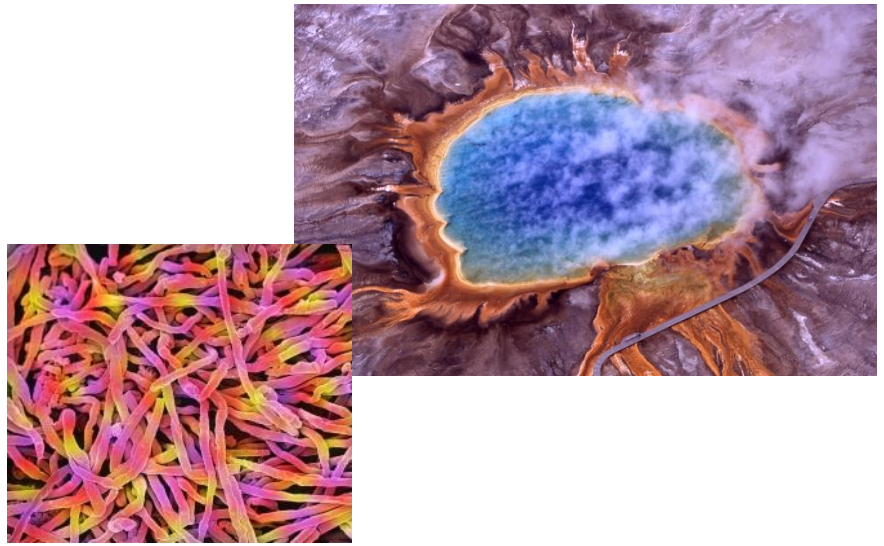
- Non-specific and collective regulation (temperature, pH, diffusion, and expression)
  - Localization (compartmentalization) and enzyme complexing
  - Expression of isoenzymes
- Regulation of enzymatic activity
  - Inhibitors
  - Conformational changes
    - Modulators
    - Reversible covalent modification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

The degree of regulation also depends on the importance and function of the enzyme. Some enzymes require very tight regulation especially when their activity has major effects on metabolism. Others may not require such strict control.

Temperature

- 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.
- Each organism has an optimal temperature for its enzymes.
  - For thermophilic bacteria, the optimal temperature is as high as 65°C.





Enzymes function within a certain temperature range, and each enzyme has an optimal temperature at which it reaches its maximum activity.

- Enzyme activity increases with temperature because enzymes find their substrates through random collisions. As temperature increases, kinetic energy of molecules increases, so collisions between enzymes and substrates become more frequent and faster and this will increase the rate of interactions and, therefore, enzyme activity.

- However, at high temperatures (higher than the optimal temperature), the structure of the protein (enzyme) will be altered, and it will lose its function.

Some bacteria live in very high temperatures such as those found in hot springs. These are called thermophiles, which means "heat- loving". Their enzymes are adapted to function and remain stable at high temperatures, which can reach around 55 or higher.

pH

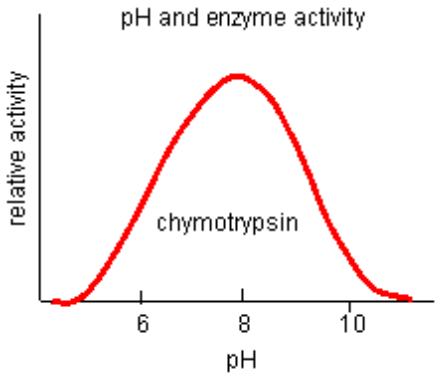
- pH alters the protonation state of the substrate and/or the enzyme and, hence, their binding.
- The effect of pH is enzyme-dependent.

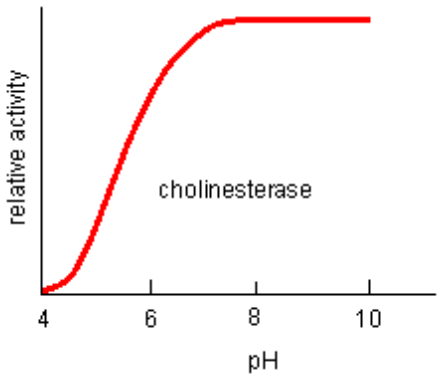
Enzymes also have an optimal pH at which they show maximum activity. For many enzymes in our body, this is around pH 7, but the optimal pH varies depending on the enzyme and its location in the body.

Changes in pH can affect the ionization and charge of amino acid side chains within the enzyme. This can change the enzyme's structure, including the active site, and therefore affect its activity.

Even a small change in charge can affect how the enzyme interacts with its substrate.

Chymotrypsin is a digestive enzyme released by the pancreas and works in the small intestine (alkaline environment), its optimal pH is 8.

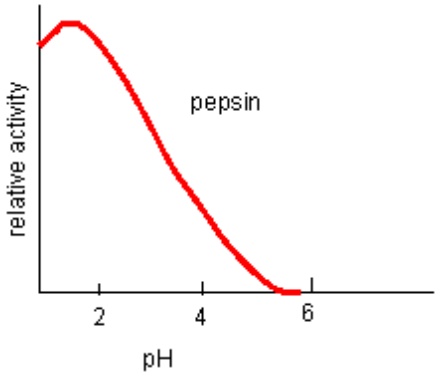


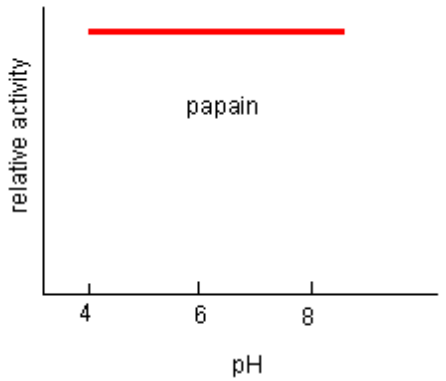


Cholinesterase is an enzyme responsible for the degradation of neurotransmitters, works at pH 7 or higher (ineffective in acidic environments).

Pepsin is a digestive enzyme in the stomach which works in acidic environments (pH=2).

Overuse of antacids affects the activity of pepsin, because it neutralizes the stomach. (increases the pH).



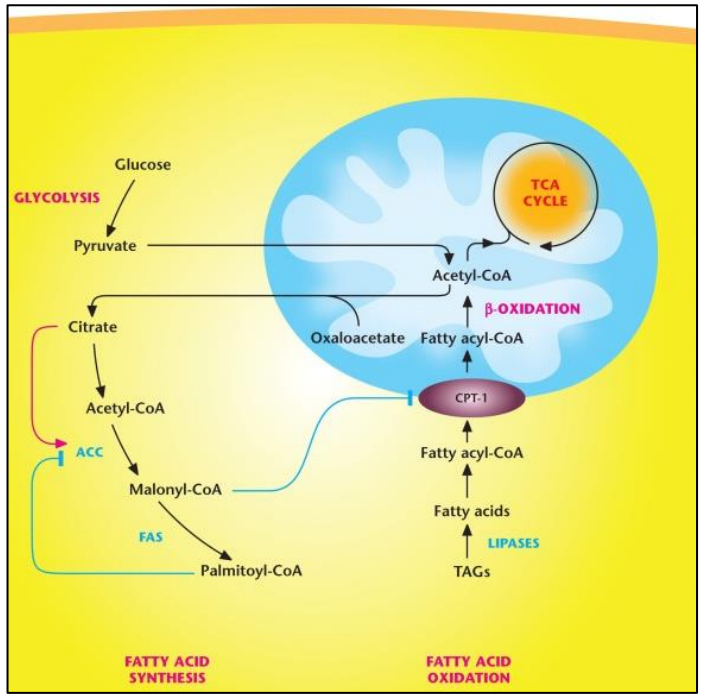


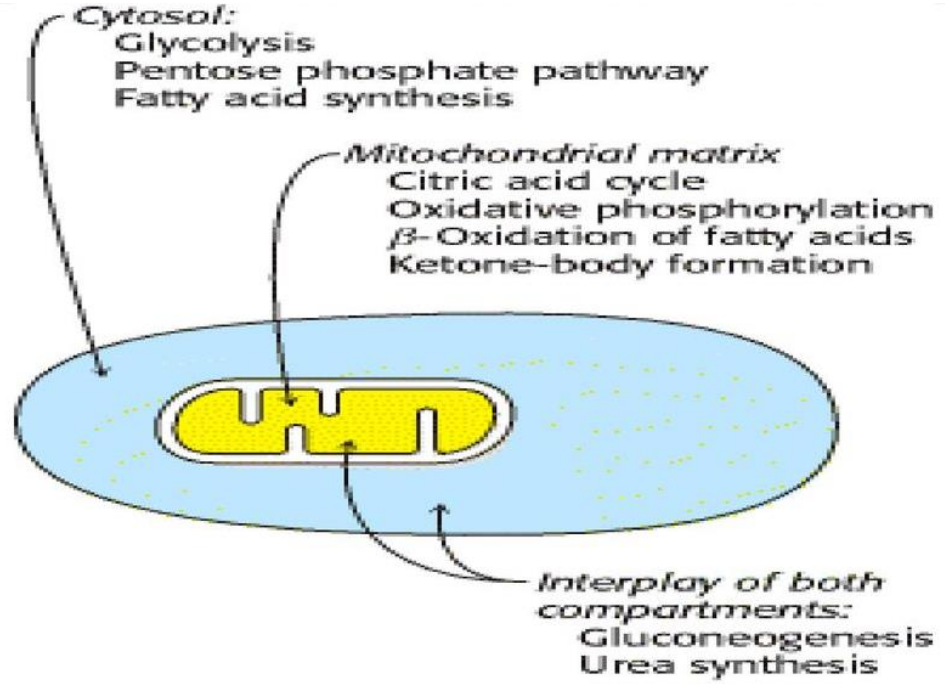
Papin is an enzyme found in papaya, works in both acidic and basic environments.

Compartmentalization Localization

- Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide.
  - Example 1: lysosomal enzymes Function optimally at pH (5.5 – 6)
  - Example 2: fatty acid metabolism
    - Synthesis occurs in cytosol, whereas break-down is mitochondrial.

Don't memorize the figures.





Compartmentalization means placing enzymes, substrates, and metabolic reactions in specific cellular compartments. This helps limit diffusion because the molecules do not need to diffuse through the entire cell to find each other.

When enzymes and their substrates are concentrated within a smaller compartment, the probability of an enzyme encountering and binding to its substrate increases. Therefore, compartmentalization can make metabolic reactions more efficient.

Another important function of compartmentalization is to separate metabolic pathways that should not occur simultaneously in the same location. For example, the cell separates fatty acid synthesis from fatty acid degradation: Fatty acid synthesis occurs mainly in the cytosol, while fatty acid degradation occurs mainly in the mitochondria and peroxisomes.

Cellular compartments can also have different internal conditions, such as different pH values, suitable for the enzymes functioning there.

Enzyme complexing

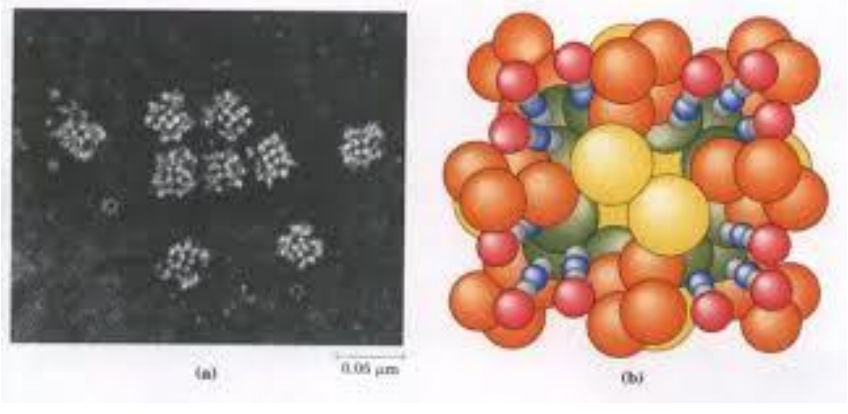
Increases the probability of the enzyme to find the substrate.

- Formation of a complex of multiple enzymes also reduces diffusion.
- Example: Pyruvate dehydrogenase complex (mitochondria) is composed of 3 enzymes (don't memorize): decarboxylation, oxidation, & transfer of the acyl group to CoA.

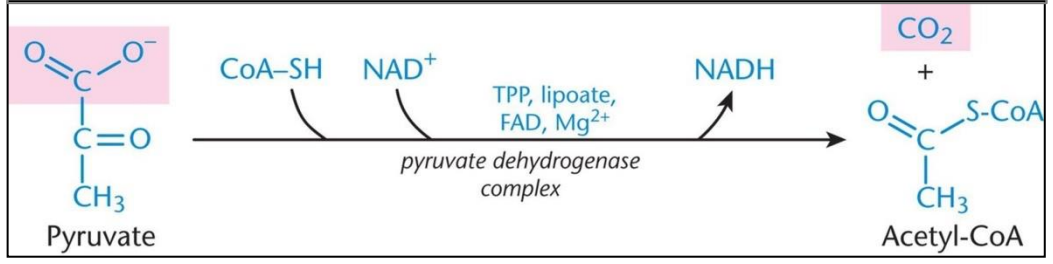
Substrate A $\xrightarrow{\text{Enzyme 1}}$ Substrate B $\xrightarrow{\text{Enzyme 2}}$ Substrate C $\xrightarrow{\text{Enzyme 3}}$ Substrate D

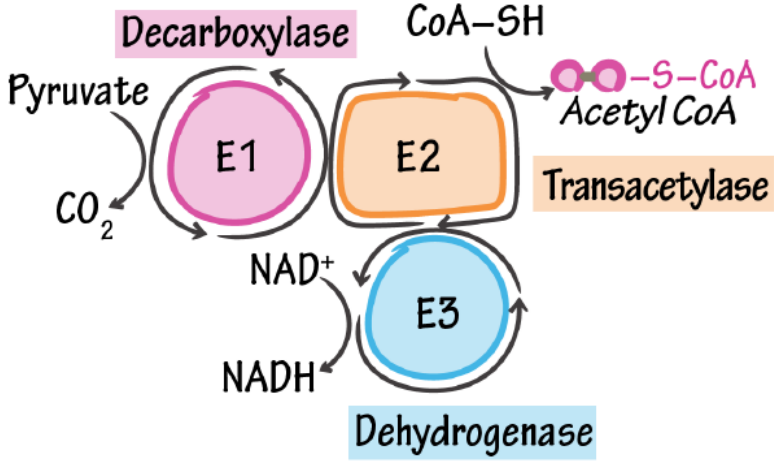
One choice for each enzyme is to bind to its substrate individually, for example: substrate A binds to enzyme 1 then the reaction is catalyzed and substrate B results and diffuses to the surrounding area and keep colliding with substrates and enzymes until it reaches the appropriate enzyme (enzyme 2) then the reaction occurs & substrate C results & it goes like that until the final product is reached, you can see that this operation will take a very long time.

Another choice, which is more efficient is to combine all three enzymes in one cluster, working faster together by passing each product to the following appropriate enzyme



Those three enzymes combined consist of 150 polypeptides , the first and second enzymes have 60 polypeptides (active sites) for each, why the third one has 30 polypeptides (active sites), so they catalyze 120 reactions per unit of time

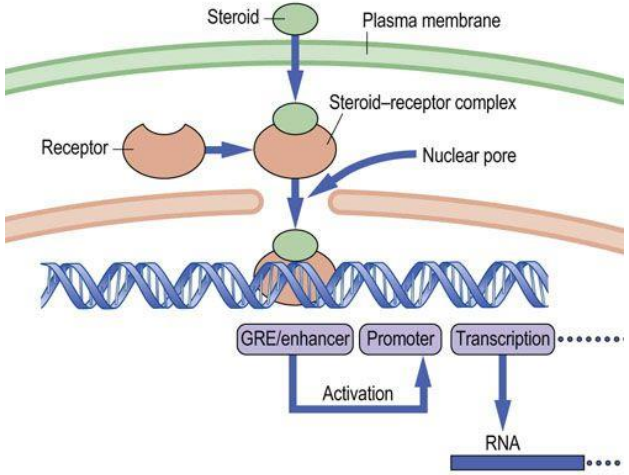




Regulation of enzyme amount Non-specific

- Three mechanisms:
  - Enzyme synthesis at the gene level
  - Enzyme degradation by proteases
  - Synthesis of isozymes

Sometimes cells increases synthesis rate or limit degradation, thus the enzyme will stay longer in the cells.



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

- When you get hungry, the pancreas secretes glucagon hormone to send a message for the liver to start synthesizing enzymes that will break down fatty acids, cholesterol, sugars.
- On the other hand, when you eat, the pancreas will secrete insulin hormone this time that will work on different cell types. Such as in the liver, so it will increase the enzymes that will contribute to fatty acid synthesis, or the ones that will add glucose to form glycogen.

Don't memorize

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

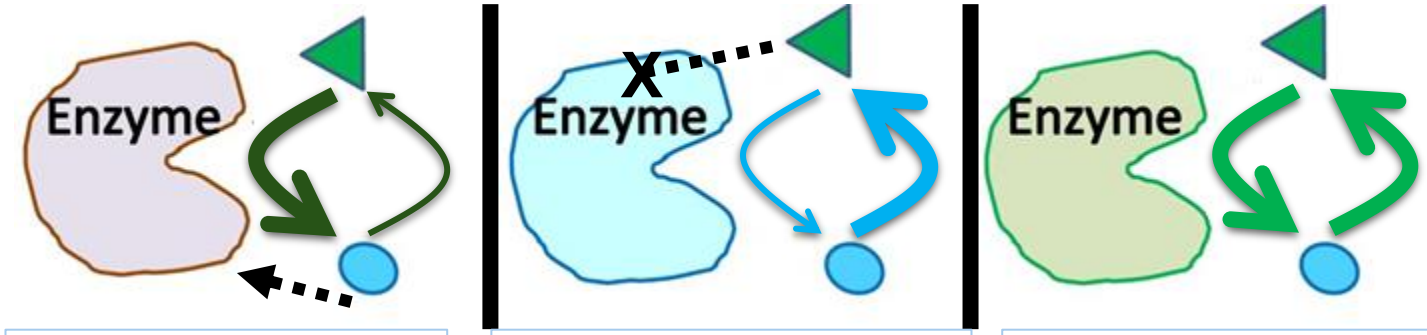
Isoenzymes (isozymes)

Different proteins produced from different genes in different tissues.

Catalyze the same reactions.

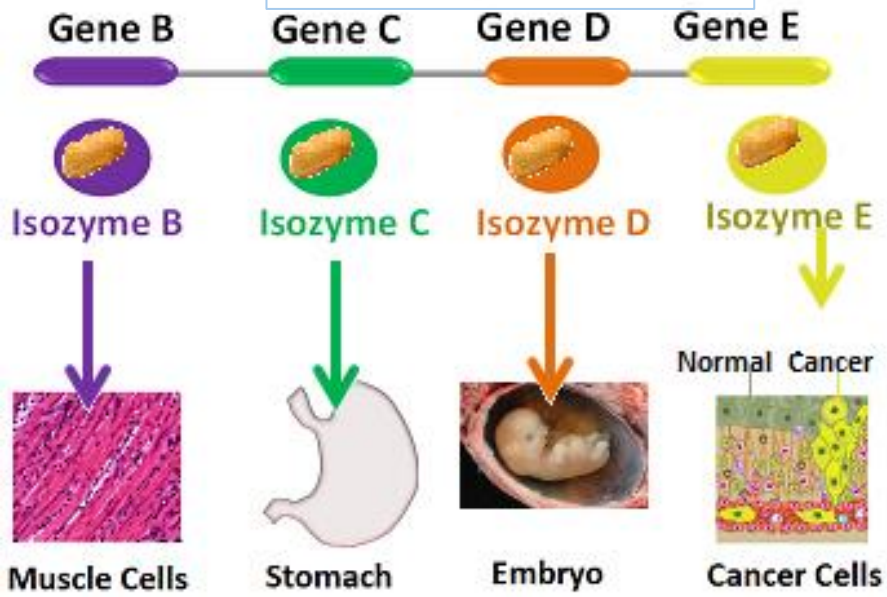
- Isoenzymes are enzymes that can act on the same substrate(s) and produce the same product(s).
- They are generated by different genes that are highly homologous (similar).
- Often, various isozymes are present in different tissues.
- They can be regulated differently.
- They can have different catalytic activities.
  Different kinetics.

The dotted arrow means inhibition (feedback, inhibition), recall biology 101



Tends to make circles. | Tends to make triangles. | No tendency to a specific shape.

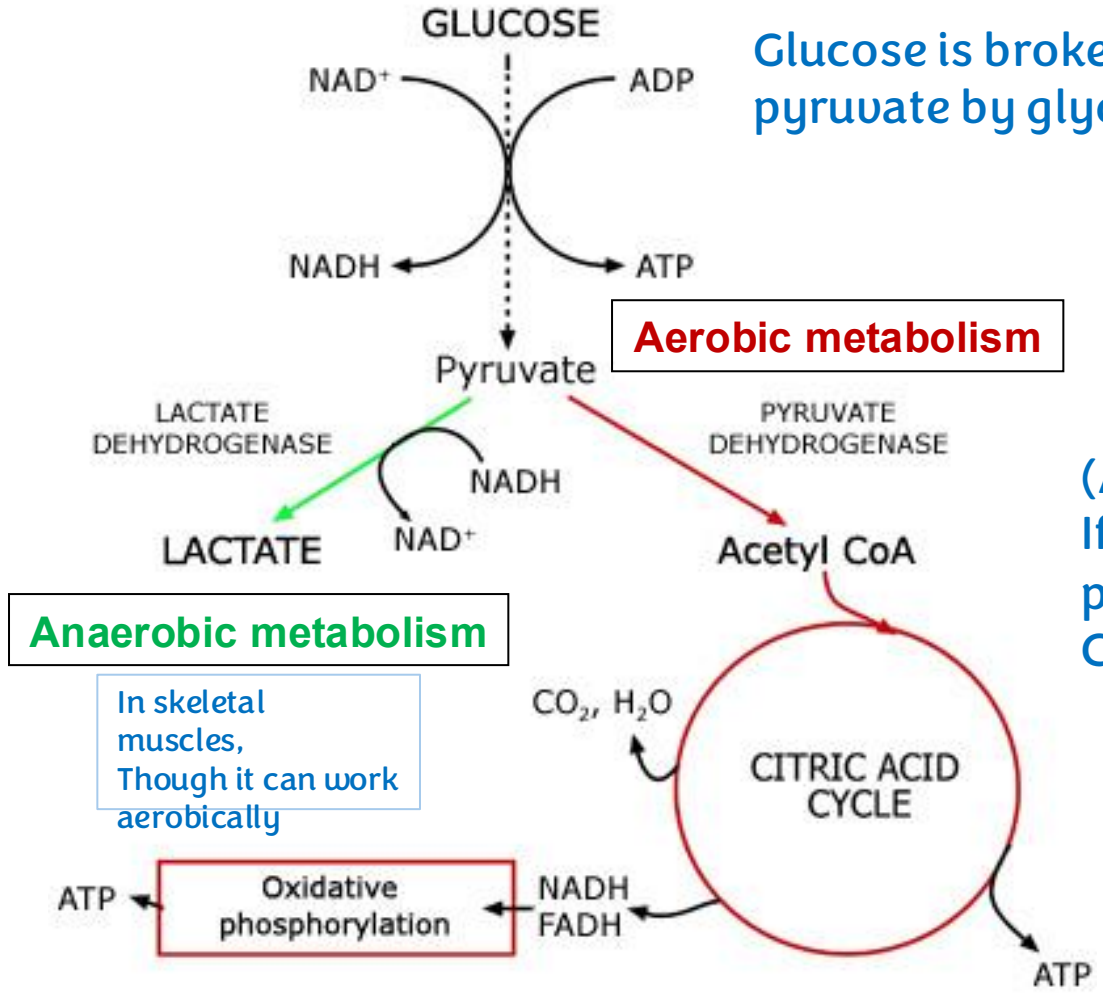
In other words, tissue specific.



Aerobic vs. anaerobic metabolism

Glucose is broken up to pyruvate by glycolysis.

(Anaerobic metabolism)
Lactate flows through the blood circulation to different organs, the heart for example. Where it will be transformed into pyruvate by lactate dehydrogenase, then to acetal co-A , that will continue into Citric acid cycle, because the cardiac muscles use aerobic metabolism.



(Aerobic metabolism)
If oxygen is present, pyruvate will turn into acetyl CoA. Then enter Citric acid cycle.

Lactate dehydrogenases (LDH)

Skeletal muscles turn pyruvate into lactate.
Cardiac muscles turn lactate into pyruvate (aerobic).

4 polypeptide chains

- LDH is a tetrameric enzyme composed of a combination of one or two protein subunits: the H (heart) subunit and M (skeletal muscle) subunit.

H stands for the heart
M stands for the muscles

These 2 polypeptides are produced from 2 genes(H/M).

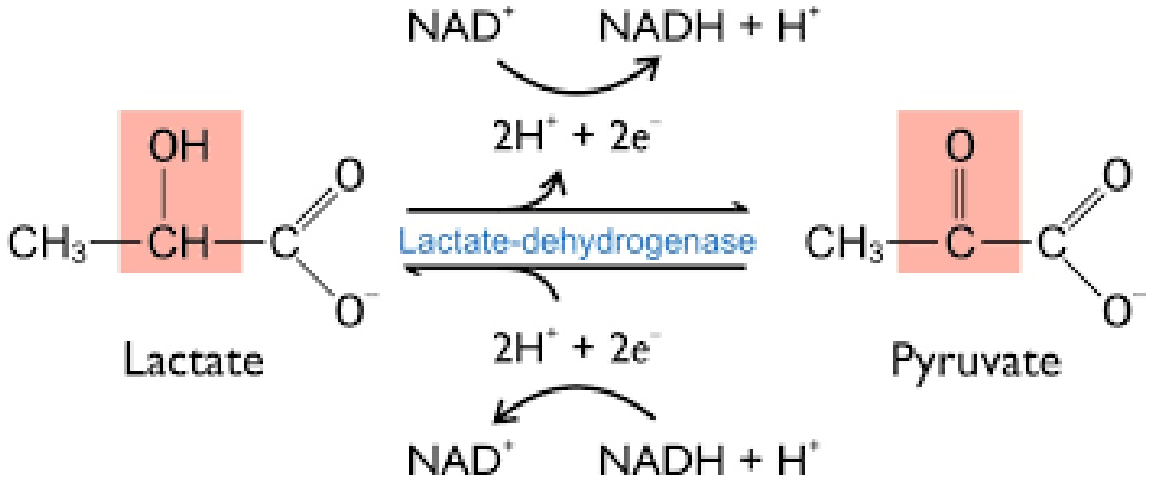
- These subunits combine in various ways leading to 5 distinct isozymes (LDH1, LDH2, LDH3, LDH4, LDH5) with different combinations of the M and H subunits (H4, H3M. H2M2, HM3, and M4).

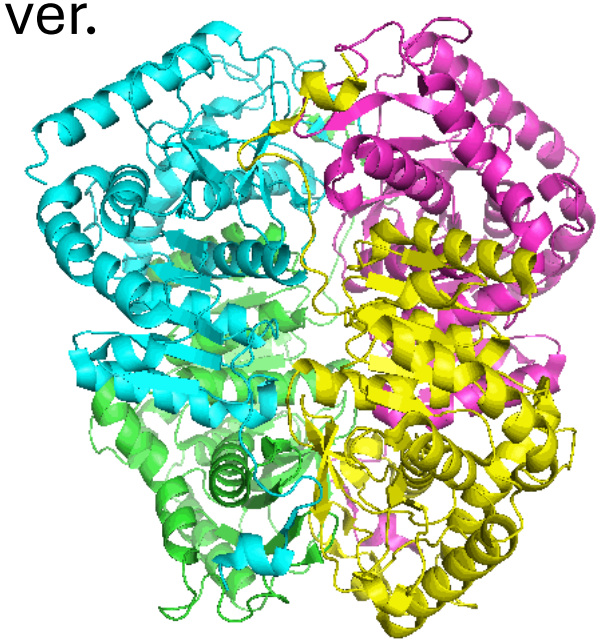
LDH1 : H4
LDH5 : M4

All these combinations are LDH and they all do the same reactions.

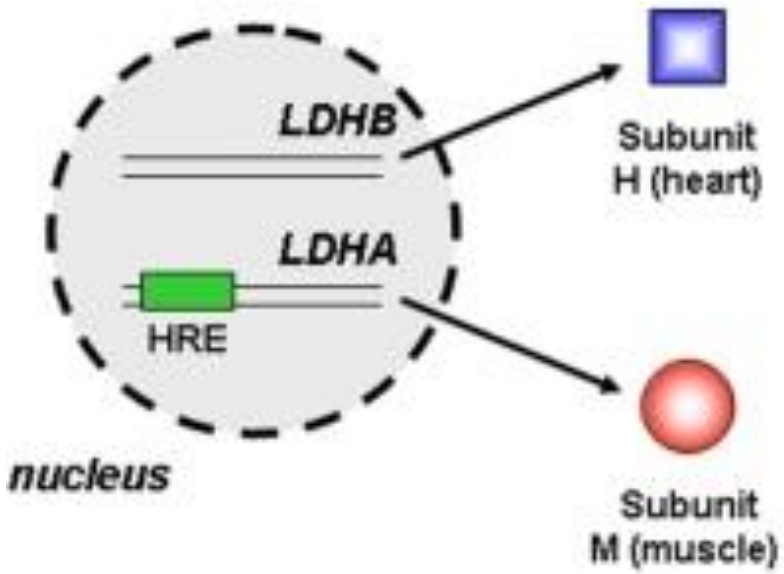
- The H4 isozyme or LDH1 is characteristic of that from heart tissue, and the M4 isozyme of LDH5 is typically found in skeletal muscle and liver.

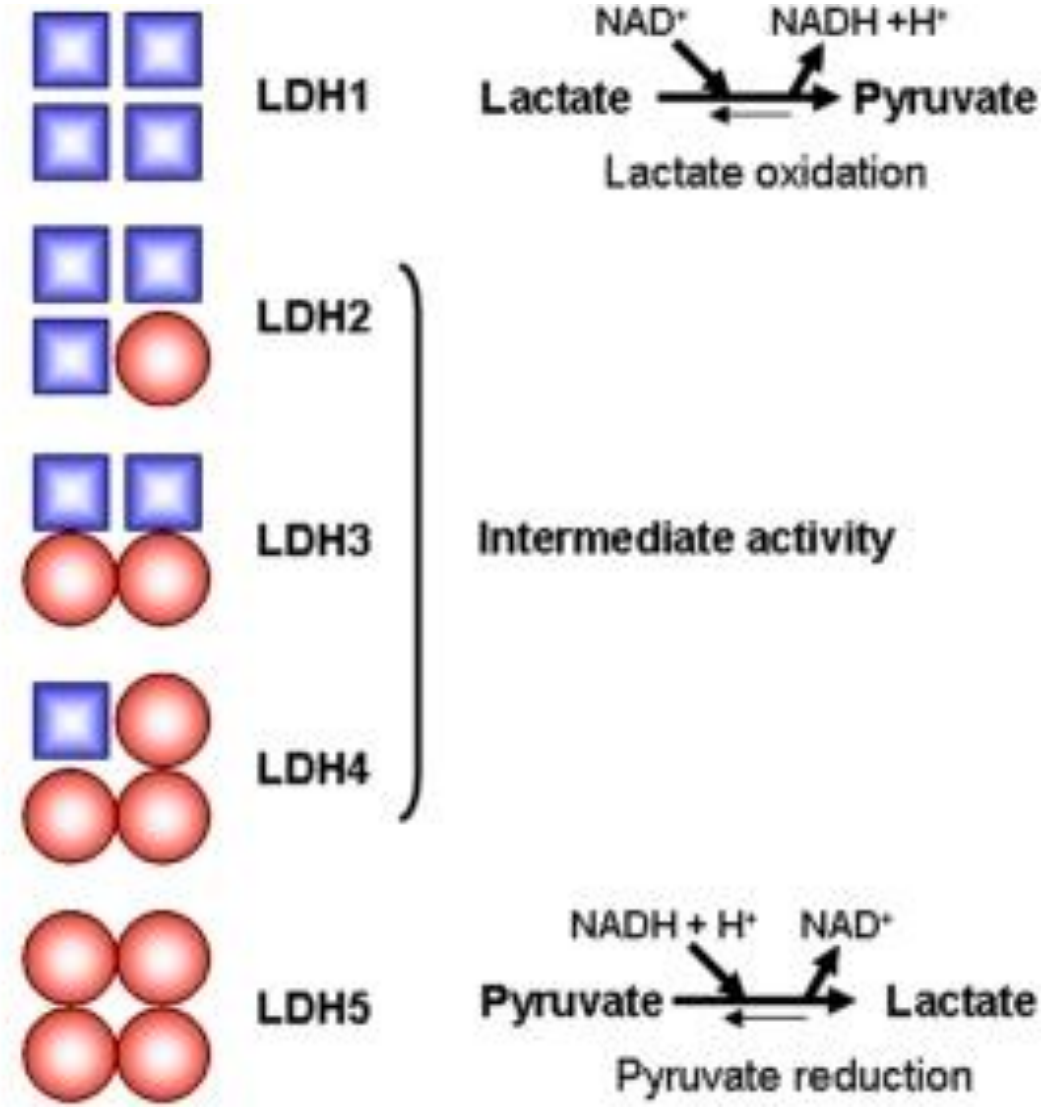
Oxidation of lactate while reduction of NAD+.





The homology (similarity in the amino acids sequence in primary structures/ similarity in genes within the DNA) in H&M is high but there are still some differences that will make regulation and catalysis different between them.

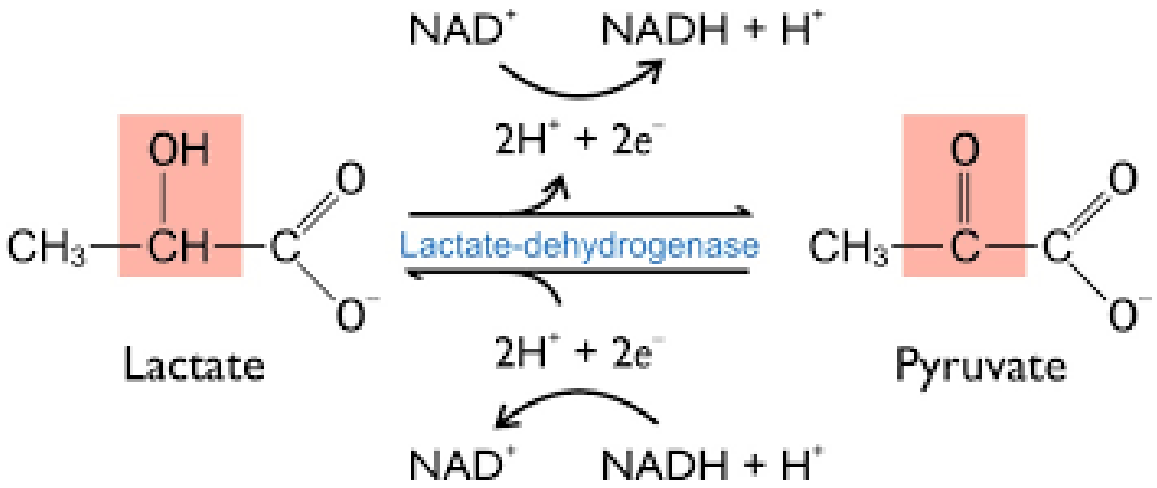




- Although the five isoforms catalyze the same reaction, they differ in their primary structure, kinetic properties, tissue distribution, affinity to the substrate (direction of the reaction), regulation, and isoelectric point.
- The M subunit has a higher affinity towards pyruvate, thus converting pyruvate 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).

H&M can still do the less favored reaction but would prefer to do the more favored one.

Logic behind tissue distribution



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**

The five isoforms are generated in different tissues.

- 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.

Same enzyme different purposes.

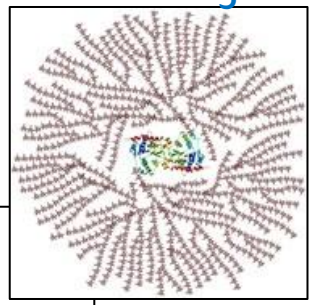
Hexokinase vs glucokinase

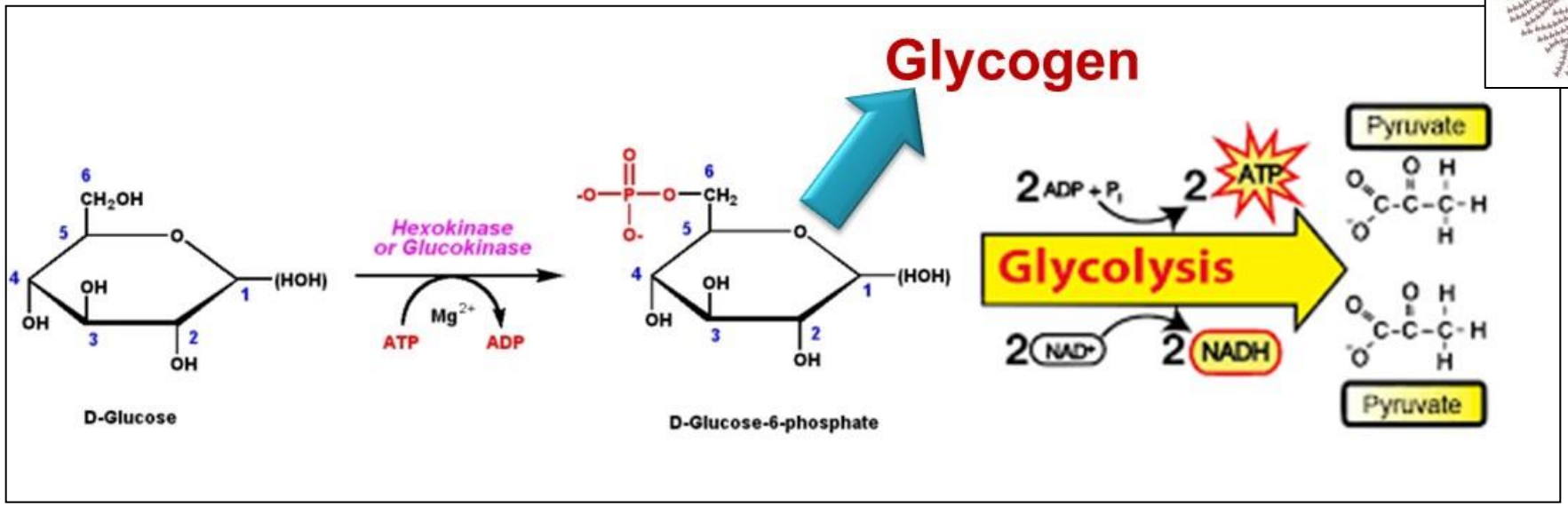
- Hexokinase and glucokinase (hexokinase IV) are allosteric isozymes that catalyze:

Glucose + ATP → Glucose-6-Phosphate + ADP

- Glucokinase is a liver (and pancreatic) enzyme, whereas hexokinase is a skeletal muscle enzyme.
  - The purpose of liver glucose is to store glucose as glycogen (or balance glucose level in the blood). when the body is in starvation mode, the liver breaks glycogen and releases glucose to the body tissues.
  - The purpose of skeletal muscle glucose is to produce energy.

    skeletal muscles phosphorylate glucose to make pyruvate (glycolysis) then to lactate.

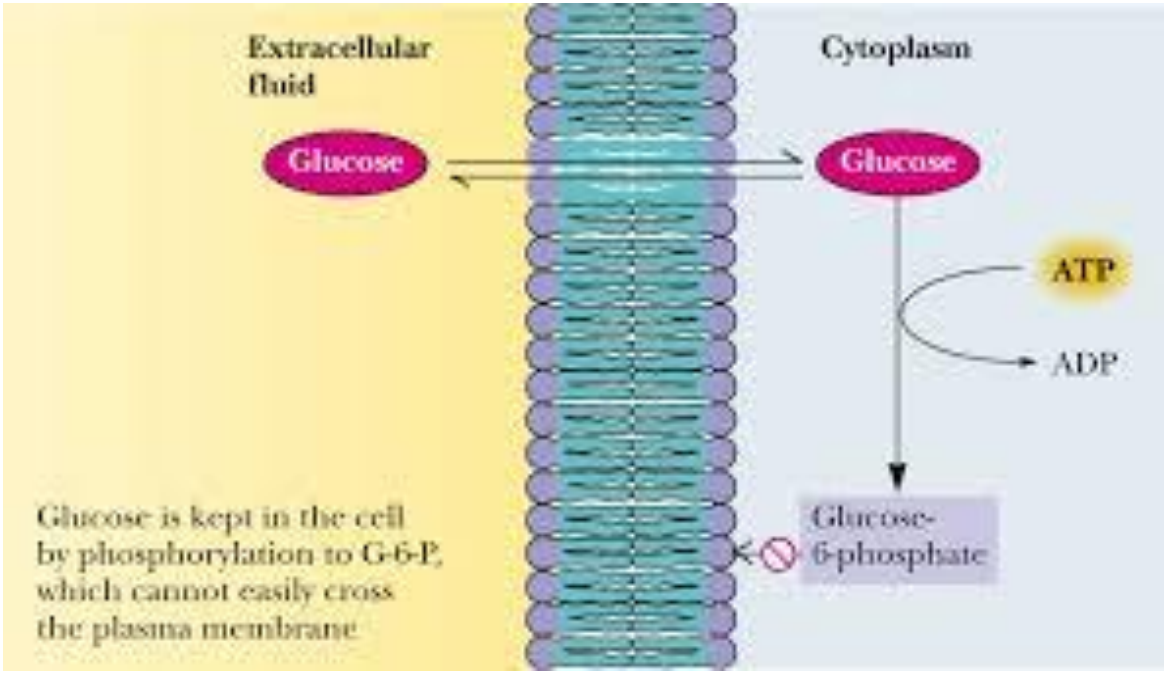




Biological significance

- Note: once glucose is phosphorylated, it cannot cross plasma membrane out of cells.
  - Liver: low efficiency enzyme to not store glucose and keep it to other organs.
  - Skeletal muscles: high efficiency enzyme to trap glucose immediately.

The efficiency of glucose phosphorylation differs between the liver and muscle. In muscle, the efficiency of phosphorylating glucose is high. In the liver, however, the efficiency of glucose phosphorylation is relatively low.



Glucose undergoes phosphorylation, which traps glucose inside the cell because the phosphorylated glucose carries a negative charge and cannot easily cross the cell membrane.

The reason is related to the characteristics of the kinase in the liver. The liver contains an enzyme that phosphorylates glucose, but its efficiency depends on the glucose concentration. When glucose levels are very high, phosphorylation occurs efficiently. However, when the cells need to take up glucose, the phosphorylation of glucose is regulated according to the metabolic state.

isozymes

Regulation of hexokinase and glucokinase

Their genes are different, their tissue distribution is different, their kinetics are different, and their regulation is different, even though they catalyze the same reaction: the phosphorylation of glucose.

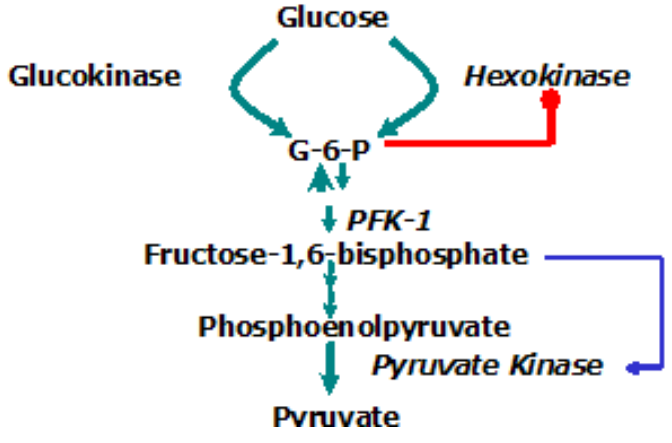
- Note Vmax and K_M values
- Regulation
  - Hexokinase is **inhibited** by glucose-6-phosphate, but glucokinase is not.
- Significance:
  - At fasting state, glucose is not stored.
  - At well-fed state, skeletal muscles do not consume all glucose in blood and liver can convert excess glucose to glycogen for storage.

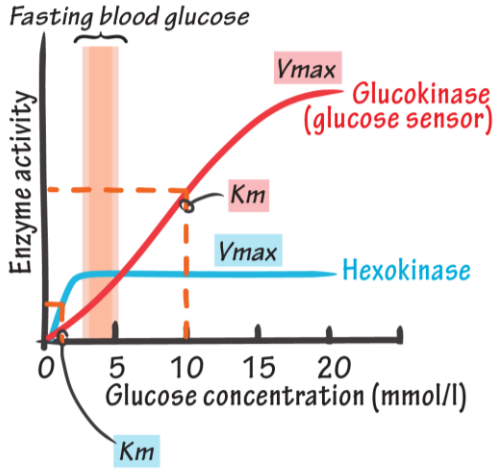
In the fasting blood glucose the amount of glucose present in the blood is relatively low. So the hexokinase works efficiently more than the glucokinase.

When glucose levels are very high, both hexokinase and glucokinase can work, but hexokinase has a much lower Vmax than glucokinase.

Hexokinase could get inhibited at some point by glucose-6-phosphate, while glucokinase can't be inhibited by it, the more the merrier of glucose in fact

The regulation and genes are different.





Km
Glucokinase > Hexokinase
Spares glucose stores for brain, muscle & other tissues.

Vmax
Glucokinase > Hexokinase
At fasting glc conc. – Hexokinase is at Vmax, Glucokinase activity varies according to glc conc.

In skeletal muscle, after glucose enters the cell, hexokinase phosphorylates glucose. When glucose-6-phosphate becomes sufficiently high, it causes inhibition of hexokinase.
So, the skeletal muscles didn't consume all the glucose by phosphorylation and keep amount to the brain

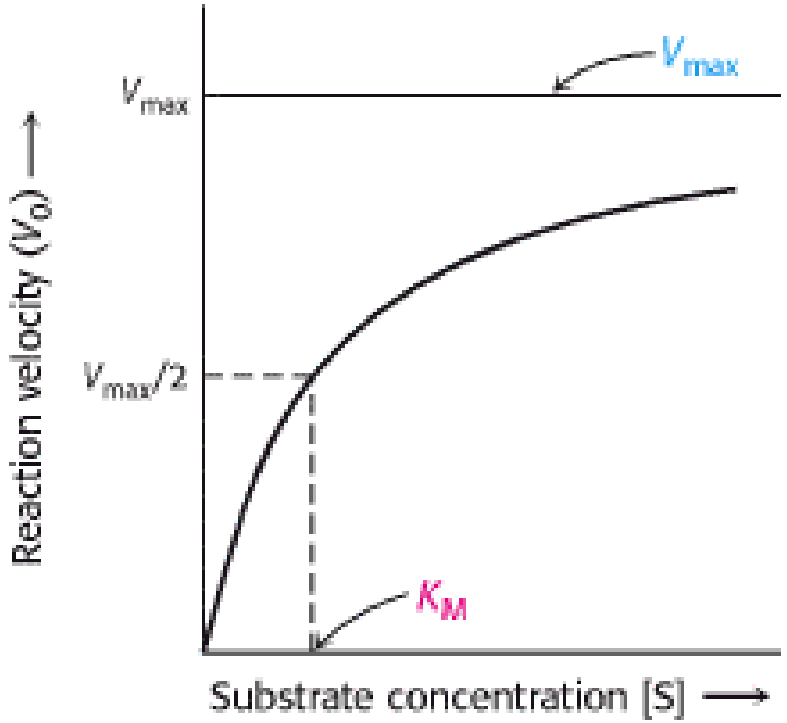
Regulation of enzymatic activity

But before, let's talk about this

A disadvantage of the Michaelis-Menten equation

- Determination of K_M from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach Vmax.
- This prevents the calculation of both Vmax and K_M.

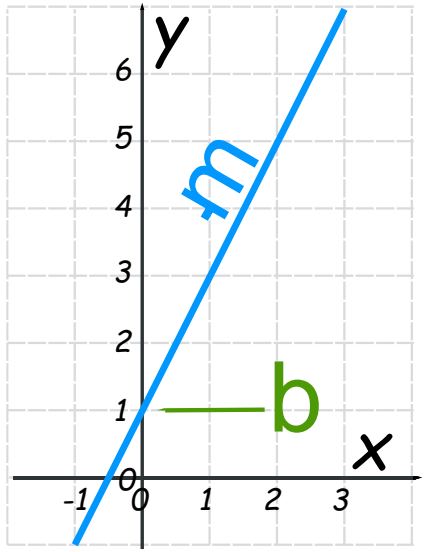
In reality, we do not reach V_{max}. Because reaching Vmax requires a high concentration of substrate. If we didn't reach V_{max}, we can't know the half of the V_{max} and K_M



$y = mx + b$	m is the slope b is the y-intercept

Solution

Lineweaver and Burk try to solve the problem by converting to linear



The Lineweaver-Burk or **double-reciprocal plot**

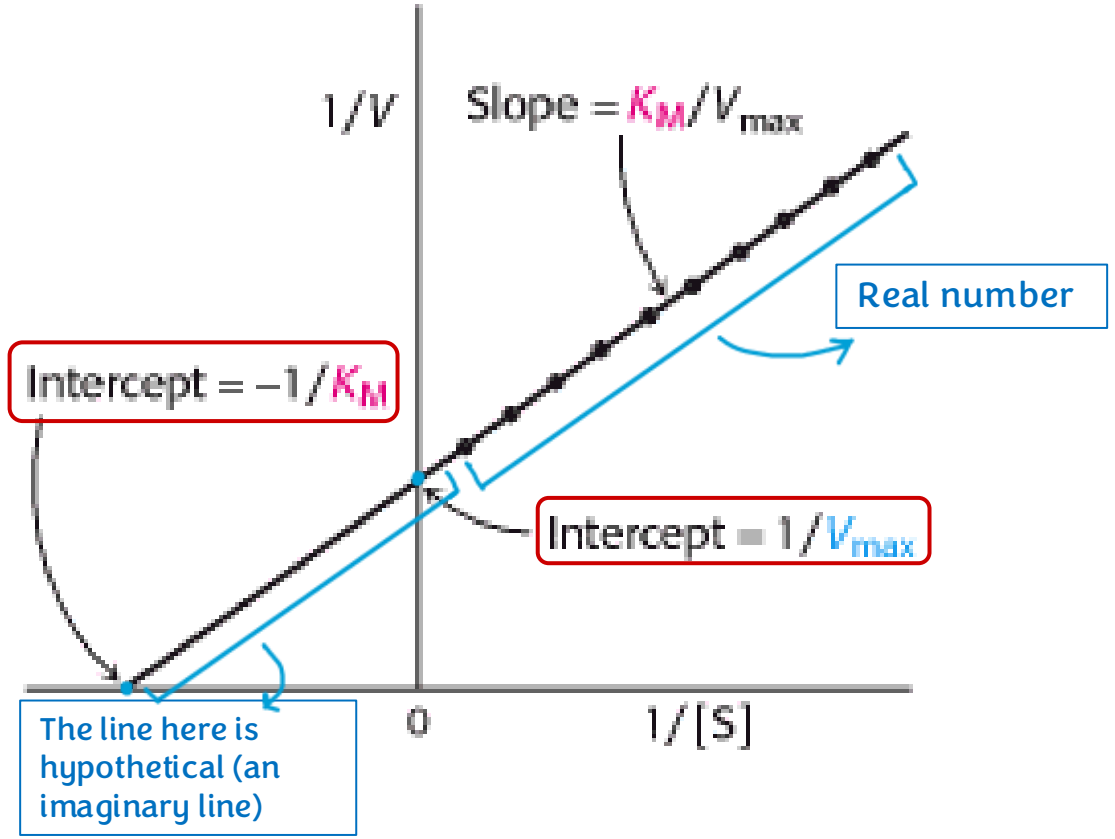
- A plot of $1/V_o$ versus $1/[S]$, called a Lineweaver-Burk or double-reciprocal plot, yields a straight line **with an intercept of $1/V_{max}$ and a slope of K_M/V_{max}.**
- The intercept on the x-axis is $-1/K_M$.

$$V_0 = V_{max}\frac{[S]}{[S] + K_M}$$

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\cdot\frac{1}{[S]}$$

$$y = b + mx$$

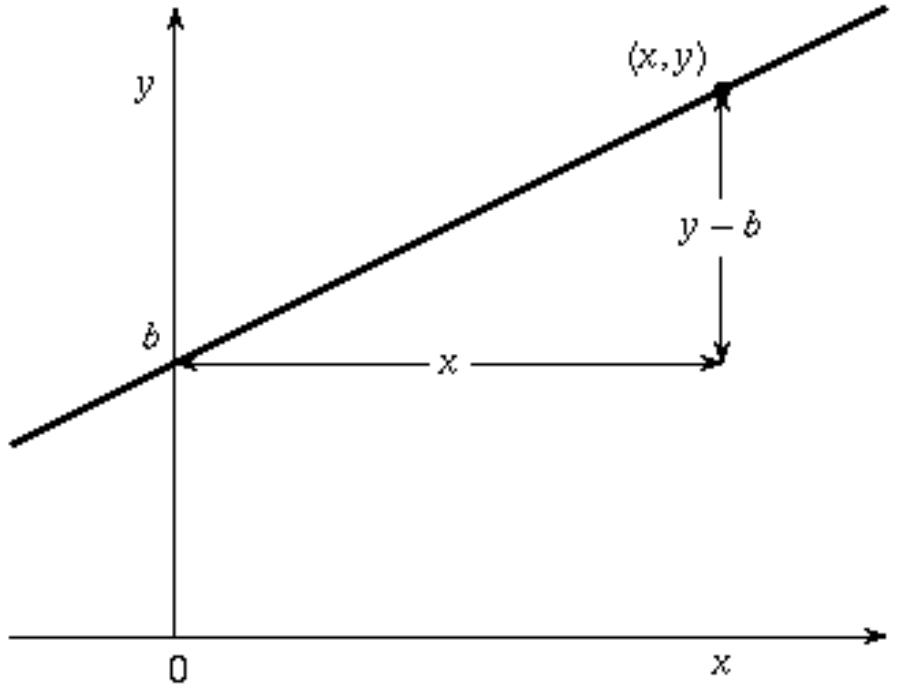
This helps in calculation the V_{max} and K_M



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

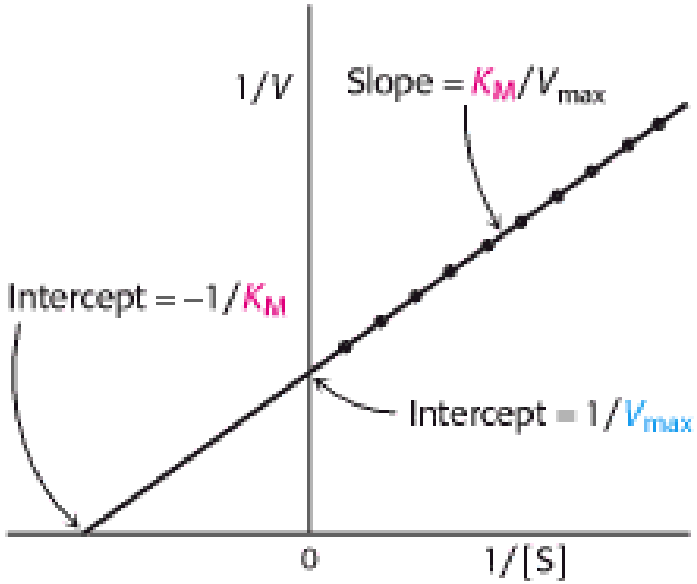
- y is y-axis = 1/ V_o
- x is x-axis = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

- If x = 0, then y = b (x-axis is 0, then y-intercept = 1/Vmax)



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

If y = 0, then mx = -b (y-axis is 0, then x-intercept = $-1/K_M$)

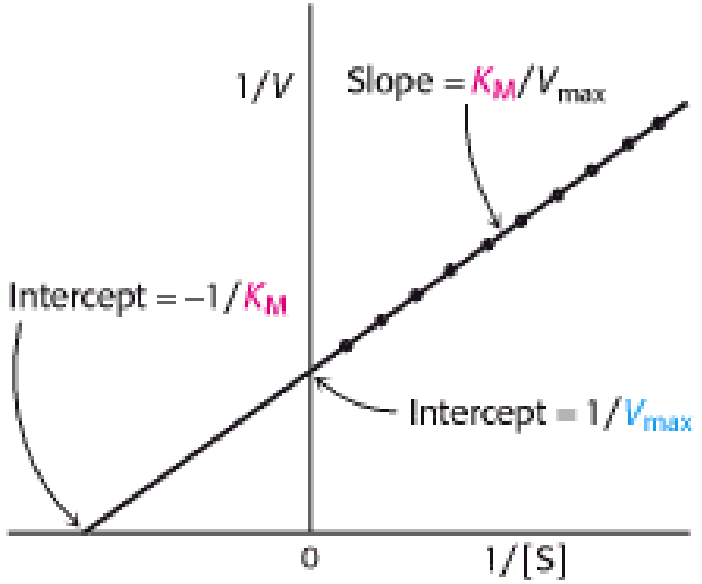
How?

0 = 1/Vmax + (K_M/Vax) . (1/[S])

-1/Vmax = (K_M/Vax) . (1/[S])

-1 = K_M . (1/[S])

-1/ K_M= 1/[S]



Test yourself

Quiz

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي:

»ذكّرته الظروف الصعبة بالأذكار،
فحفظها وواظب عليها ..
زالت الظروف لكن لسانه ظلّ رطبًا«

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			