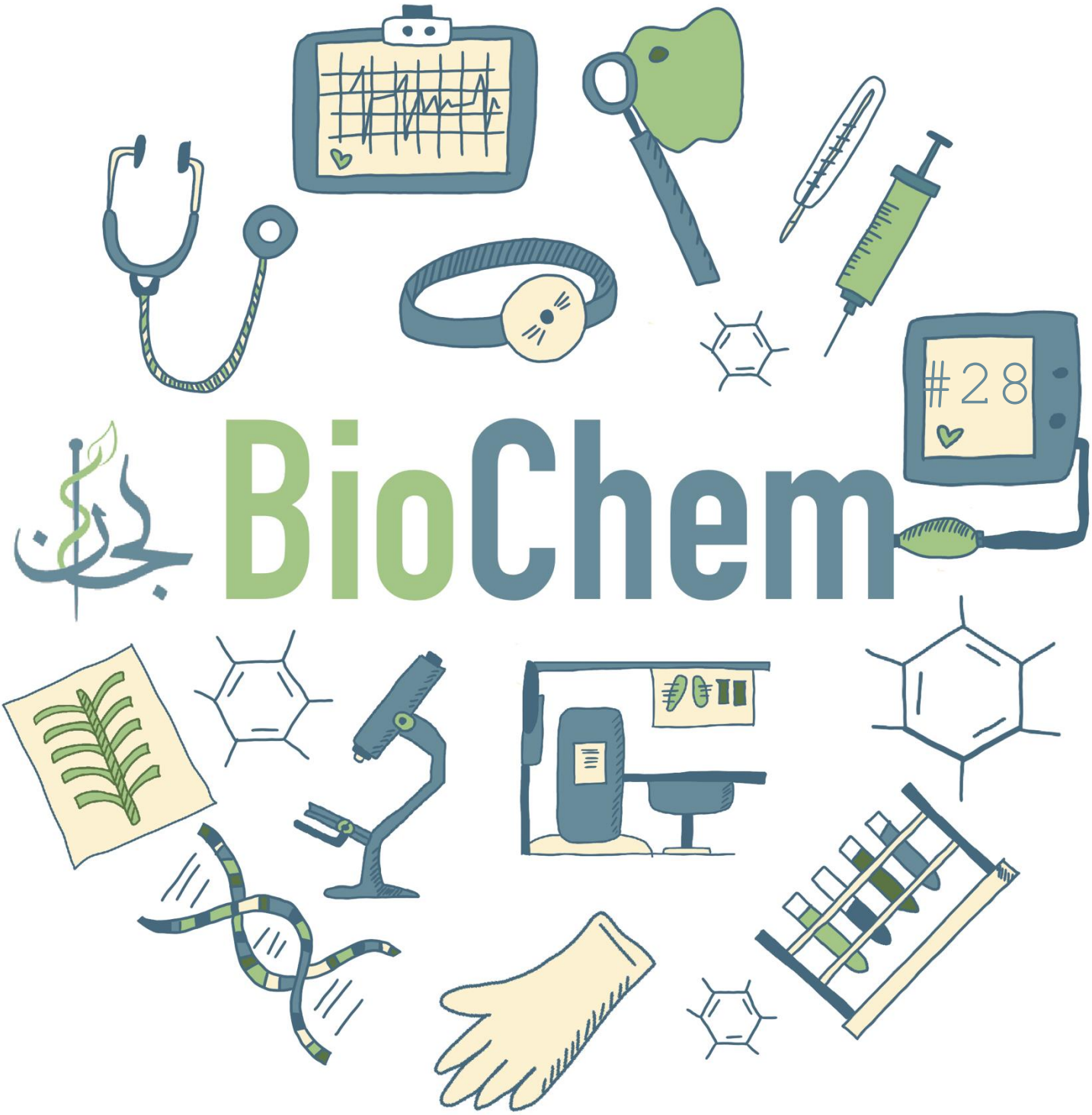

BioChem

Writer: Sara Abdalla

Science: Nada odeh

Grammar: Nada odeh

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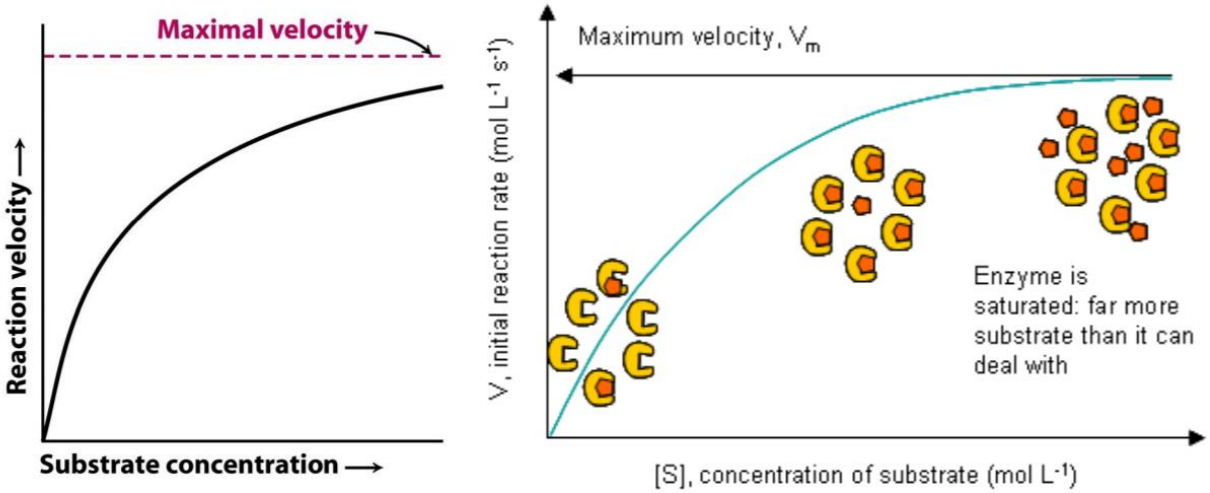
■REMEMBER:-

1) Enzymatic reactions may either have a simple behavior (HYPERBOLIC MANNER "SATURATION") or complex (allosteric) behavior (SIGMOIDAL MANNER).

2) Simple behavior of enzymes: as the concentration of the substrate rises, the velocity rises until it reaches a limit (MAXIMUM VELOCITY Vmax for each enzyme).



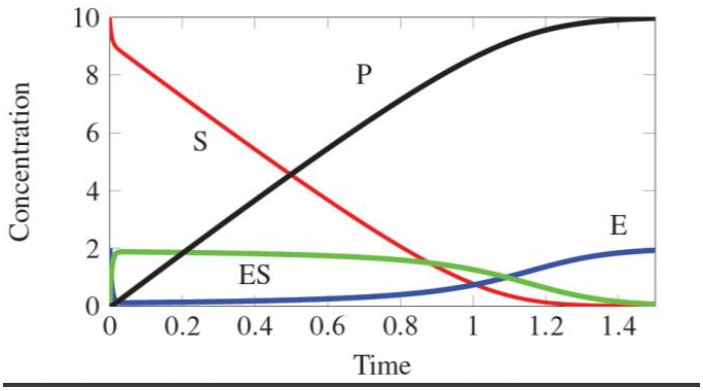
3) Maximum velocity **(CONSTANT PROPERTY FOR EACH ENZYME):** reveals **the turnover number** of an enzyme, which is the number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is **fully saturated** with substrate, at Vmax the reaction is in zero-order rate.

4) The **Michaelis-Menten equation** is built on two assumptions which are :

A) The production of the product is irreversible, the reaction goes in one way, accordingly there is no **K-2.**

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

B) **The Steady State Assumption,** the formation and degradation of the enzyme-substrate complex (ES complex) are in the same rate, ES is in a steady state.



▪ Notice that the concentration of ES seems to be constant, as a result the change in the concentration of ES at a unit of time is ZERO, from which the equation occurred.

$$\frac{dES}{dt} = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

$$0 = k_1\ E\ .\ S - k_{-1}\ ES - k_2\ ES$$

3) The final equation of **Michaelis-Mentem** is used for any **SIMPLE** enzyme, at any substrate concentration, which is:

$$v = \frac{Vmax\ S}{K_m + S} \qquad V_0 = V_{max}\frac{[S]}{[S] + K_M}$$

4) The Km, (Michaelis constant) is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex, so it describes the **affinity** of enzyme for the substrate.

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

- Although, it is not an ACTUAL measure for the affinity, because of the presence of **K2** which leads to product formation, the actual measure for affinity is **KD** , which equals (K-1 / K1), Km is a simple measure inside the lab (easy and cheap), as Km includes the reaction of product formation(K2), which in turn leads to a slight of inaccuracy in the affinity, as it increases the rate of degradation (ES → E + P), by increasing the concentration of (E) and decreasing the concentration of (ES), therefore, Km gives us a LOWER affinity when compared to the actual one, which only depends on K-1 AND K (KD), so we can determine KD using complex machines that measure it in millisecond but they are very expensive that's why Km is more common.

KD : dissociation constant, The actual measure of the affinity, KD = (k-1 /k1).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

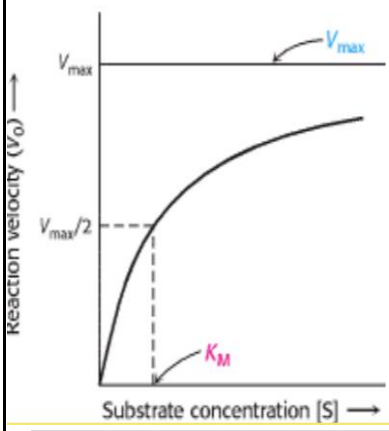
▪ THE MICHAELIS CONSTANT (Km)

The lower the Km of an enzyme towards its substrate, the higher the affinity, and when **more than one substrate** is involved each will have a unique Km and these values have a wide range(mostly between (10-1 & 10-7 M)). BUT using the same enzyme, in the same reaction, the product will be the same, even though we have two different substrates with different Km values we have the same product and therefore, **no change in Vmax.**

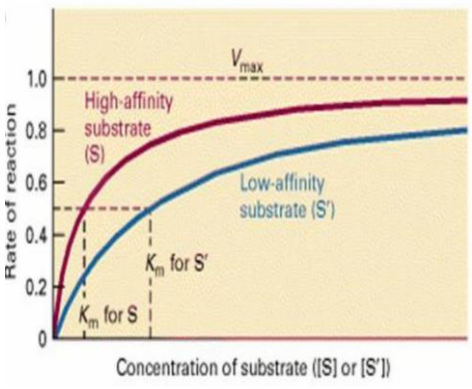
table 8–6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0



One substrate



Two substrates

- For the substrate that has higher Km (lower affinity), higher concentration of it are needed to achieve Vmax, BUT the same Vmax value will be achieved by both substrates at the end .

For example, glucokinase (transferase: transfers one group from one molecule to another), this enzyme transfers the phosphate group from ATP to glucose, there is two substrates, so two different Km values (different affinities), BUT , as there is one single product (glucose-6-phasphate) there is one Vmax.

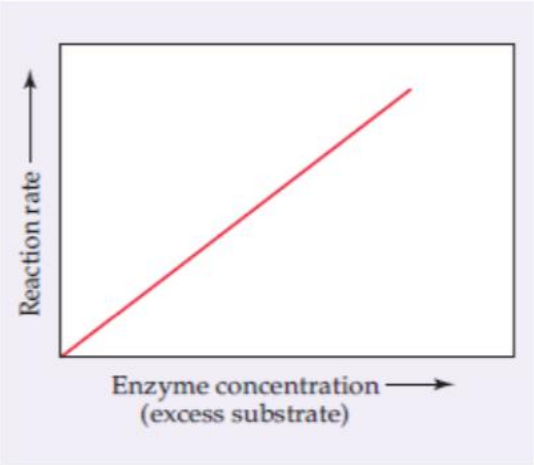
Glucokinase can also use fructose as a substrate rather than glucose, which is a different substrate, with a different Km value, and different affinity (but the second substrate will be the same which is ATP), and glucose is more specific/reactive for glucokinase than fructose (Km for glucose < Km for fructose). Accordingly , there will be a different reaction, a different product (fructose-6-phosphate), so different Vmax.

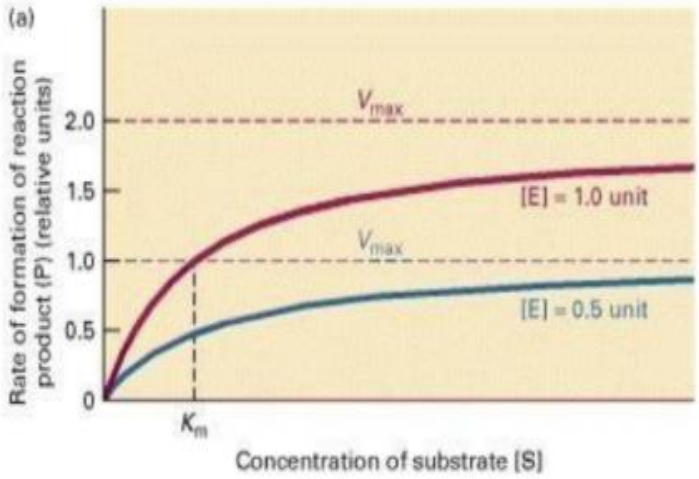
▪ Effect of the enzyme concentration on Km and Vmax.

When you increase the enzyme concentration, what will happen to Vmax & Km?

As we know, Vmax is a constant property of the enzyme (The best efficiency for the enzyme).As a way to understand, we can compare it to the maximum velocity of a car, each car has its own unchangeable Vmax. BUT, changing the car's engine capacity would result in a different Vmax, so same for enzymes, **using a higher concentration of the enzyme would result in a higher Vmax, increasing the concentration of an enzyme will increase the Vmax in a linear manner.**

However, Km value will not change, as the affinity of the enzyme to the substrate is constant.





▪TO SUM UP, answer these questions:

1) We started a reaction with 0.5 microM concentration of a specific enzyme, with a MW 3 g/mol, in a 25 C, then we have repeated the experiment using 1 microM concentration of the same enzyme, how much the change in Vmax will be?

Ans: it will be doubled, the other information are not important, as Vmax only depends on the enzyme concentration.

2) We have repeated the experiment using one third of the enzyme concentration, how much the change in Vmax will be?

Ans: it will decrease by a third.

3) We have two experiments each with a different enzyme, in the fist experiment the enzyme velocity was 10 microM/S , and in the second experiment the velocity was 20 microM/S , can we determine which enzyme is more efficient?

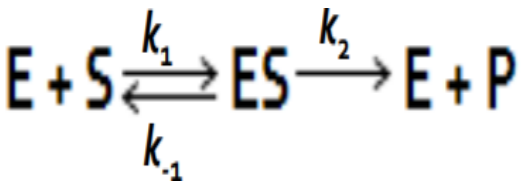
Ans: keep in mind that Vmax represents the efficiency of an enzyme at **specific concentration,** different concentration result in different Vmax values , BUT in this case the concentrations are unknown, therefore, we cant determine the efficiency as the concentrations may be different.

4) We have two experiments each with a different enzyme, in the first experiment 1 microM of the enzyme is used , and the velocity was 10 microM/S , in the second experiment 4 microM of the enzyme was used ,and the velocity was 20 microM/S , which enzyme is more efficient?

Ans: the enzyme used in the first experiment, because in the second experiment each 1microM had a Vmax of 5 microM/S (20/4), However in the first experiment each 1microM had a Vmax of 10 microM/S.

▪ Vmax & Kcat :-

Now, according to the last two questions, scientist had came up with a new concept to measure the efficiency of an enzyme, according to both Vmax and enzyme concentration which is Kcat.



Turnover Numbers (k_{cat}) of Some Enzymes		
Enzyme	**Substrate**	**k_{cat} (s^{-1})**
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

▪ The maximal rate, Vmax, is equal to the product of **K2 , also known as kcat,** and the total concentration of enzyme.

$$V_{max} = k_2 [E]_T$$

▪ kcat, **the turnover number**, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated.

$$k_{cat} = V_{max} / [E]_T$$

▪In other words, the maximal rate, Vmax, reveals the **turnover number** of an enzyme if the total concentration of active sites **$[E]_T$** is known.

▪ EXAMPLE:

1) a 10-6 M solution of carbonic anhydrase catalyzes the formation of 0.6 M H2CO3 per second when it is fully saturated with substrate. A) Calculate kcat, B) How much time does each single reaction need?

Sol: A) Vmax= 0.6 M , $[E]_T$= 10-6 M , $k_{cat} = V_{max}/[E]_T = 6 \times 10^5$ **s -1 (per second)** =3.6 X 107 min-1.

B) in each second there is 6×10^5 reactions, therefore, one reaction needs $(1/6 \times 10^5)$ second, which equals 1.7 µs.

So, each catalyzed reaction takes place in a time equal to 1/k2 ,

▪ The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second.

▪ Reaction Rate (V), Enzyme Activity, Specific Activity, Turnover Number.

1) **Reaction rate (velocity):** measures the concentration of substrate consumed (negative) (or product produced(positive)) per unit time (mol/{L.s} or M/s).

2) **Enzyme activity:** measures the number of moles of substrate consumed (or product produced) per unit time (mol/s).

Enzyme activity = rate of reaction × reaction volume

3) **Specific activity**: measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.g}), more specific , used in comparison between different enzymes in different masses.

Specific activity = enzyme activity / actual mass of enzyme

→ This is useful in determining enzyme purity after purification.

4) **Turnover number:**measures moles of substrate converted per unit time per moles of enzyme (min-1 or s-1).

Turnover number = specific activity × molecular weight of enzyme

▪ So, there is two ways to measure Kcat/ turnover number :

1) Vmax/[E]T

2) Specific activity x molecular weight of enzyme.

▪ Example:

A solution contains initially 25X10^-4 mol L -1 of peptide substrate and 1.5 µg chymotrypsin in 2.5 ml volume. After 10 minutes, 18.6X10^-4 mol L -1 of peptide substrate remain. Molar mass of chymotrypsin is 25,000 g mol-1, Calculate:

A) How much is the rate of the reaction? ▪ (conc./time)

B) How much is the enzyme activity? ▪ (mol./time)

C) How much is the specific activity? ▪ (enz. Act. / enz. Mass)

D) How much is the turn over number? ▪ (sp. Act. X enz. molar mass)

Sol: concentration of product produced = second concentration – first concentration = 25X10^-4 mol L -1 - 18.6X10^-4 mol L -1 = 6.4X10^-4 mol L -1

A) rate of the reaction = conc/time = 6.4X10^-4 mol L -1/ 600 = 1.067X10^-6 mol/s.L

B) Activity = rate X volume = 2.67 X 10^-9 mol/s

C) Specific activity = Activity/mass= 2.67 X 10^-9 mol/s / 1.5 X 10^-6 g = 1.78 X 10^-3 mole/(s.g)

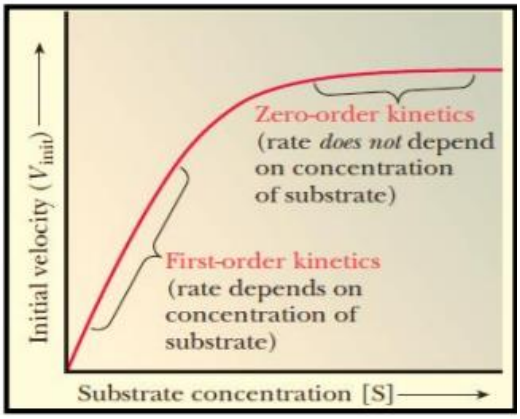
D) Turn over number = specific activity X molar mass = = 1.78 X 10^-3 X 25000 = 44.44 s-1

▪ Disadvantages of Michaels-Menten equation & lineweaver-Burk or double reciporal plot.

1) What are the disadvantages of Michaels-Menten equation?

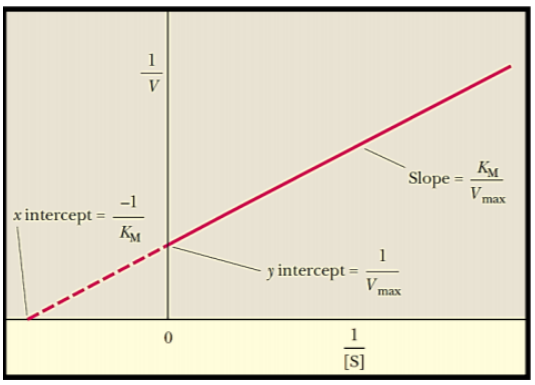
A) We need to use a **huge amount (excess) of substrate** in order to reach the Vmax, which needs a lot of experiments, power, time and money.

B) Determining the Km from hyperbolic plots is not accurate, Km equals the concentration of the substrate at ½ Vmax, so the position of ½ Vmax is actually **approximated**, which makes this method inaccurate , the same thing when determining Vmax, the values are not accurate.



These two disadvantages prevent the calculation of both Vmax & Km accurately.

2) Then, two scientists invented a new method, by taking the **reciprocal** of both sides of the Michaelis-Menten equation, which give a new plot which is **LINEAR**, and because the plot is linear, we need at least two points **(two experiments),** different velocities(Y) depending on their concentrations (X) to draw the line easily.



- **Lineweaver-Burk plot:** A plot of $1/V_0$ versus $1/[S]$ (double reciprocal plot), yields a straight line with an y-intercept of **1/Vmax** and a slope of **KM/Vmax**, the intercept on the x-axis is **-1/KM.**

$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

ص=أس+ب

- This equation made a revolution in the enzymology science, because it decrease the amount of substrate, money, power, and number of experiments, but it is historical now, as **computers** can calculate all the parameters easily and quickly.

$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}}\right]$$

Examples:

1) A biochemist obtains the following set of data for an enzyme that is known to follow **Michaelis-Menten kinetics**. **Approximately**, Vmax of this enzyme is … & Km is …?

A. 5000 & 699

B. 699 & 5000

C. 621 & 50

D. 94 & 1

E. 700 & 8

Substrate Concentration (µM)	Initial velocity (µmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

Sol: **(E)** , the word approximately gives us an indication that the question must be solved without obtaining accurate numbers mathematically, also the info " Michaelis-Menten kinetics" provides us with tell us that the enzyme is **simple** ,as a result it reacts in a hyperbolic manner, so according to the plot the Vmax doesn't depend on the substrate concentration (zero-order), therefore, to find Vmax we search for two concentrations which are largely different (huge change in the concentration) , with a very small change in velocities, as you can see from 1000 to 5000 (5 folds) the velocity approximately doesn't change (from 698 to 699), which tells us that Vmax is approximately 699, therefore the correct answer till now may be B or E.

Km is the concentration of the substrate when the velocity equals ½ Vmax, and ½ Vmax is approximately 349 (699/2), the concentration at this point is 8, so Km is 8, and the answer is **E** .

2) You are working on the enzyme "Medicine" which has a molecular weight of 50,000 g/mol. You have used 10 µg of the enzyme in an experiment and the results show that the enzyme converts 9.6 µmol per min at 25˚C. the turn-over number (kcat) for the enzyme is:

A. 9.6 s -1

B. 48 s -1

C. 800 s -1

D. 960 s -1

E. 1920 s -1

Sol : (C)

Firstly: calculate the enzyme moles = mass /MW = 2 X 10^-10 moles of the enzyme.

Secondly: kcat = Vmax(in moles /s) / moles of the enzyme = 800 s-1

▪Enzyme regulation:

There are many **MODES** for regulation which are:

1) Isozymes. 2) inhibition. 3) Conformation. 4) Amount. 5) None- specifically.

▪ In this sheet we will talk about isozymes and irreversible inhibitors.

You should never feel guilty about taking a break. ;)

1) Isozymes(isoenzymes) the differential **Km values**

- They are enzymes with different sequences of amino acids, different structures BUT**, ALL** catalyze the same reaction, each one in a different tissue in the body, same substrate & product, different gene, different localization, different parameters **(Km, Vmax, kcat)**

- So, the same reaction occurs in different tissues in the body, each tissue has a specific isozyme with a specific structure that catalyze the reaction in a specific efficiency, affinity, rate ..etc

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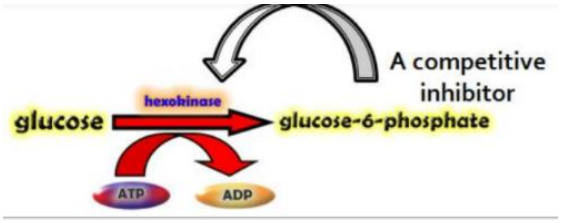
A) Hexokinase:



- Let's take hexokinase as an example, as we know whenever glucose enters a cell it will be immediately phosphorylated into glucose-6-phosphate, because phosphate group is a bulky group with a **negative charge,** so glucose will be trapped inside the cell, and the enzyme responsible for this process (phosphorylation) is **GLOCUOKINASE(Catalyzes the first step in glucose metabolism)**, so we need glocukinase in all cells, because phosphorylation occurs in all cells by this enzyme, but with different mechanisms, affinities , efficiencies , abilities to be inhibited and different Km, Vmax values .. etc, so glucokinase works differently in different tissues.

- *There are **two isozymes** for glucokinase which are :*

1) **Hexokinase I** (RBCs).

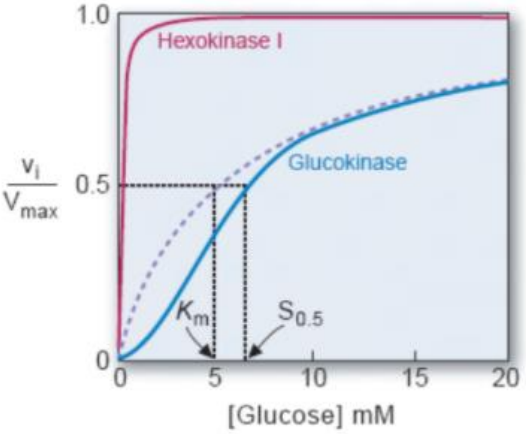
2) **Hexokinase I V** (glucokinase, liver, pancreas).

- *What are the differences between the RBCs and the liver cells?*

Ans:1) RBCs don't have **mitochondria** (most energy production occurs in mitochondria) so RBCs solely depends on glycolysis (in cytosol) for energy production (few energy) so it needs glucose to exist permanently inside the cells to provide enough energy, however ,liver cells have mitochondria as a result they produce good amounts of energy so no need for glucose to be available all the time.

2) Liver cells consume and also stores glucose as **glycogen** , However RBCs only cosume glucose by converting it to energy by glycolysis.

▪ *How do these isozymes differ?*



1) In the Km values:

Hexokinase I (RBCs): KM (glucose) **≈ 0.1 mM (higher affinity, the absence of mitochondria and therefore the production of low amounts of energy, makes these cells in need for glucose all the time)**

Hexokinase I V (glucokinase, liver, pancreas) **≈ 10 Mm(lower affinity, because of the presence of the mitochondria and the production of a good amount of energy, these cells don't need glucose all the time)**

‚So what happens when blood glucose falls below its normal fasting level (≈ 5 mM)?

A) RBCs : They will keep phosphorylating glucose by hexokinase glucose at rates near Vmax, because Km < glucose concentration in the fasting level, so glucose will bind hexokinase effectively, and this is needed by the body as RBCs depends solely on glycolysis, glucose must be present inside them all the time.**(Km < fasting value → high effectiveness)**

KEEP IN MIND:

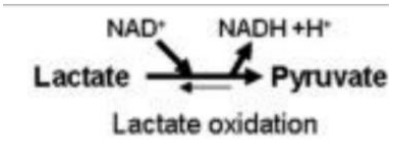
When the concentration of the substrate is HIGHER than the Km the substrate will binds the enzyme effectively, however when the substrate concentration is LOWER than Km the substrate will binds ineffectively.

B) Liver: rate of phosphorylation ONLY increases above fasting levels (after a high carbohydrate meal), but while fasting the body doesn't need to store glucose because there is no excess amount to be stored, so to start storing glucose, there must be an excess amount of it**(Km > fasting level→ low effectiveness).**

So,High KM of hepatic glucokinase promotes storage of glucose

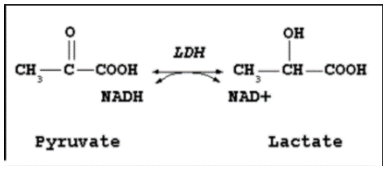
2) Feedback inhibition: when the concentration of the **product (glucose-6-phosphate)** increases, it inhibits the enzyme itself **(hexokinase)**, and as the liver acts as a storehouse, any increase in the concentration of the product should not inhibit the enzyme because excess glucose can be stored as glycogen. On the other hand, when RBCs take the energy needed from glucose, the enzyme must be inhibited. In pancrease, glucokinase must not be inhibited because it acts as the mirror of the blood, pancreatic cells must always sense the concentration of the glucose in the blood regardless of its concentration, so all the glucose that enters blood cells must be converted to glucose-6-phosphate, so it would be trapped and depending on its concentration, pancreatic cells then secret **insulin or glucagon** in certain amounts with respect to this concentration.

B) Lactete Dehydrogenase **(LDH):**



- Lactate dehydrogenase is an enzyme that interconverts between lactate and pyruvate.

- When glucose molecules enter the cell they will be converted into pyruvate (glycolysis), then there will be two pathways, which depend on the presence of **oxygen (O_2).**

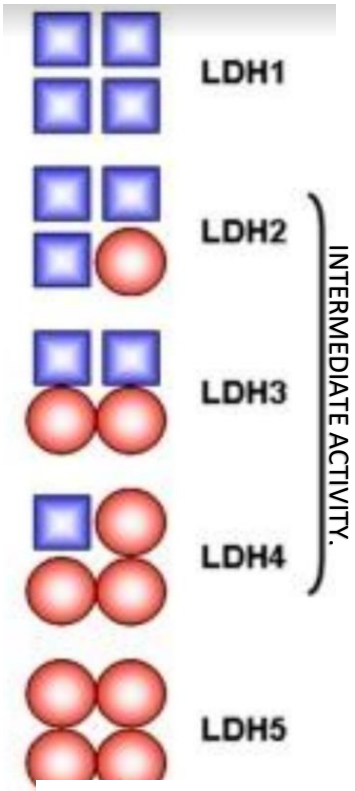


1) Aerobic respiration **(in the presence of oxygen),** this pathway occurs in mitochondria, where pyruvate is converted into acetyl-coA, then Kreps cycle , oxidative phosphorylation, and production of ATP.

2) anaerobic respiration **(NO oxygen),** pyruvate will be reduced to form lactate by lactate dehydrogenase(LDH) , which will cause fatigue, and when the oxygen is present again, lactate molecules will be oxidized to form pyruvate molecules again by lactate dehydrogenase, and pyruvate molecules will go through the aerobic respiration because we have oxygen now.

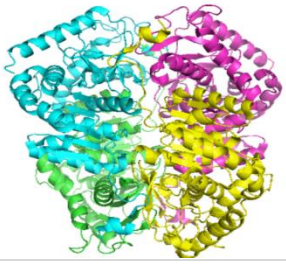
- **Muscle fatigue** is the decline in ability of a **muscle** to generate force, caused by the LACK OF OXYGEN, fatigue occurs in skeletal muscles as a sign to get rest after a vigorous exercise, however, fatigue DOESN'T take place in cardiac muscle (it keeps working all the time).

Actually dehydrogenase exists also in cardiac muscle, so HOW could that fatigue doesn't occur even with the presence of LDH↓?



- LDH is a tetramer, which means it has **four** subunits, it has **five** isozymes which come in different combinations of subunits (look at the following figure).

- The **BLUE SQUARE** is called (H), therefore, LDH1 is named **H4,** it presents in the **HEART**, H4 has the ability to convert any lactate molecule to a pyruvate molecule, BUT, it can't convert pyruvate molecules into lactate molecules, that's why there's no lactate accumulation in heart muscle, and therefore no fatigue.



- The RED CIRCLE is called (M), therefore, LDH5 is named M4, it's present in the **SKELETAL MUSCLES,** M4 has the ability to

interconvert between both lactate and pyruvate, which gives skeletal muscles the ability to get fatigued, so whenever this happens , a rest is needed .

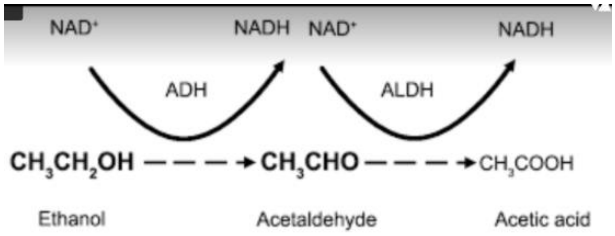
So, what distinguish H4 from M4 to give them these different features?

1) Km value for pyruvate in the heart >>> Km value for pyruvate in skeletal muscles, accordingly pyruvate must be in a very high concentration in the heart to be reduced into lactate, however in M4 a lower concentration of pyruvate may be converted to lactate.

2) Inhibition: In the heart, if there is a high concentration of pyruvate and the enzyme started binding pyruvate molecules, the active site is designed in a way that after binding the enzyme will be broken "substrate inhibition", so the enzyme won't go in the forward direction, however, when lactate binds to H4(in the heart), there will be no inhibition and it will be converted into pyruvate.

In skeletal muscles:M4 is not inhibited, any pyruvate that binds to the enzyme will be converted to lactate.

C) Aldehyde dehydrogenase(ADLH):



▪ALDH Oxidizes acetaldehyde to acetate, it has **four tetrameric** isozymes (I-IV)

▪ After the digestion of alcohol**(Ethanol),** ethanol travels to the liver cells, then it is oxidized by the enzyme **alcohol dehydrogenase (ADH)** into **ACETALDEHYDE**, which is a toxic material (the toxicity of alcohol), then aldehyde dehydrogenase **(ALDH)** oxidizes acetaldehyde into acetic acid, so this enzyme is actually neutralizing the toxicity of alcohol. There are **two** isozymes for ALDH in our hepatic cells:

1) ALDH II **(higher Km, cytosolic),** needs high amount of acetaldehyde to stat oxidizing.

This isozyme is present in the cytosol, and starts working when there is a high concentration of acetaldehyde, and coverts it to acetic acid.

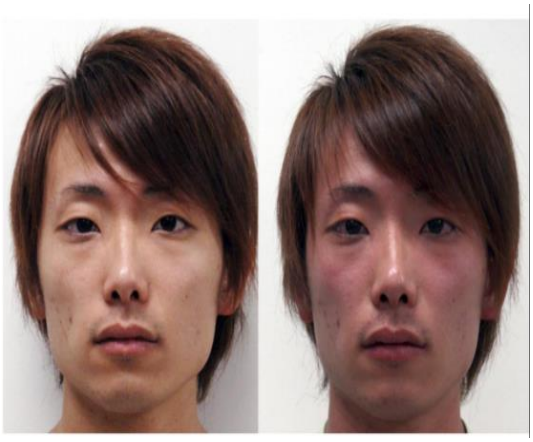
2)ALDH I **(Low Km, mitochondrial),** low amounts of acetaldehyde are enough for the oxidizing to occur .

This isozyme is present in the mitochondrion, and works with a high affinity, converting acetaldehyde into acetic acid.

▪For some people the mitochondrial (ALDH I) isozyme is mutated ,as a result the Km value increases (increase the amount of substrate needed for the enzyme to go through and start

oxidizing), and the affinity decreases ,so **the TOTAL affinity** to acetaldehyde will decrease because now, we have two isozymes with high Km values and low affinities.

- Those people who have this mutation will show strong symptoms **(tachycardia and flush response)** after drinking alcohol, as the affinity for acetaldehyde is lower, the concentration of acetaldehyde will increase, leaving the cells and heading to the blood stream therefore the toxicity increases in the blood and the symptoms take place.



- 50% of Japanese & Chinese are unable to produce ALDH I (not observed in Caucasian & Negroid populations)

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2) Inhibition.

- Inhibitors are classified into two different categories:

1) reversible, which are considered physiological, normally take place in our bodies.

2) irreversible, they are obtained (acquired), our bodies don't synthesize them normally, such as toxins, poisons, drug.

A) Irreversible inhibitors, mechanism- based inhibitors.

These inhibitors interferes with the mechanism of the enzyme, due to this , enzymes won't be able to catalyze reactions . These inhibitors mimic or participate in an intermediate step of the catalytic reaction,

- **The kinetic effect** of irreversible inhibitors is to **decrease the concentration of active enzymes.**

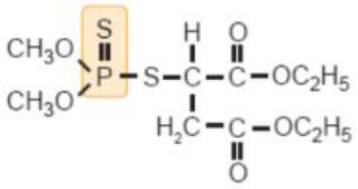
- ***They are classified into:***

A. Covalent inhibitors.

When a covalent inhibitor (drugs, toxins) comes to the enzyme it will **INITIALLY** bind to the active site amino acids **COVALENTLY,** or by an extremely tight bond and it won't dissociate from the enzyme killing it (will be degraded at the end)

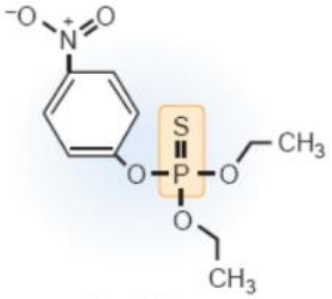
EXAMPLE:1) The lethal compound **[DFP]** is **organophosphorus** as it contains phosphate group **that serves as a prototype for**:

1) **Insecticides** such as: malathion, parathion, shown in the figure.



Malathion

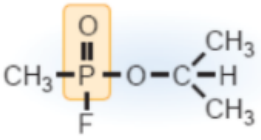
2) **Nerve gases** used in wars such as: salin gas, shown in the figure, breathing this gas causes suffocation, within a short amount of time.



Parathion

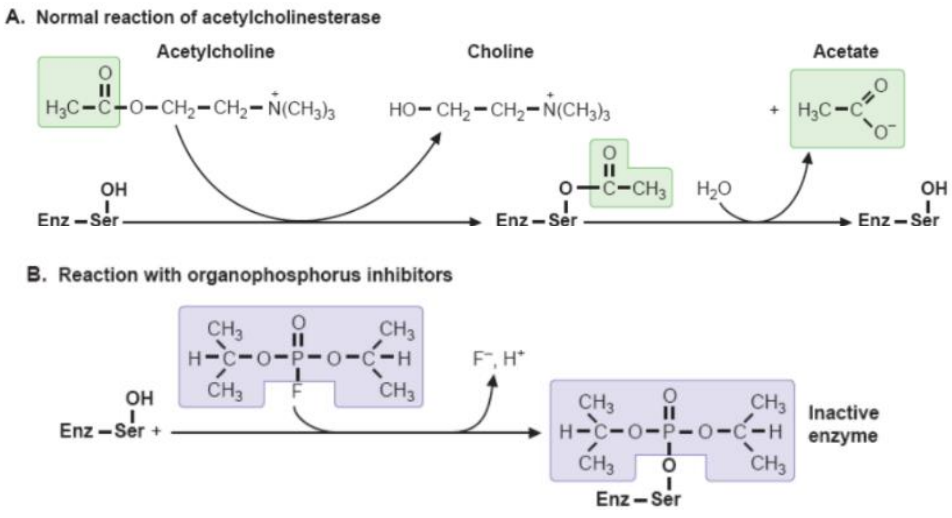
So, What exactly happens?

Salin gas molecules and the other organophosphorus compounds derived from (DFP) compound, bind to the active side of a key enzyme in the central nervous system which is: **Acetylcholinesterase ,** it is the enzyme that is responsible for breaking down the binding acetylcholine molecules (that cause contraction in the muscle) ,so it comes out of the nerve to the acetylcholine receptors of the muscle, and breaks down acetylcholine, causing relaxation in the muscle, look at figureA.



Sarin

- As these **organophosphorus compounds** target acetylcholinesterase, and bind covalently with its active site through **serine,** as a result, the enzyme will be inactive, as shown in the figure B.



- As acetylcholinesterase became inactive, it will lose its ability to break down acetylcholine, and **acetylcholine** will keep binding to its receptors on different muscles, therefore, muscles will keep contracting with no relaxation, until it reaches the **tetanus** state.

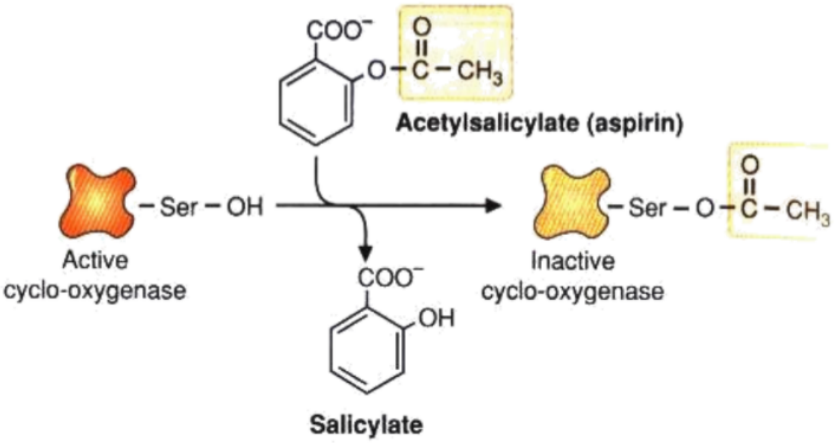
- Tetanus of muscles means that the muscle is unable to work and function as a result of **energy depletion**, so the effect on the skeletal muscles is not dangerous, however, the

danger is in the energy depletion of the **diaphragm**, this will cause respiratory arrest, and the patient will die.

- **DFP** also inhibits other enzymes that use serine ex:(serine protease) but the inhibition is not as lethal.

2) Aspirin(acetylsalicylic acid):

As you know, aspirin inactivates the enzyme that is responsible for converting **arachidonic fatty acid**, into different prostaglandins, leukotriene, thromboxane..etc (inflammatory agents), which is **cyclooxygenase (prostaglandin endoperoxide synthase),** by a covalent acetylation for serine amino acid in its active site.



- Aspirin is made up of two portions (Acetyl and salicylic acid), it resembles a portion (acetyl group) of the prostaglandin precursor (arachidonic fatty acid) that is **a physiologic substrate for the enzyme,** and the salicylic acid leaves the reaction, as shown in the figure.

- So aspirin prevent the synthesis of clotting agents (thromboxane),that's why people who take aspirin daily, must stop taking it for a week before getting in a surgery, so as the cyclooxygenase will be regenerated.

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2)**Transition- state** analogs & compounds that resemble intermediate stages of the reaction.

- **The Transition state molecule** is more fit to the active site than the substrate because it is the formulation of the substrate so that the reaction can go through, therefore it binds to it very **tightly,** so the idea is to synthesize transition state analogs, so as now they bind very tightly to the enzyme due to its higher affinity to them when compared to that of substrates , however drugs cannot be designed in a way that precisely mimics the transition state! (highly unstable structure).

- So, transition-state analogs: extremely potent **inhibitors (bind more tightly),** these drugs are also substrate analogs, as they represent the state between the substrate and transition state (it looks like both of them, however there is a modification in their structures that the

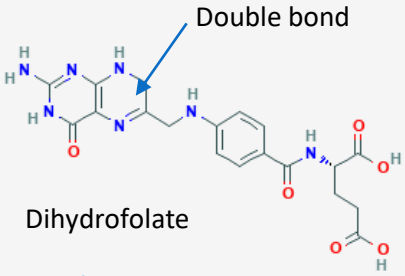
reaction will not go through) so, substrate analogs bind more tightly (with higher affinity) than substrates, but with no reaction taking place(inhibition).

- Because the enzyme itself binds the analog, it seems that it chooses to kill itself, therefore they called these inhibitors suicide inhibitors.

- **EXAMPLES:**

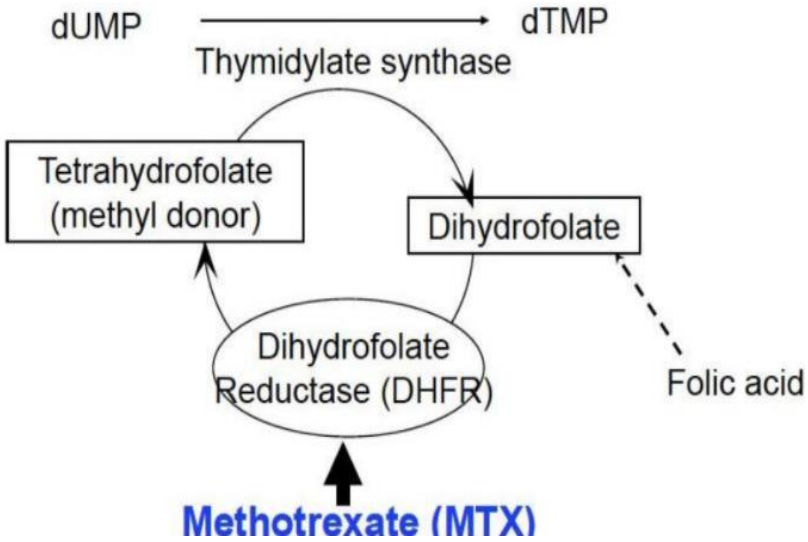
1) Methotrexate(synthetic inhibitor), the most common drug used for breast cancer (anticancerous).

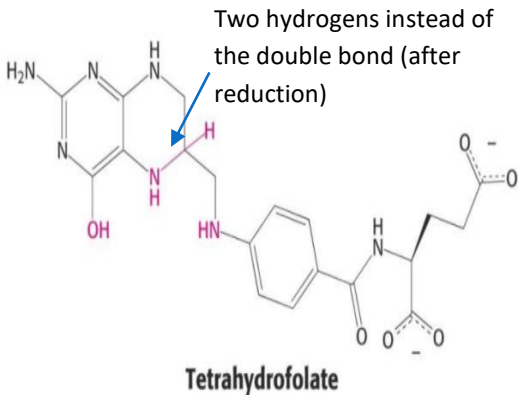
- It targets the enzyme **dihydrofolate reductase (DHFR),** it is an enzyme that reduces dihydrofolate into tetrahydrofolate (methyl donor), look at the figure.

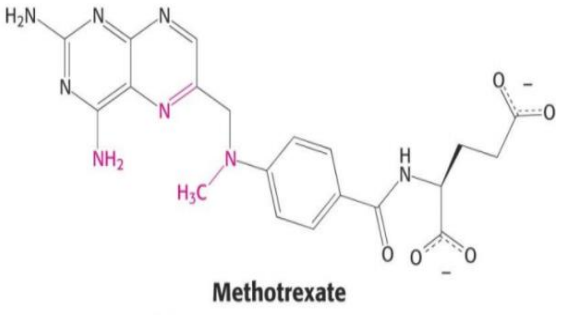


Not from slides, but the doctor explained it's structure.

- This reduction reaction is essential for **thymidylate synthesis,** which is a nitrogenous base required for the synthesis of DNA and RNA, look at the following figure.





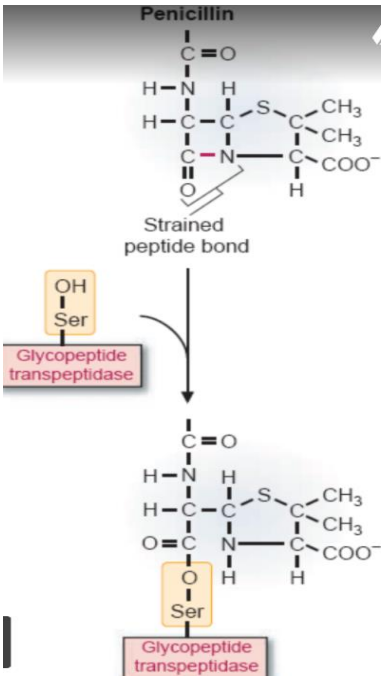


- **Methotrexate** mimics dihydrofolate with some modifications(differences are highlighted in pink), look at the figure, the active site has higher affinity to it than to the substrate , it binds 1000 fold more tightly, it will block the enzyme**(dihydrofolate reductase)** and the reduction reaction can't go through, as a result there will be no thymidylate synthesis, and therefore it inhibits nucleotides synthesis, and DNA, RNA synthesis, and accordingly there will be no **new cells** and this is how it fights cancer.

2) Penicillin(antibiotic)

It contains **beta lactam ring,** which has a nitrogen atom connected to a carbonyl group in an amide bond, which looks like a peptide bond, this drug is a transition state analog to an enzyme that is required for the synthesis of **the bacterial membrane** which is **glycopeptidyl transferase or transpeptidase,** (from it's name (peptidase) it breaks down peptide bonds).

- The reaction is favored by the strong resemblance between the **peptide bond** in the beta-lactam ring of penicillin & the transition- state complex of the natural **transpeptidation reaction,** so the enzyme will bind penicillin with much higher affinity than the original substrate, and it will break down the strained peptide bond in penicillin thinking that it is a peptide bond but it is not, so the reaction will not take place, the enzyme will be inhibited and accordingly, the bacterial membrane won't be built, which kills the bacteria.



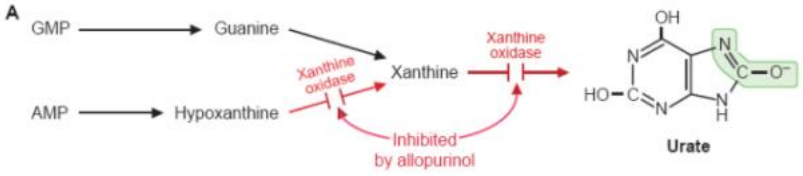
Note: Inhibitors that undergo **partial reaction** to form irreversible inhibition in the active site are sometimes termed suicide inhibitors.

3) Allopurinol: a drug used to treat gout.

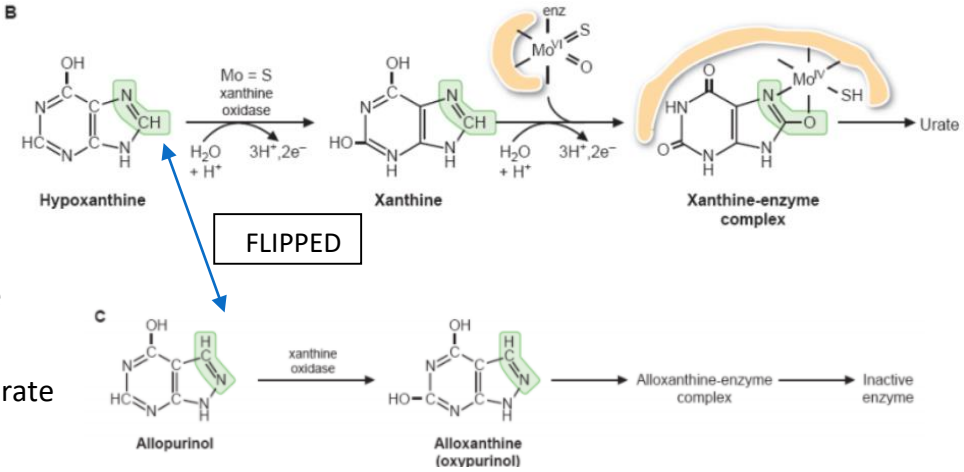
Gout: disease that is caused by the accumulation of urate crystals (insoluble) in joints mainly in the big toe, which causes inflammation of the joint and friction leading to **pain**

How are urate molecules produced?

- Purines present normally in our bodies and in many types of food (meat, chicken), as you can see in the figure, GMP will be converted to xanthine by deamination, and AMP will be converted to hypoxanthine by deamination then hypoxanthine will be converted to xanthine by xanthine oxidase , when we eat food with high content of purines, the amount of xanthine increases, then the oxidation of xanthine by xanthine oxidase increases which will produce urate crystals.



▪ The enzyme contains a molybdenum–sulfide (Mo-S) complex that binds the substrates and transfers the electrons required for the **oxidation reactions.**



▪ We can treat gout disease by the inhibition of the enzyme xanthine oxidase, and therefore decrease urate crystals production, the enzyme commits suicide(inhibition) by providing the drug transiton-state analog (Allopurinol which analog hypoxanthine (substrate))

▪ The active site affinity to allopurinol is much higher than it is to hypxanthine, therefore the Xanthine oxidase oxidizes the drug allopurinol to oxypurinol, which binds **very tightly** to a molybdenum–sulfide complex in the active site.

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3)Heavy metals:

▪ Tight **binding of a metal** to a functional group in an enzyme such as: Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe). Metals are **relatively nonspecific for the enzymes** they inhibit, particularly if the metal is associated with high-dose toxicity.

Over normal values of **mercury** and **lead** causes serious abnormalities, because they bind to the enzymes with a **very high affinity** compared to the metals that are normally present inside the enzymes.

1) lead: Lead provides an example of a metal that inhibits through replacing the **normal functional metal in an enzyme**, such as calcium, iron, or zinc, it may work in a reversible manner, competing with zinc in the active site of the enzymes that synthesize the heme, or it may work in an irreversible manner, Its developmental & neurologic toxicity maybe its ability to replace **Ca+2** in several regulatory proteins(have higher affinity to lead compared to calcium) that are important in the central nervous system and other tissues.Lead is used in walls painting production , and this is dangerous on children ,because as we know, kids eat whatever they find , so if pieces of this dry painting were eaten by them , and as their blood brain barriers aren't fully tightened, lead will reach their brains and accumulate there leading to an irreversible retardation .

2) Mercury: binds to so many enzymes, often at **reactive sulfhydryl groups (cysteine in the active site)** in the active site, mercury binds covalently to the thiol group, in a **ligation bond**, which is a very strong bond.

BEST WISHES ☺

SHORT QUIZ

TEST BANK

1) Malonate is a competitive inhibitor of succinate dehydrogenase, a key enzyme in theKrebs tricarboxylic acid cycle. The presence of malonate will affect the kinetic parameters of succinate dehydrogenase in which one of the following ways?

(A) Increases the apparent Km but does not affect V max
(B) Decreases the apparent Km but does not affect V max
(C) Decreases V max but does not affect the apparent Km
(D) Increases v max but does not affect the apparent Km
(E) Decreases both V max and Km

2) The pancreatic glucokinase of a patient with MODY had a mutation replacing a leucine with a proline. The result was that the Km for glucose was decreased from a normal value of 6 mM to a value of 2.2 mM, and the Vmax was changed from 93 U/mg protein to 0.2 U/mg protein. Which one of the following best describes the patient's glucokinase compared with the normal enzyme?
A. The patient's enzyme requires a lower concentration of glucose to reach 1⁄2Vmax

A. The patient's enzyme requires a lower concentration of glucose to reach 1⁄2Vmax
B. The patient's enzyme is faster than the normal enzyme at concentrations of glucose <2.2 mM.
C. The patient's enzyme is faster than the normal enzyme at concentrations of glucose >2.2 mM.
D. At near-saturating glucose concentration, the patient would need 90 to 100 times more enzyme than normal to achieve normal rates of glucose phosphorylation.
E. As blood glucose levels increase after a meal from a fasting value of 5 to 10 mM, the rate of the patient's enzyme will increase more than the rate of the normal enzyme

3) A runner's muscles use glucose as a source of energy. Muscle contains glycogen stores that are degraded into glucose 1-phosphate via glycogen phosphorylase, which is an allosteric enzyme. Assume that an allosteric enzyme has the following kinetic properties: a Vmax of 25 U/mg enzyme and a Km of 1.0 mM. These kinetic parameters were then measured in the presence of an allosteric activator. Which one of the following would best describe the findings of that experiment?

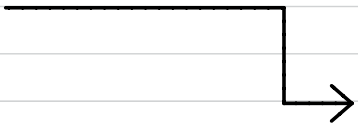
A. A Vmax of 25 U/mg enzyme and a Km of 0.2 mM
B. A Vmax of 15 U/mg enzyme with a Km of 2.0 mM
C. A Vmax of 25 U/mg enzyme with a Km of 2.0 mM
D. A Vmax of 50 U/mg enzyme with a Km of 5.0 mM
E. A Vmax of 50 U/mg enzyme with a Km of 10.0 mM

4. An enzyme-catalyzed reaction was carried out with the substrate concentration initially a thousand times greater than the Km for that substrate. After 9 minutes, 1% of the substrate had been converted to product, and the amount of product formed in the reaction mixture was 12 mmol. If, in a separate experiment, one-third as much enzyme and twice as much substrate had been combined, how long would it take for the same amount (12 mmol) of product to be formed?

A) 1.5 min
B) 13.5 min
C) 27 min
D) 3 min
E) 6 min

5. The following data were obtained in a study of an enzyme known to follow Michaelis-Menten kinetics:
Whats the km?

A) 1 mM.
B) 1,000 mM.
C) 2 mM.
D) 4 mM.
E) 6 mM.



tics:

V_0 (μmol/min)	Substrate added (mmol/L)
217	0.8
325	2
433	4
488	6
647	1,000

7. The double-reciprocal transformation of the Michaelis-Menten equation, also called the Lineweaver-Burk plot , To determine Km from a double- reciprocal plot, you would:

A) multiply the reciprocal of the x-axis intercept by -1.
B) multiply the reciprocal of the y-axis intercept by -1.
C) take the reciprocal of the x-axis intercept.
D) take the reciprocal of the y-axis intercept.
E) take the x-axis intercept where V0 = 1/2 Vmax.

8.The number of substrate molecules converted to product in a given unit of time by a single enzyme molecule at saturation is referred to as the:

A) dissociation constant.
B) half-saturation constant.
C) maximum velocity.
D) Michaelis-Menten number.
E) turnover number.

9.Vmax for an enzyme-catalyzed reaction:
A) generally increases when pH increases.
B) increases in the presence of a competitive inhibitor.
C) is limited only by the amount of substrate supplied.
D) is twice the rate observed when the concentration of substrate is equal to the Km.
E) is unchanged in the presence of a uncompetitive inhibitor.

10. A transition-state analog:

A) is less stable when binding to an enzyme than the normal substrate.
B) resembles the active site of general acid-base enzymes.
C) resembles the transition-state structure of the normal enzyme-substrate complex.
D) stabilizes the transition state for the normal enzyme-substrate complex.
E) typically reacts more rapidly with an enzyme than the normal substrate.

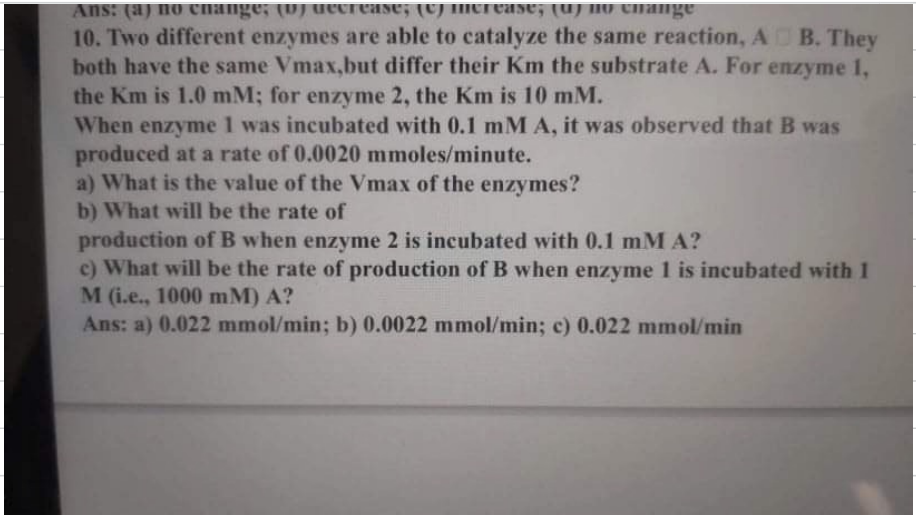
11. Penicillin and related drugs inhibit the enzyme ; this enzyme is produced by

A) b-lacamase; bacteria
B) transpeptidase; human cells
C) transpeptidase; bacteria
D) lysozyme; human cells
E) aldolase; bacteria

12. For a reaction that can take place with or without catalysis by an enzyme, what would be the effect of the enzyme on the:

(a) standard free energy change of the reaction?
(b) activation energy of the reaction?
(c) initial velocity of the reaction?
(d) equilibrium constant of the reaction?

Q13)


Ans: (a) no change; (b) decrease; (c) increase; (d) no change
10. Two different enzymes are able to catalyze the same reaction, A ⇌ B. They both have the same Vmax,but differ their Km the substrate A. For enzyme 1, the Km is 1.0 mM; for enzyme 2, the Km is 10 mM.
When enzyme 1 was incubated with 0.1 mM A, it was observed that B was produced at a rate of 0.0020 mmoles/minute.
a) What is the value of the Vmax of the enzymes?
b) What will be the rate of
production of B when enzyme 2 is incubated with 0.1 mM A?
c) What will be the rate of production of B when enzyme 1 is incubated with 1 M (i.e., 1000 mM) A?
Ans: a) 0.022 mmol/min; b) 0.0022 mmol/min; c) 0.022 mmol/min

a) What is the value of the Vmax of the enzymes?
b) What will be the rate of production of B when enzyme 2 is incubated with 0.1 mM A?
c) What will be the rate of production of B when enzyme 1 is incubated with 1 M (i.e., 1000 mM) A?

Q1	Q2	Q3	Q4	Q5		Q7	Q8	Q9	Q10	Q11
A	A	A	C	C		A	E	D	C	C

Q12 ⟶ (a) no change; (b) decrease; (c) increase; (d) no change

Q13 ⟶ a) 0.022 mmol/min; b) 0.0022 mmol/min; c) 0.022 mmol/min