Doctor 022

Biochemistry

Sheet no.

Kinetics + Regulation

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Kinetics Part:

Rate of reaction (velocity)

Rate of reaction is calculated as concentration of substrate disappearing (or concentration of product appearing) per unit time (mol L–1 . sec–1 or M . sec–1).

Rate of reaction=Molarity(M)/Time(T)

Concentration (M) = mol / vol

Enzyme activity

In order to measure an enzyme's activity, we measure the number of moles of substrate disappearing (or products appearing) per unit time (mol . sec–1)

Side note: in biomedical experiments, they add a substrate to a specific enzyme then they calculate the products by a fluorescence or a colour that comes out.

****Eliminate volume**

In other words,

enzyme activity (mol.sec-1)= rate of reaction × reaction volume

Specific activity

Specific activity means the moles of products produced or the moles of substrates converted per unit of time per unit of mass, the sample should be isolated because sometimes samples are contaminated (impure), and the amount of proteins (enzymes) isolated must precisely reflect the enzyme.But, if it was contaminated, the mass of the enzyme would be less than the

sample's mass due to the presence of other substances that increase the mass.

Specific activity is usually a measure of enzyme purity and quality in a sample.

It is described as moles of substrate converted *per unit time per unit mass of enzyme* (mol . sec−1 . g-1).

*Eliminate weight of enzyme

Specific activity =enzyme activity / mass of enzyme (grams)

This is useful in determining enzyme purity after purification.

It is also used when the molar enzyme concentration is not known.

(If the moles of enzyme present is unknown, it is impossible to calculate kcat).

Turnover number

Turnover number (kcat) is related to the specific activity of the enzyme where it is

Turnover number = specific activity × molecular weight of enzyme

It is expressed as moles of substrate converted per unit time (usually per

second)/*moles of enzyme* (min-1 or sec-1)

Specific Activity was per mass of an enzyme

Remember: Kcat= V max/ [E]t

Sample calculations:

A solution contains initially **25.0 × 10^{-4} mol L^{-1}** of peptide substrate and **1.50 µg chymotrypsin**, in **2.5 mL** volume. **After 10 minutes, 18.6 × 10^{-4} mol L^{-1}** of peptide substrate remain. Molar mass of chymotrypsin is **25,000 g mol^{-1}**.

peptide substrate consumed	= 6.4 x 10^{-4} mol L^{-1} in 10 minutes
Rate of reaction $= \frac{M}{T}$	**= 6.4 x 10^{-5} mol L^{-1} min^{-1}**
Enzyme activity (rate × volume)	= 6.4 x 10^{-5} mol L^{-1} min^{-1} x 2.5 x 10^{-3} L **= 1.6 x 10^{-7} mol min^{-1}**
Specific activity (activity / mass)	= 1.6 x 10^{-7} mol min^{-1} / 1.50 µg **= 1.1 x 10^{-7} mol $µg^{-1}$ min^{-1}**
Turnover number (sp. act. × molar mass)	= 1.1 x 10^{-7} mol $µg^{-1}$ min^{-1} x 25,000 g mol^{-1} x 10^{6} µg g^{-1} = 2.7 x 10^{3} min^{-1} = **45 s^{-1}**

A disadvantage of the Michaelis-Menten equation:

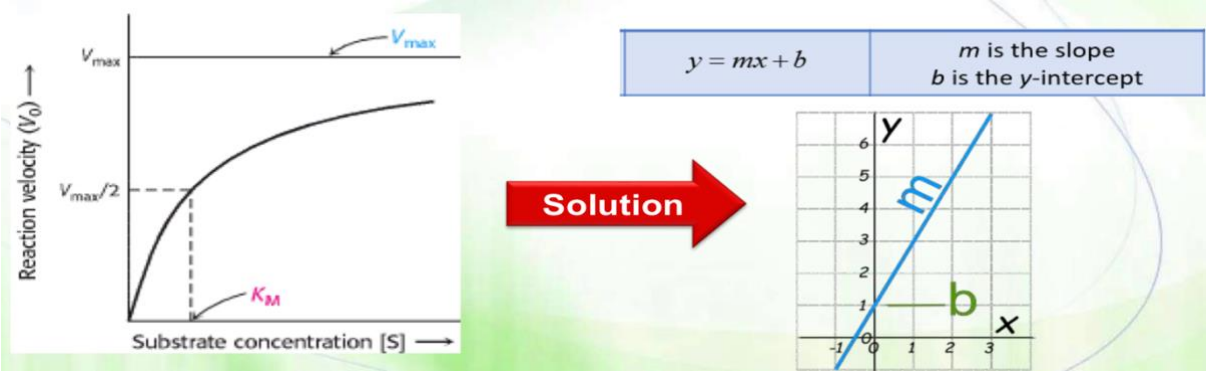
Determination of Km from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach Vmax.

This prevents the calculation of both Vmax and KM

Realistically,we can't reach Vmax, thus we can't calculate Km.
-Remember, Km=Vmax/2

-To reach Vmax ,we need a HUGE amount of substrate.
A similar analogy, Imagine that you dissolve 1 kg of sugar in a cup of tea. It is impossible.

To solve this proplem, the Michaelis-Menten equation was converted into a linear equation.



The Y intercept (المقطع الصادي) is equal to *1/Vmax , But it's hypothetical since we can't have a value for 1/Vmax when the subtrate concentration is equal to Zero.*

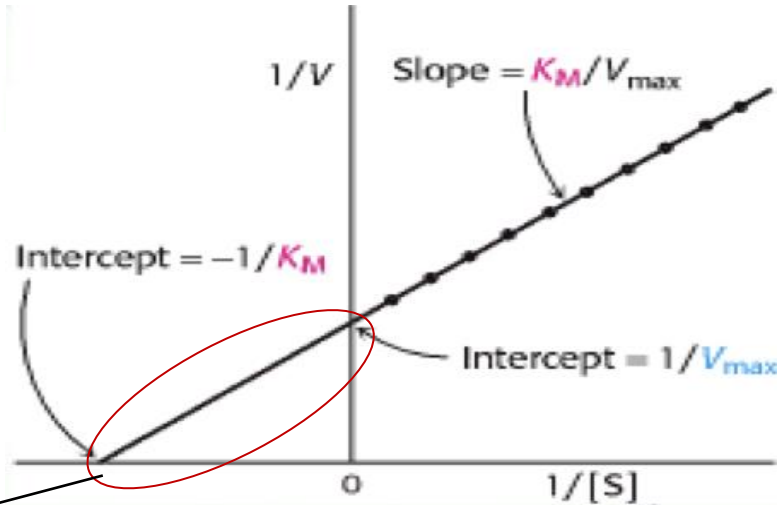
While the X intercept (المقطع السيني) is equal to *-1/Km. That's hypothetical too because we can't have a negative value for the substrate concentration.*

The Lineweaver-Burk or double-reciprocal plot

How to obtain this plot? We do an experiment, at certain concentrations of substrates, we start to calculate what the rate is at a specific [S]. We draw that liner plot from the values obtained.Since it os a linear function, 3 constants determine it, 1. The slope (الميل) 2. The X-intercept. 3. The Y-intercept

- **A plot of 1/ V. versus 1/[S], called a Lineweaver-Burk or double-reciprocal plot, yields a straight line with an intercept of 1/Vmax and a slope of Km /Vmax.**
- **The intercept on the x-axis is -1/ Km.**

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

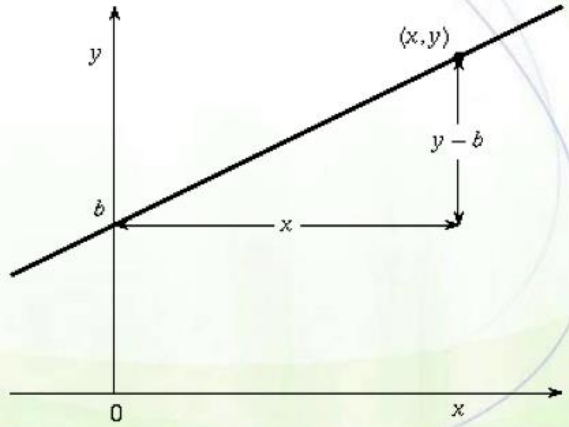


This line is hypothetical

When the line intercepts with the X-axis, this means that [1/V] is equal to Zero, and [1/S] equals [-1/Km]

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



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

If y = 0, then mx = -b (y-axis is 0, then x-intercept = -1/Km). How??
0 = 1/Vmax + (Km/Vax) . (1/[S])

- **-1/Vmax = (Km/Vax) . (1/[S])**
- **-1 = Km• (1/[S])**
- **-1/Km= 1/[S]**

For example, if the X intercept was -10 , this means that (-1/Km) = -10. Then Km would equal 0.1.

"As simple as that"

Enzymes Regulation (inhibition)

We talked about 3 types of inhibitors that affect enzymes that follow the *Michaelis-Menten plot* (hyperbolic) which are 1. Competitive, 2.non-competitive 3. Uncompetitive.

Enzyme inhibition can be either reversible or irreversible.
Reversible inhibitors rapidly dissociate from enzymes (e.g. non-covalent binding).

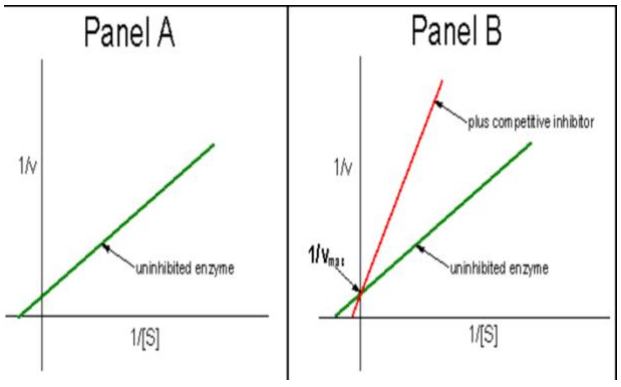
- Competitive, noncompetitive, or uncompetitive inhibition.
  An irreversible inhibitor is tightly bound (e.g. covalently) to the enzyme.
- Lower concentration of active enzyme.

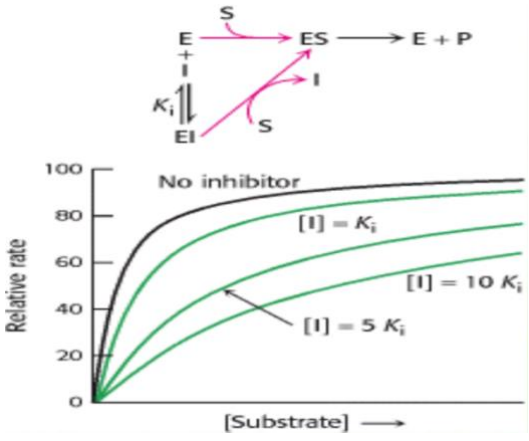
Let's Consider the differences between each type of inhibitors:

Competitive

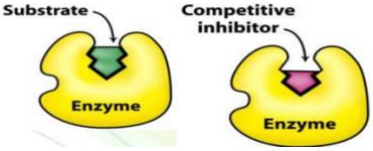
Competitive inhibitors compete with the substrate for the active site.

- Increasing substrate can overcome inhibition.
- Same Vmax, but higher Km (higher concentration of substrates is needed to reach half of the Vmax)





The Y- interception is the same as Vmax is unchanged. While the X- interception is changed (Km has increased) thus (1/Km) has decreased but (-1/Km) has increased.

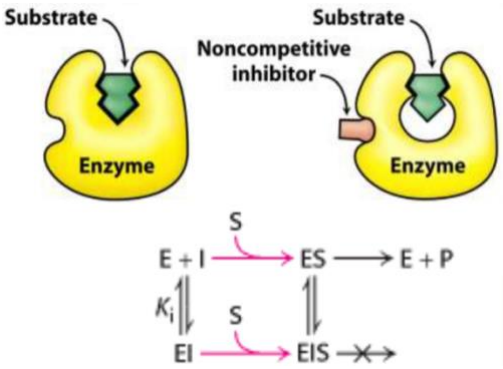


Non-competitive inhibitors

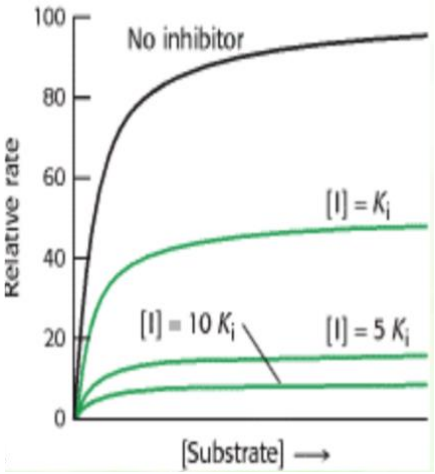
Affect the activity but doesn't affect the binding affinity between the substrate and the enzyme.

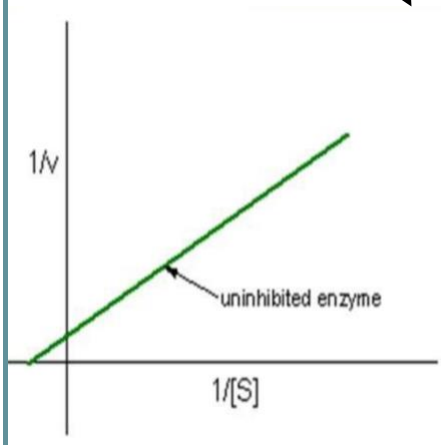
Noncompetitive inhibitors bind E or ES complex at a site other than the catalytic site. Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product.

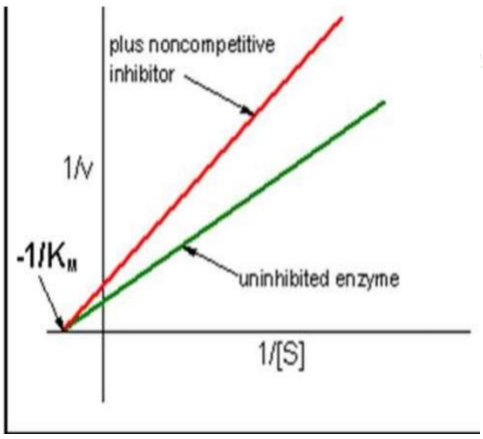
- Lower Vmax, but same Km



The X-interception is unchanged, but the Y-interception has changed (Vmax is lower) thus (1/Vmax is higher) so the plot has shifted

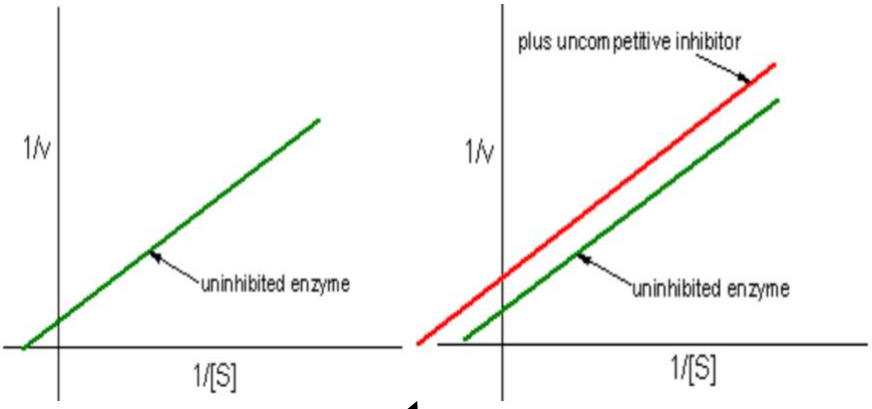


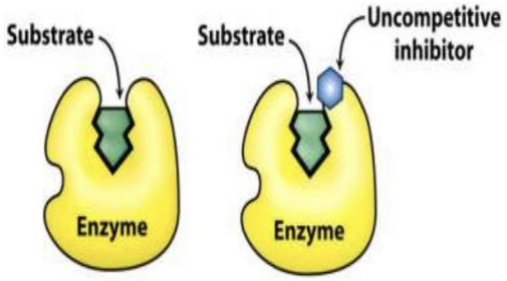




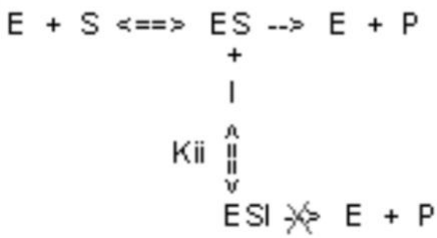
Un-competitive inhibitors

Uncompetitive inhibitors bind to the enzyme-substrate complex only reducing both Vmax and Km. These inhibitors bind with the active site only after when the substrate bounds, so the reaction is ended.

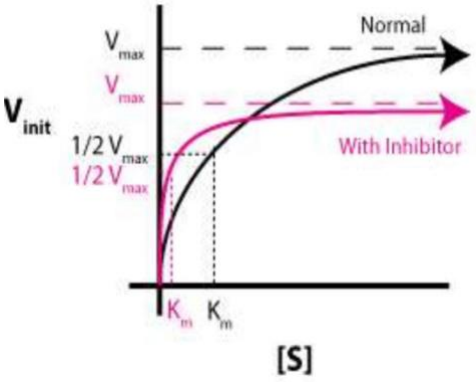




UNCOMPETITIVE



The affinity has increased, Km is decreased thus -1/Km is lower. Vmax has also decreased, the enzyme is inhibited so its activity has reduced, thus 1/Vmax has increased



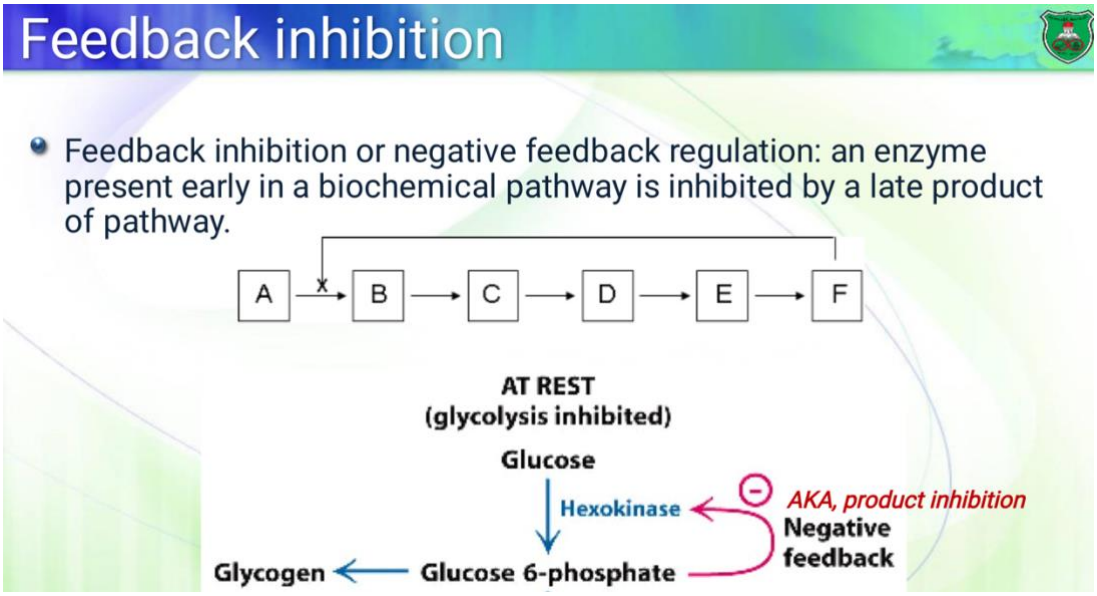
The doctor said: 'I can be very creative as you have noticed, so try to exercise and calculate different variables among different plots'

Modes of metabolic regulation:

- ## Feedback inhibition

In feedback inhibition regulation, the end product of a metabolic pathway acts as a "feedback signal" and binds to an enzyme involved in an earlier step of the pathway. This binding inhibits the activity of the enzyme, reducing the production of the end product. It helps regulate and balance the levels of substances in the body. The end product tells the enzyme: "stop, there is an enough amount of me, don't waste more".

Ex: In the glycolytic pathway, Glucose 6-phosphate , the final product, can bind to an early enzyme which is Hexokinase in the pathway. When Glucose 6-phosphate binds to the Hexokinase , it inhibits its activity, preventing further synthesis of Glucose 6-phosphate. This helps regulate the glycolytic pathway



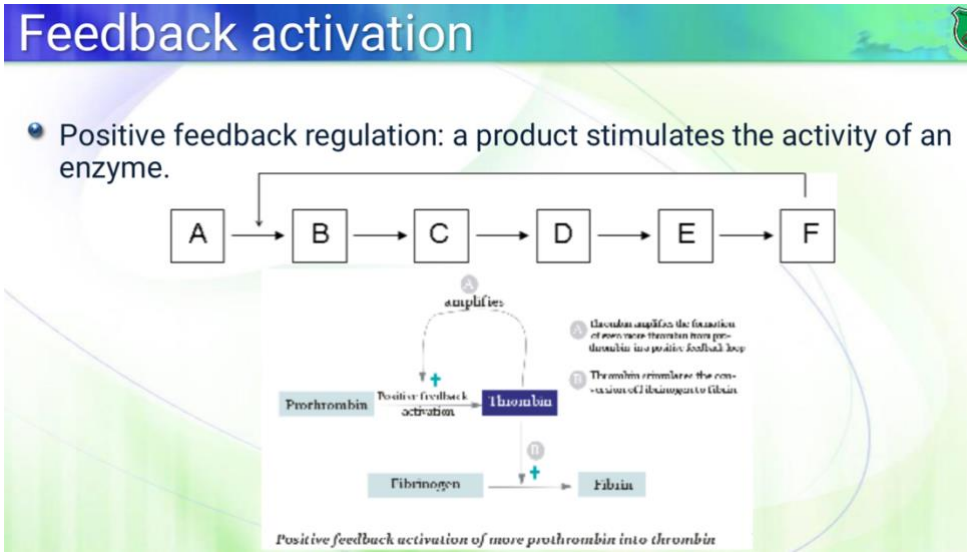
- ## Feedback activation

When an intermediate or a product of a certain metabolic pathway activates an earlier step in the pathway to enhance its own production. This mechanism helps increase the efficiency and speed of the pathway. They say to the enzyme "make more of me, be more active"

Ex:

1) *Blood clotting: When products are produced, it signals the enzyme that there is an injury and bleeding. The enzyme then makes more clots to close the wound.*

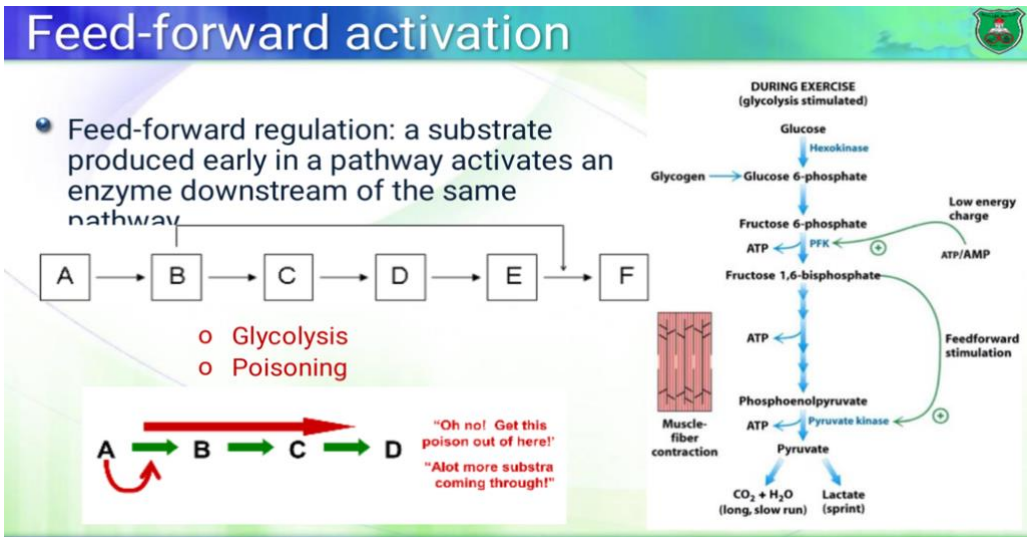
2) Poison: When poison is detected. This can lead to the production of more specific enzymes or molecules that help neutralize or eliminate



the poison from our bodies

- ## Feed-forward activation

In feed-forward regulation, it involves the activation of an enzyme downstream in a metabolic pathway by a substrate that is produced earlier in the same pathway. This activation occurs in order to enhance and optimize the overall efficiency and speed of the pathway. It's like a signal that helps the pathway operate more effectively and efficiently.



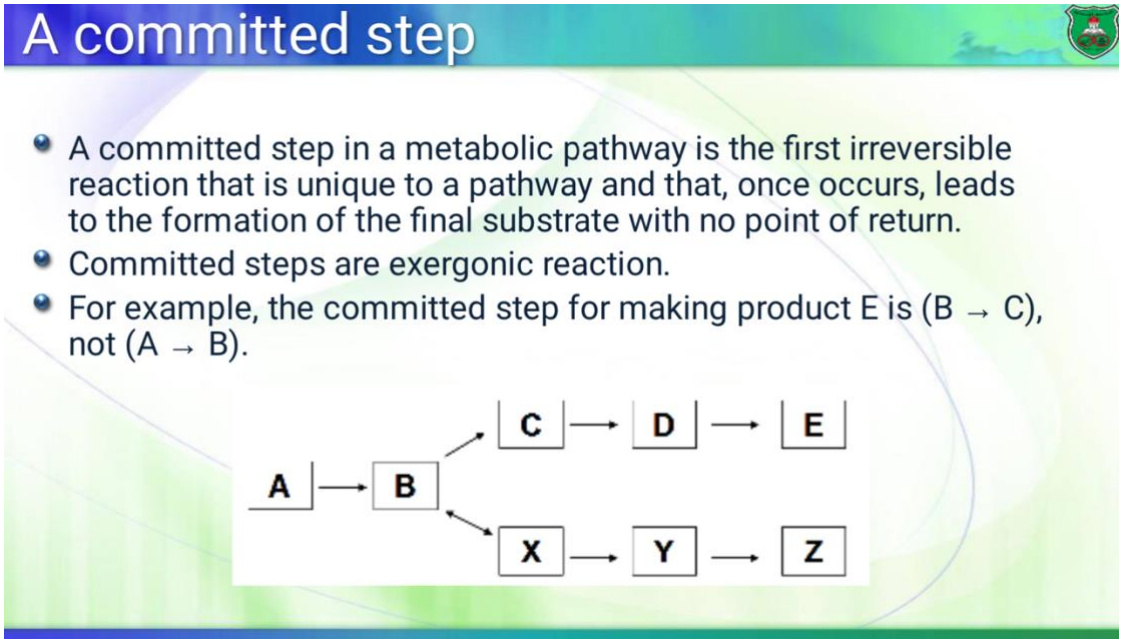
Ex: Fructose 1,6-bisphosphate activates the final enzyme in the pyruvate manufacturing pathway. When the level of Fructose 1,6-bisphosphate increases, it indicates having low energy levels in the cell (insufficient ATP). This triggers an accelerated reaction and increased ATP production.

In the previous example, why does the quantity of Fructose 1,6-bisphosphate control the reaction? Why not the glucose 6-phosphate?

Because it is classified as a committed step.

A committed step:

The committed step refers to a specific step in a metabolic pathway that is considered irreversible and highly regulated. It is often the first unique step in the pathway that commits a molecule to a particular pathway, and subsequent steps are dependent on it. In the context of the previous example, the activation of the final enzyme by Fructose 1,6-bisphosphate can be considered a committed step because it determines the direction and rate of the reaction.

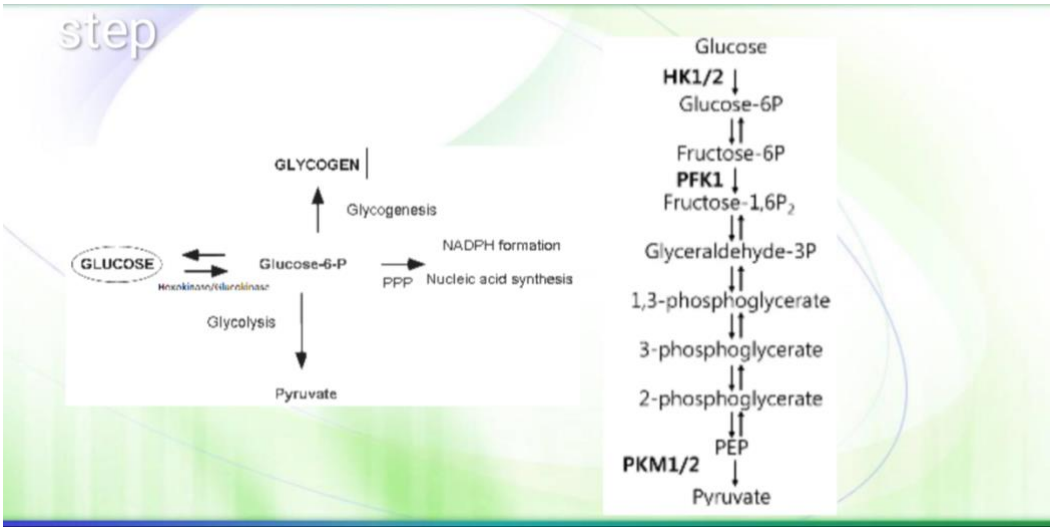


In the previous example, What is the committed step in converting?

A⟶E: the committed step is B⟶C

A⟶Z: the committed step is X⟶Y (Notice that it is not B → X because this step is reversible.)

- **PFK, not HK/GK, is the committed step**



In this reaction, why do we consider Fructose 1,6-bisphosphate as the committed step and not Glucose 6-phosphate, even though Glucose 6-phosphate undergoes an irreversible reaction? The reason is that the cell can utilize Glucose 6-phosphate for various pathways other than just converting it to pyruvate. (As A → B in the previous slide, it can be converted to C or X. For this reason, it cannot be considered as the committed step.) So, once Fructose 1,6 bisphosphate is produced, the cell is signaled that there is a need of ATP.

- *Rate-limiting reactions*

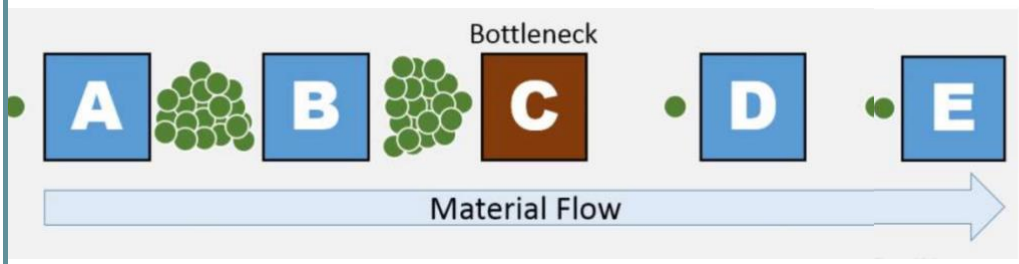
The rate-limiting reactions are the slowest steps in a chemical reaction that determine the overall rate of the reaction.

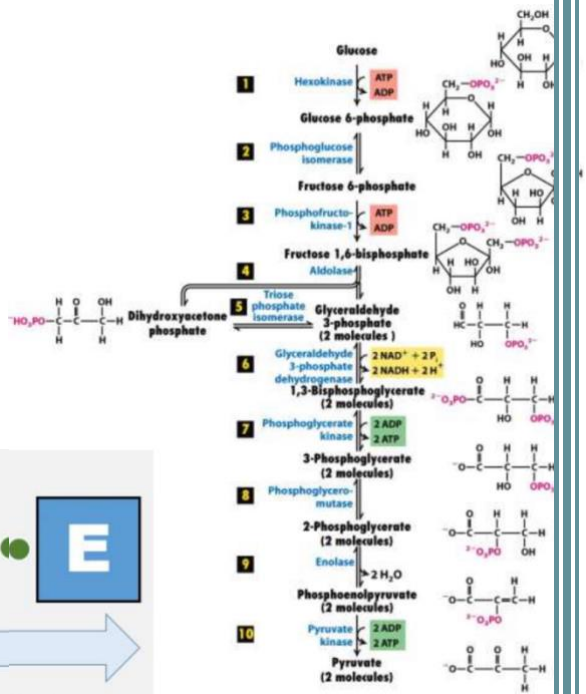
Rate-limiting reactions slow down rate of reactions because:

- *requirement for high amount of energy*, **because it often involves the formation of high-energy intermediates or the overcoming of a high activation energy barrier. This energy requirement slows**

down the reaction and makes it the limiting factor in determining the overall rate.

- *strict regulation of enzymes*, because some enzymes are highly regulated and have a significant influence on controlling the speed of the reaction. They can either speed up or slow down the rate-limiting step, which ultimately affects the overall reaction rate.
- *high Km values of enzyme towards its substrate*, Enzymes with high Km values have low affinity for their substrate, meaning there is less attraction between them so they have a harder time binding to their substrate, which ultimately slows down the reaction.
- *These reactions are also usually, but not necessarily, committed steps.*





Enzymes in Disease Diagnoses:

Enzymes are used in many biomedical examinations that test for certain diseases. They are specifically used as biomarkers; which are indicators for biological condition/status. How?

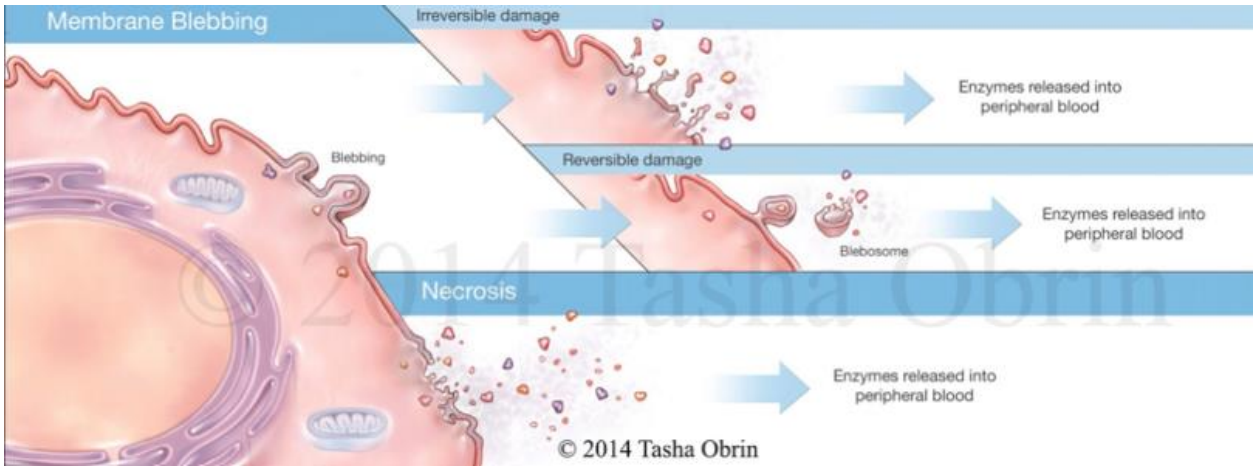
- **The presence of enzymes in serums taken from patients indicates tissue or cellular damage**
- **The measurement of the enzyme amount in serum is of diagnostic significance**
- **Examples :**
  - The Amino Transferases: Alanine Transaminase (ALT), Aspartate aminotransferase (AST)
  - Lactate Dehydrogenase (LDH)
  - Creatine Kinase (CK) which is also called Creatine Phosphokinase (CPK)

When examiners are attempting to diagnose an issue with, for example, the liver, they'd test a blood sample within the liver, then they begin looking for enzymes; because if enzymes are found within that sample at an irregular rate, it would hint at some type of tissue or cellular damage. It's important to note that Liver enzymes or Cardiac enzymes can be found circulating the blood system without there being any damage; because cells always die and release their contents into the bloodstream. So, normally, there'd be a 'regular' level for these enzymes in the blood. However, in the case of, for example, liver damage (eg: due to an overconsumption of alcohol), or bacterial or viral infection, this would cause for cellular damage, and the enzymes would escape from the dead cell, then the examiners would detect this irregular increase in enzyme concentration, leading them to believe that some issue has happened. Furthermore, detection of Isozymes is more helpful since they are a lot more tissue-specific.

We'll talk about the different Enzymes mentioned and how we could use them in disease diagnoses now:

• AST and ALT in Liver Damage:

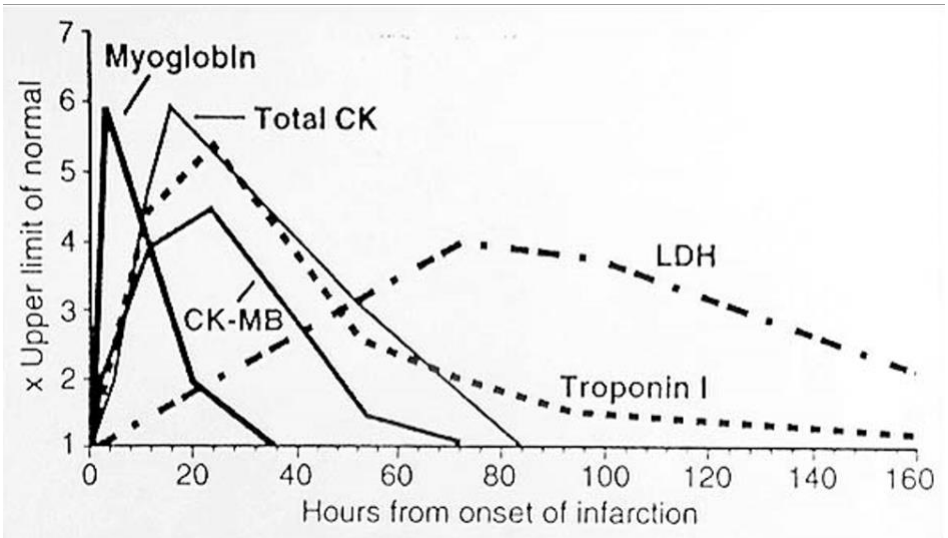
- **The typical liver enzymes measured are AST and ALT**
- **ALT is predominantly in Hepatocytes**
- **The ratio between both (ALT/AST) has diagnostic significance**
  - **Liver disease/damage (not of viral origin) ALT/AST<1**
  - **Viral hepatitis ALT/AST > 1**



The professor didn't focus on this image at all

Proteins Profile in Myocardial Infarction

Dr. mamoun uses the following image to give general information about the protein/enzyme changes after Myocardial Infarction. Afterwards, he goes into details about each one like in the slides. Also he uses the phrases "Cardiac Arrest", "Heart Attack", and "Myocardial Infarction" synonymously, so don't get confused :)

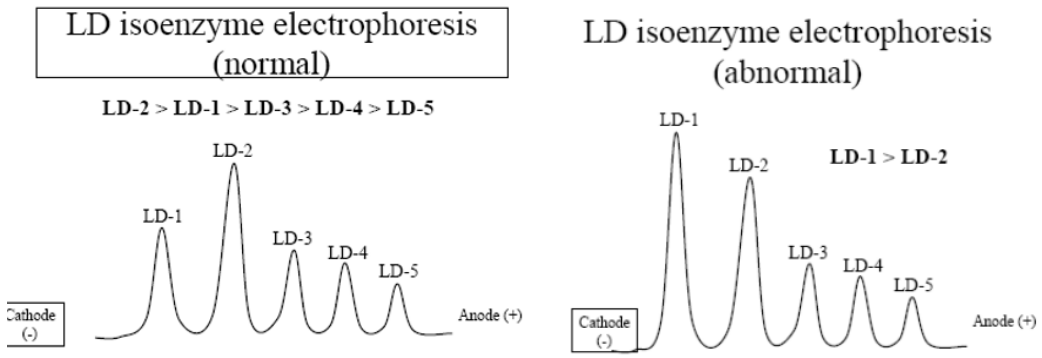


If a patient suffers a heart attack; which means that cardiac tissue was damaged, we could expect a release of the proteins found in the cardiac muscle, a prominent one is the Myoglobin found in cardiac tissue, its concentration is expected to increase because of the muscle damage that happens with the infarction. Though, it's important that Myoglobin also is found in high levels when we an excessive exercise is done (which causes the skeletal muscles to damage, which would release Myoglobin), so Myoglobin levels is not a specific biomarker of Heart Attack. However, we have other enzymes, like Creatine Phosphokinase, and its more specific Isozyme, CK – MB, which is only found in cardiac tissue; so if its concentration increases, this means that we have an issue in the cardiac muscle.

Notice also the timing (which is represented on the X-Axis المحور السيني), doctors examine the protein concentrations and their correlations with time to indicate the exact timing of when the heart attack happened. For example, a patient can come in 3 days after having chest pain, the doctors test for LDH (which peaks after 3 days) and can see that he had a heart attack

Note that doctors examine for the protein Troponin I in most cases which is only found in cardiac tissue.

• LDH in Myocardial Infraction



- **A comparison of serum levels of the LDH-1/LDH-2 ratio is used for diagnosis for myocardial infarction (heart attacks)**
- **Normally, this ration is less than 1**
- **Following an acute myocardial infarction, the LDH ration will be more than 1. Why?**

Because LDH-1 is found mainly in the cardiac tissue, while LDH-2 is found in Red Blood Cells. Normally (since RBCs undergo hemolysis regularly), LDH-2 would be found more abundantly than LDH-1, however, if the patient suffers from a myocardial infraction, the cardiac tissue would tear, which would cause for LDH-1's concentration to increase rapidly, making its concentration higher than LDH-2.

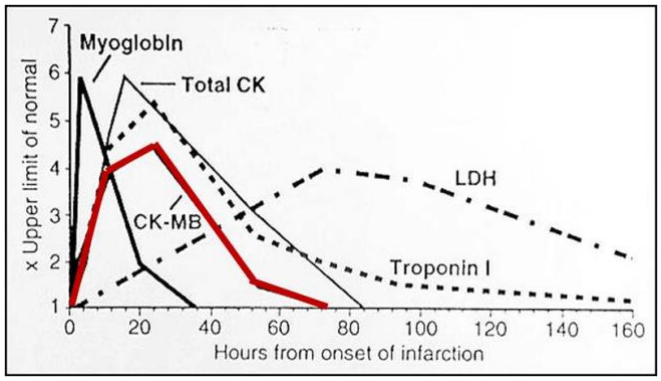
• CPK in Myocardial Infarction

- **CPK is found primarily in heart and skeletal muscle, as well as the brain**
- **There are three tissue-specific isozymes of CPK**
  - **CKP3 (CPK-MM) —> Skeletal Muscle**
  - **CPK2 (CPK-MB) — > Cardiac Tissues as well as other tissues**
  - **CPK1 (CPK-BB) — > Brain**

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

As we could notice from the table, we can use the different percentages of different isozymes of CPK across different tissues to our advantage in diagnosing Cardiac Infarction; because the concentration of CPK-MB would increase a lot.

-In the cardiac muscle we have more MM, but the amount of MB is relatively high, if a cardiac damage happens, there will be a release of MB so its level will increase (although MM will increase too but MB is our indicator in the case of the cardiac muscle). While if a damage occurs in the brain, BB is going to be released.



The advantage of CPK is that it gets cleared relatively quickly (notice that the peak is after 24 hours but it gets cleared after two days); so if a patient complains of a small chest pain but tells the he/she had the same pain two or three days ago we examine the CPK (of course we examine the other things like Troponin too), if the Troponin is high and the CK-MB is low this is an indication that the patient had a heart attack two (or three) days ago and there is a possibility of a having another heart attack because there is still some chest pain so we keep examining CK-MB and Troponin.

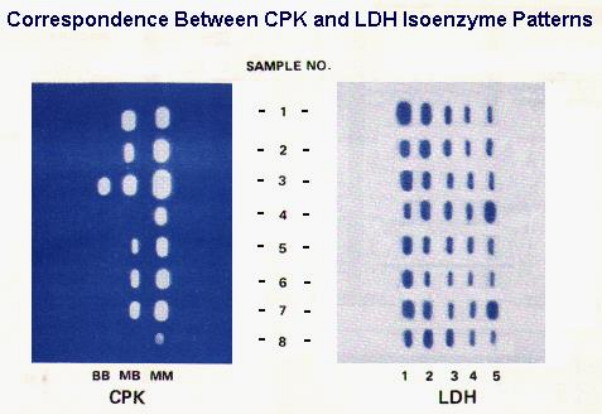
- *A significant amount of CPK-MB is released after MI leading to increased CPK-MB/total CPK ratio (diagnostic of an acute infarction).*

- *CPK-MB disappears in 1-3 days, so another elevation is indicative of another event (reinfarction).*

Here is an example *using Gel Electrophoresis technique*:

The Interpretation:

- **Sample #3 represents results for standard proteins (a sample that contains proteins in certain levels used as a ruler to know that the first column represents LDH1 and the second represents LDH2 and so on).**
- **Sample #8 results are from a normal specimen we always have cell damage so it's normal to see these enzymes in the blood.**
- **Sample# 1 MI patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the LDH flip and the high relative activity of the MB isoenzyme (notice that LDH2 has lower intensity than LDH1 whereas in the normal sample LDH2 is higher).**
- **Sample# 2 MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated.**
- **Sample# 6 MI patient (the 1st day post MI); CK level is elevated with a high relative MB isoenzyme activity and the LDH flip is evident.**
- **Sample# 5 MI patient (2 days post MI) is like sample #6, but lower CK levels.**
- **Sample# 7 MI patient with passive liver congestion or the patient was involved in an accident as a consequence of the MI and suffered a crushing muscle injury (this patient had a heart attack as a result of a liver disease so LDH1 is higher than LDH2 and LDH5 is high).**
- **Sample# 4 a patient with liver disease (the ratio between LDH2 and LDH1 is normal but LDH5 is very high).**



Troponins in MI:

- **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 infarction if it occurred only days after the first.

-We use Troponin as a standard in clinic meaning that we stopped using LDH.

The End of SHEET 24

(وَفِي السَّمَاءِ رِزْقُكُمْ وَمَا تُوعَدُونَ)