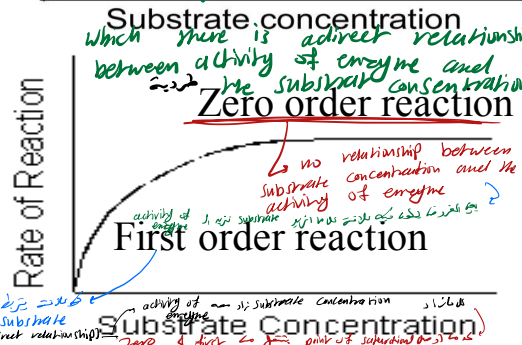
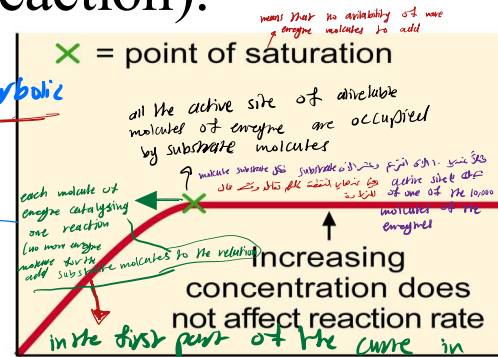


# Effect of substrate concentration

- At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.
- Higher concentrations cause more collisions between the molecules.
- The rate of reaction increases (First order reaction).
- The maximum velocity of a reaction is reached when the active sites are almost continuously filled.
- Increased substrate concentration after this point will not increase the rate.
- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).

The shape of the curve that relates activity to substrate concentration is hyperbolic.

*The curve is hyperbolic*  
*consist of two parts*  
*steady state*  
*in the activity of enzyme in response to the increase the substrate concentration*



# Michaelis-Menten Kinetics →

معادلة بيمونج الملائم بين  
substrate concentration and  
rate of enzymatic activity

↪ zero → There is no response  
in the enzymatic activity to any  
increase in the substrate  
concentration

- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction  $[V_i]$ , the concentration of substrate  $[S]$  and two constants,  $V_{max}$  and  $k_m$  (which are set by the particular equation).
- The symbols used in the Michaelis-Menten equation refer to the reaction rate  $[V_i]$ , maximum reaction rate ( $V_{max}$ ), substrate concentration  $[S]$  and the Michaelis-Menten constant ( $k_m$ ).

# Michaelis-Menten equation

-The dependence of initial reaction velocity on [S] and  $K_m$  may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

$$V_1 = \frac{V_{max}[S]}{K_m + [S]}$$

*Handwritten notes:*  
 -  $V_1$ : initial in the begin of the reaction  
 -  $V_{max}$ : maximum activity of the enzyme  
 -  $[S]$ : the substrate concentration  
 -  $K_m$ : Michaelis-Menten constant  
 -  $V_1$  is rate of enzymatic activity and the substrate concentration  
 -  $V_1$  is very little compare to  $V_{max}$  the  $K_m$  (very little compare to  $V_{max}$  the  $K_m$ )  
 -  $V_1 = k[S]$  (directly proportional to substrate concentration)

1- When [S] is much less than  $k_m$ , the term  $k_m + [S]$  is essentially equal to  $k_m$ .

$$V_1 = \frac{V_{max}[S]}{k_m}$$

Since  $V_{max}$  and  $k_m$  are both constants, their ratio is a constant (k).

In other words, when [S] is considerably below  $k_m$ ,  $V_{max}$  is proportionate to  $k[S]$ .

The initial reaction velocity therefore is directly proportionate to [S].

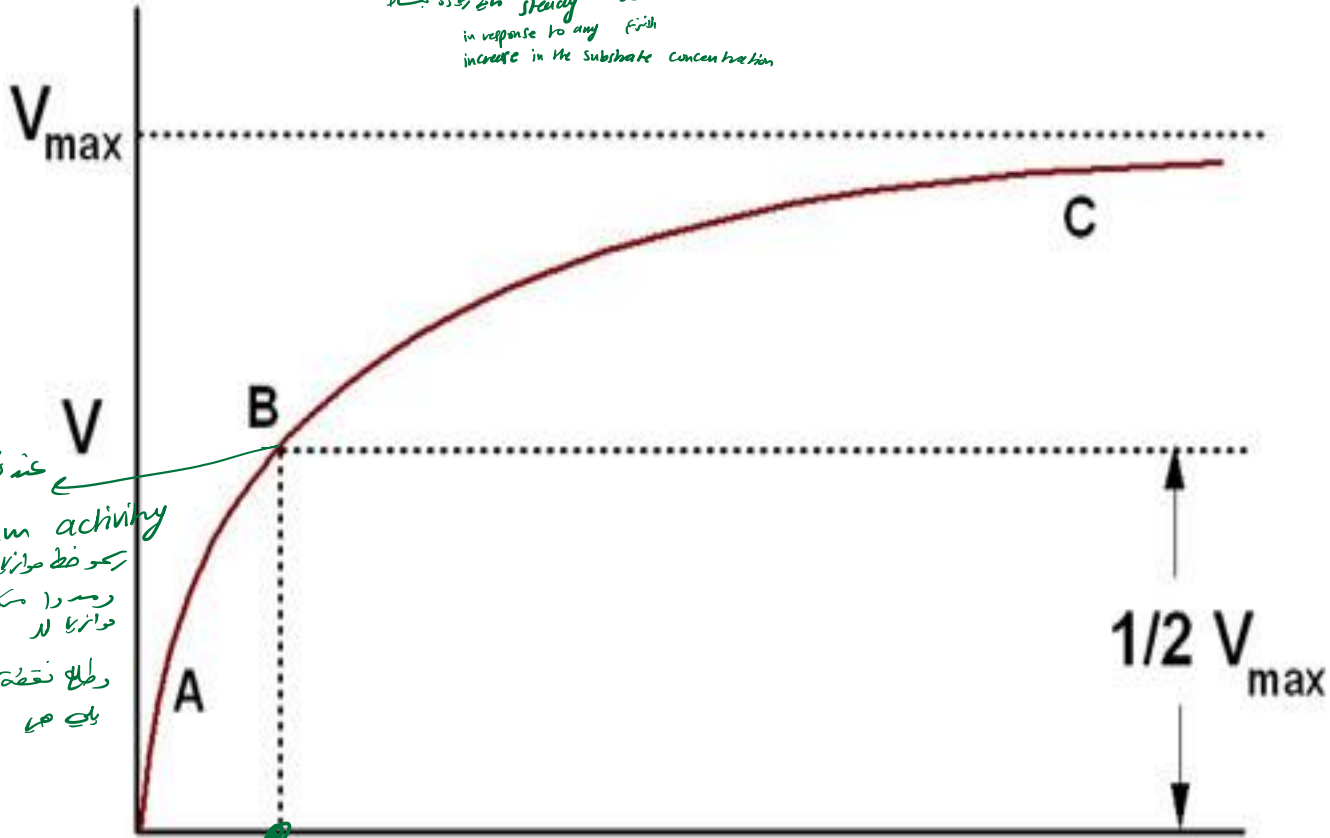
*Handwritten notes:*  
 -  $V_{max}$  is constant (ثابت)  
 -  $k_m$  is constant (ثابت)  
 -  $V_1$  is directly proportional to [S] (مستقيم)  
 -  $V_1$  is directly proportional to [S] during the first order reaction (مستقيم)  
 -  $V_1$  is directly proportional to [S] (مستقيم)  
 -  $V_1$  is directly proportional to [S] (مستقيم)



# Plot of substrate concentration versus reaction velocity

Substrate Concentration (مركب التفاعل) في الأنبوب المختبر، و  $V_{max}$  هي أقصى سرعة تفاعل

activity of enzyme (النشاط الإنزيمي) is equal to half of  $V_{max}$  (نقطة نصف التفاعل) (point of saturation) ←  $K_m$  (مركب التفاعل) steady state curve (منحنى الحالة المستقرة) in response to any further increase in the substrate concentration



$V_{max}$  maximum activity (السرعة القصوى للنشاط)  
 $x$ -axis (محور  $x$ )  
 $y$ -axis (محور  $y$ )  
 $K_m$  (مركب التفاعل) (نقطة نصف التفاعل)

Substrate concentration (مركب التفاعل)  $K_m$  (مركب التفاعل) is the concentration when the activity of enzyme is half max (النشاط الإنزيمي يساوي نصفه الأقصى).  $K_m$  is the substrate concentration at which the activity of enzyme is equal to half of  $V_{max}$ .

[S]

# Lineweaver-Burk Plot

اندازه المفعول  $v_i$  ←  
 خطی بود که  $v_i$  و  $[S]$  را نشان می‌دهد  
 خطی است که  $v_i$  و  $[S]$  را نشان می‌دهد  
 ← hyperbolic curve  
 ← S-shaped curve  
 sigmoidal curve  
 ← (1/v<sub>i</sub>) vs [S]

- A Linear Form of the Michaelis-Menten Equation is used to determine  $k_m$  &  $V_{max}$ .

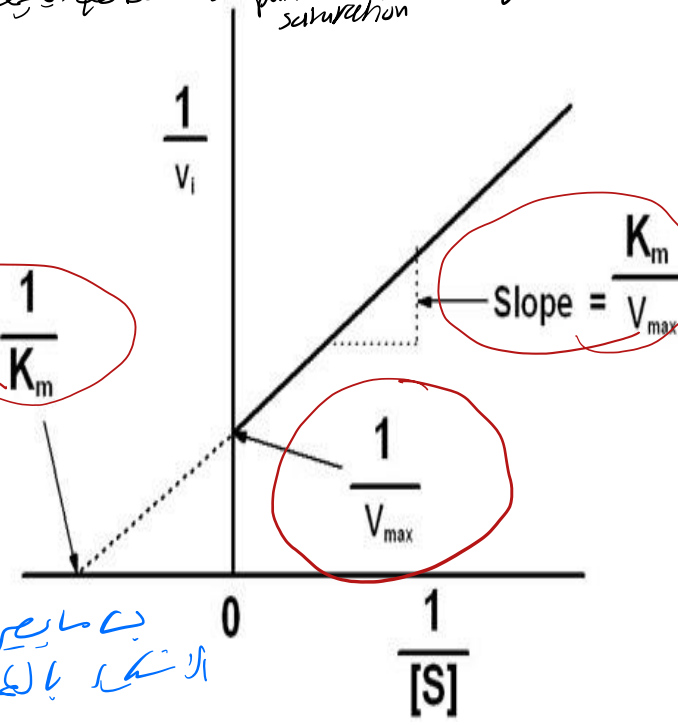
$$v_i = \frac{V_{max}[S]}{K_m + [S]} \quad \text{Invert} \quad \frac{1}{v_i} = \frac{K_m + [S]}{V_{max}[S]} \quad \text{factor} \quad \frac{1}{v_i} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

# Lineweaver-Burk Plot

- A plot of  $1/v_i$  as  $y$  as a function of  $1/[S]$  as  $x$  therefore gives a straight line whose  $y$  intercept is  $1/V_{max}$  and whose slope is  $k_m / V_{max}$ .
- Such a plot is called a double reciprocal or Lineweaver-Burk plot.

نقطه اشباع  $v_i$  →  $1/v_i$  →  $1/V_{max}$   
 point of saturation  
 line hyperbolic



به سبب این که  $v_i$  و  $[S]$  را نشان می‌دهد  
 این شکل با خطی است که  $v_i$  و  $[S]$  را نشان می‌دهد

# Km and its significance

- The Michaelis constant  $K_m$  is the substrate concentration at which  $V_i$  is half the maximal velocity ( $V_{max}/2$ ) attainable at a particular concentration of enzyme

- It is specific and constant for a given enzyme under defined conditions of time, temperature and pH

acting in all hexoses include glucose ← hexokinases enzyme and acting in glucose ← glyceraldehyde enzyme

-  $K_m$  determines the affinity of an enzyme for its substrate, lesser the  $K_m$  for is the affinity and vice versa, it is inversely proportionate to the affinity

affinity → substrate concentration → maximum activity

-  $K_m$  value helps in determining the true substrate for the enzyme.

by the knowledge of  $K_m$  we can detect a particular enzyme and we can detect a particular substrate

# **Enzymology- An overview-3**



very little amount of inhibitor can inhibit the activity...  
inhibitor in a high concentration  
it doesn't mean completely stopping the activity of the enzyme  
activity is less than that of the enzyme

# Enzyme Inhibition

→ doesn't mean stopping the activity of the enzyme but mean reducing the activity of enzyme by chemical molecule

- Inhibitors are chemicals that reduce the rate of enzymatic reactions.
- They are usually specific and they work at low concentrations.
- They block the enzyme but they do not usually destroy it.
- Many drugs and poisons are inhibitors of enzymes in the nervous system.
- Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for study of the mechanism of enzyme action.

# The effect of enzyme inhibition

- Irreversible inhibitors: combine with the functional groups of the amino acids in the active site, irreversibly. *if the inhibitor is binding to a functional group in the active site of enzyme by covalent bond or non-covalent bond stopping the activity of enzyme is irreversible*
- Reversible inhibitors: these can be washed out of the solution of enzyme by dialysis. *binding with loosely bond*

## Classification: based on:

- Their site of action on the enzyme, *صحة inhibition في ال active site*
- Whether they chemically modify the enzyme, *ان تترك ال enzyme ال active site*
- The kinetic parameters they influence. *تغير ال kinetic parameters*

## Types of enzyme inhibition

- Competitive inhibition
- Non Competitive inhibition
- Uncompetitive inhibition
- Suicidal inhibition
- Allosteric inhibition
- Feed back inhibition

# Competitive enzyme inhibition

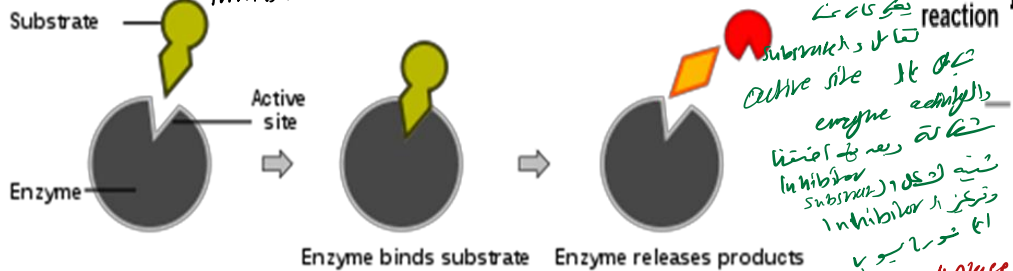
**A competitive inhibitor** → it has denoted appreciation → inhibitor as target for researchers to act for inhibiting certain enzyme and can be use clinically

- Has a structure similar to substrate (structural Analog)
- Occupies active site
- Competes with substrate for active site
- Has effect reversed by increasing substrate concentration
- $V_{max}$  remains same but  $K_m$  is increased

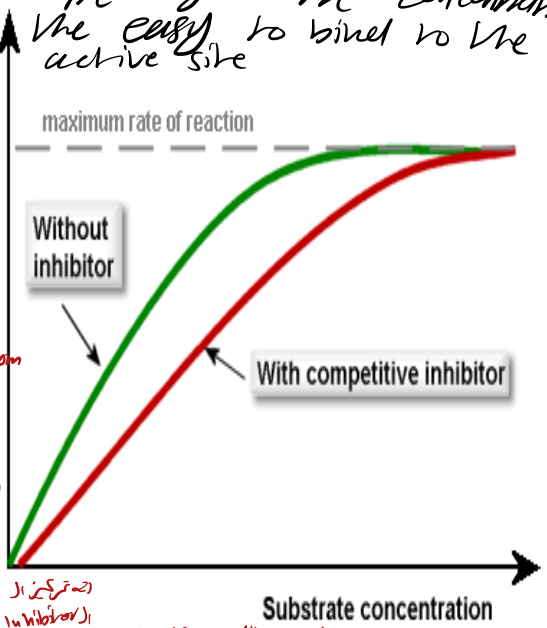
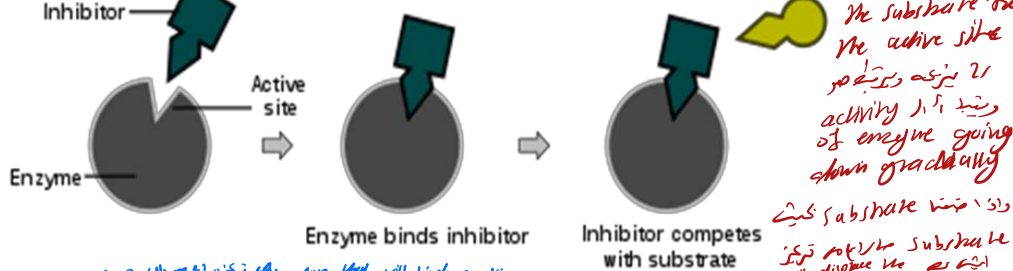
The inhibitor must be similar to the shape of substrate and both can bind to the active site the higher the concentration the easier to bind to the active site

if we have an enzymatic reaction and the substrate is binding to the active site and we start to add an inhibitor similar to the structure to the substrate

(a) Reaction



(b) Inhibition



Rate of reaction  
 active site  
 enzyme  
 substrate  
 inhibitor  
 it will displace the substrate from the active site  
 activity of enzyme going down gradually  
 and both can bind to the active site  
 the higher the concentration the easier to bind to the active site  
 it will displace the substrate from the active site and going up to the action again

دو تریزوں کے درمیان ایک

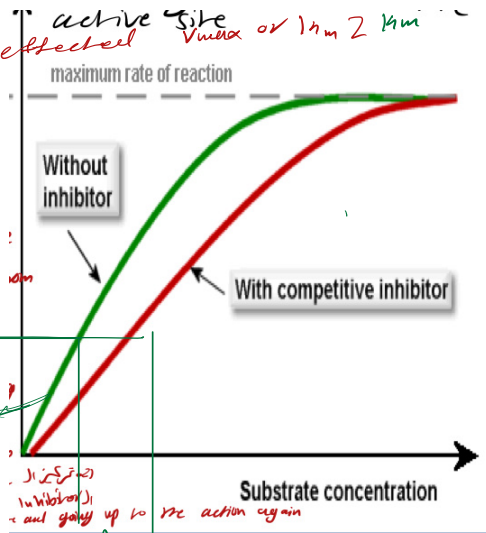
انہیں ایک جگہ جانا پڑے گا اور انہیں جگہ جگہ سے ہٹانا پڑے گا

وہاں سے انہیں ہٹانا پڑے گا اور انہیں جگہ جگہ سے ہٹانا پڑے گا

وہاں سے انہیں ہٹانا پڑے گا اور انہیں جگہ جگہ سے ہٹانا پڑے گا

- competitive inhibitor
- 1- similar to the structure of substrate
  - 2- they are binding to the active site and there is displacement

which type of kinetic will be affected



there is increase in the activity of enzyme and response to the increase in the substrate concentration

$K_m$  without inhibitor is less than  $K_m$  with the inhibitor

Competitive inhibitor is acting in one kinetic the action will go to the same level

which means that inhibitor is decreasing to its substrate activity to the original level

the present of the affinity of enzyme

# Clinical significance of competitive enzyme inhibitors

Drug	Enzyme Inhibited	Clinical Use
Dicoumarol	Vitamin K Epoxide Reductase <i>it will inhibit the process of blood coagulation</i>	Anticoagulant <i>blood coagulation</i>
Sulphonamide	Pteroid Synthetase <i>أو الازترجات إلى يتسامم بالخصية الفولوية اسم الحاد من completion V B-12 لا ينتج م الكبار</i>	Antibiotic
Trimethoprim	Dihydrofolate reductase	Antibiotic
Pyrimethamine	Dihydrofolate reductase <i>is an essential enzyme in cell division</i>	Antimalarial
Methotrexate	Dihydrofolate reductase <i>responsible for activating the folic acid to the active form (tetrahydro folate)</i>	Anticancer <i>chemotherapy</i>
Lovastatin	HMG CoA Reductase	Cholesterol Lowering drug
Alpha Methyl Dopa	Dopa decarboxylase	Antihypertensive
Neostigmine	Acetyl Cholinesterase	Myasthenia Gravis

تتعلق  
بثقب  
الانتركم

it is participating in production of the glutates which are needed for the DNA replication and cell division

HMG CoA Reductase is the main enzyme in the cholesterol synthetic pathway

it will inhibit the HMG CoA Reductase enzyme and this will reduce the production of cholesterol and it can be use for a treatment of cases hypercholesterolemia

# Non competitive enzyme inhibition

There is no similarity in the structure between the inhibitor and the substrate. The inhibitor is going to bind in another site.

- Noncompetitive inhibitors bind enzymes at sites distinct from the substrate-binding site.
- Generally bear little or no structural resemblance to the substrate.

its particular site and this is not preventing binding to the active site → enzyme-substrate-inhibitor complex

Substrate is not preventing the binding of substrate to the active site. Inhibitor is not preventing the binding of substrate to the active site.

- Binding of the inhibitor does not affect binding of substrate.
- Formation of both EI and EIS complexes is therefore possible.

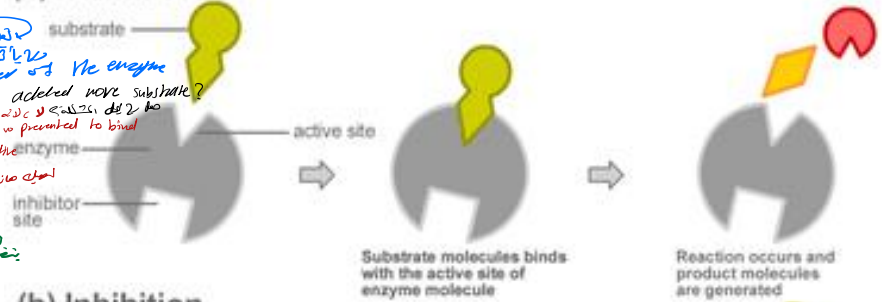
increasing the efficiency of the enzyme to convert substrate into product

- The enzyme-inhibitor complex can still bind substrate, its efficiency at transforming substrate to product, reflected by

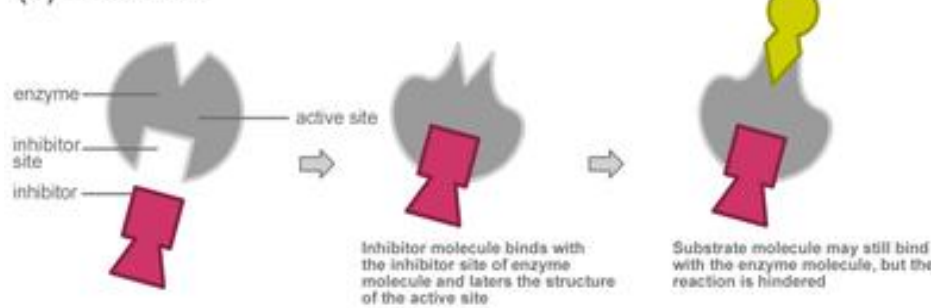
$V_{max}$  is decreased.

related to the activity of enzyme and the turn over number. Inhibitor will not affect the rate of reaction.

(a) Reaction



(b) Inhibition



the activity of enzyme will be decreased

# Examples of non competitive enzyme inhibitors

- Cyanide inhibits cytochrome oxidase.

هذا من سلسلة التنفس (المتنفس الخلوي) انه يفر بالبروتينات ومواد كيميائية اخرى ولا يتكون من واحد بل من عدة مكونات

- Fluoride inhibits enolase and hence glycolysis.

electron transport chain

في الجهاز الهضمي لا يستطيع ان يتكون من اكثر من مكون اخر وان كان مكونا من عدة اشياء لا يتكون من واحد بل من عدة مكونات  
Cytochrome oxidase enzyme if cyanide is a non competitive inhibitor of cytochrome oxidase enzyme it will decrease the energy production

- Iodoacetate inhibits enzymes having SH groups in their active sites.

وهذا لا يستطيع ان يتكون من عدة اشياء لا يتكون من واحد بل من عدة مكونات

The Effects of Inhibition on Enzyme Kinetics

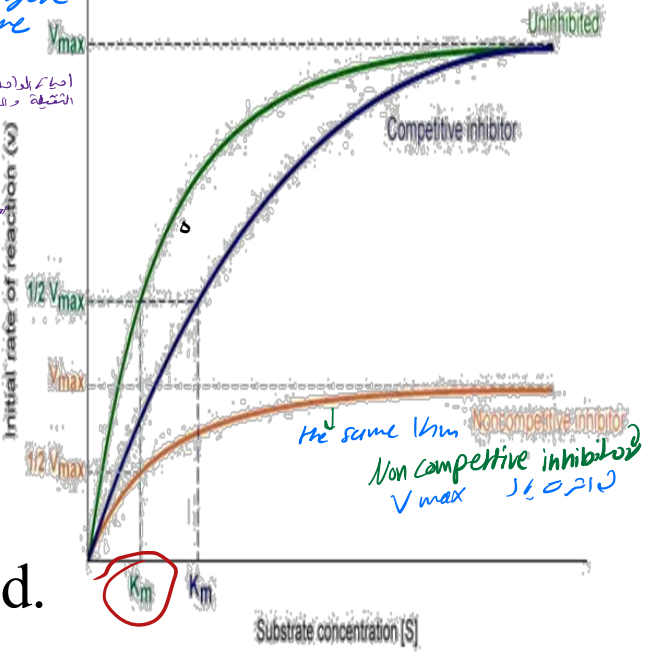
- BAL ( British Anti Lewisite, dimercaprol) is used as an antidote

glycerol dehydrogenase enzyme

phosphat dehydrogenase

for heavy metal poisoning

انما الوباء يسبب بالمعادن الثقيلة والمعادن الثقيلة تلتصق بالبروتينات وتكون الديمر كبريتول BAL لها القدرة على ان تجذب ايون المعادن الثقيلة الى مجموعة SH في انزيم وتكون الديمر كبريتول BAL لها القدرة على ان تجذب ايون المعادن الثقيلة الى مجموعة SH في انزيم



- Heavy metals act as enzyme poisons by reacting with the SH groups, BAL has several SH groups with which the heavy metal ions bind and thereby their poisonous effects are reduced.

# Uncompetitive enzyme inhibition

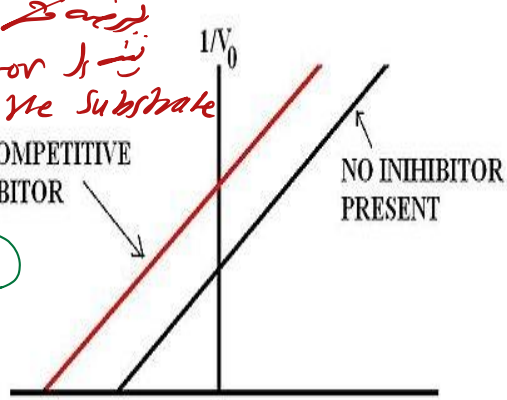
→ *نوع من التثبيط*  
*تثبيط* *في* *مركب* *الإنزيم* *والمركب*  
*والركب*

- Inhibitor binds to enzyme-substrate complex

*is not going to bind to the active site and it will bind at another site*

UNCOMPETITIVE INHIBITOR

NO INHIBITOR PRESENT



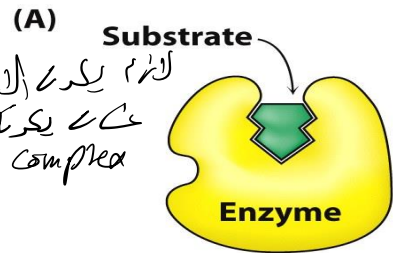
- Both  $V_{max}$  and  $K_m$  are decreased

Such as ; Inhibition of placental alkaline phosphatase

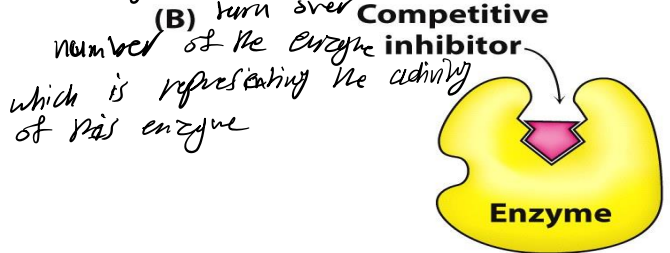
(Regan isoenzyme) by phenylalanine

in this case both  $1/[S]$  will go down  
 $V_{max}$  will go down and  $K_m$  will go down because it will affect the number of the enzyme inhibitor which is representing the activity of this enzyme

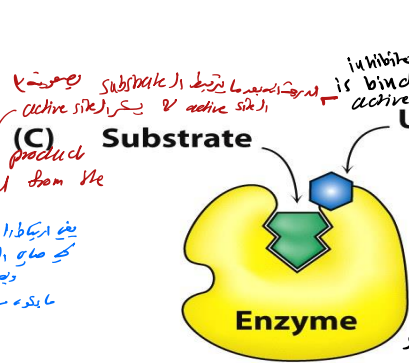
Competitive V/S  
 non competitive V/S  
 uncompetitive  
 enzyme inhibition



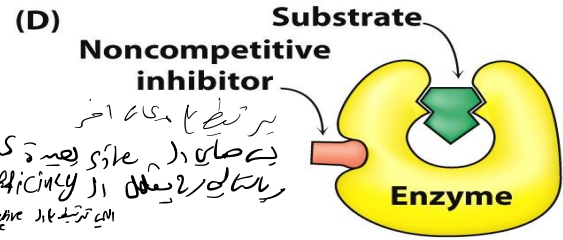
*Substrate يركب في الموقع النشط للإنزيم*  
*enzyme substrate in inhibitor complex*



number of the enzyme inhibitor which is representing the activity of this enzyme

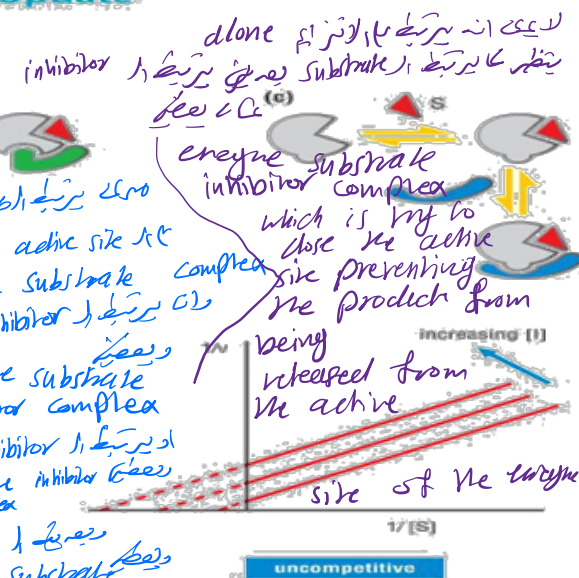
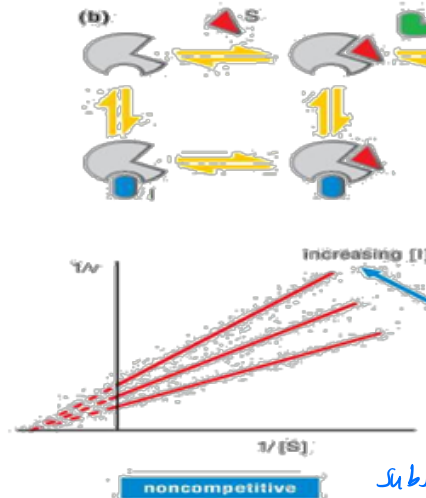
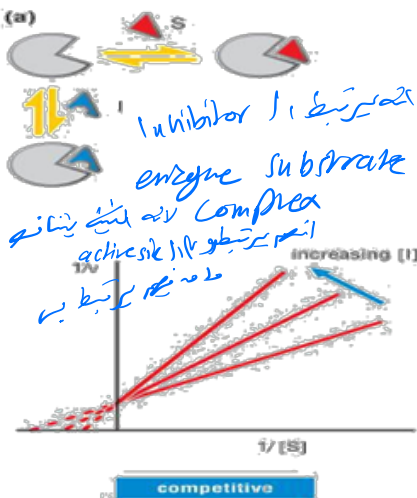


*inhibitor يركب في الموقع النشط للإنزيم*  
*is binding very close to the active site*  
*preventing the product to be released from the active site*  
*active site: لا يستطيع الركيب في الموقع النشط للإنزيم*  
*active site: لا يستطيع الركيب في الموقع النشط للإنزيم*  
*inhibitor يركب في الموقع النشط للإنزيم*  
*is not binding to enzyme alone*

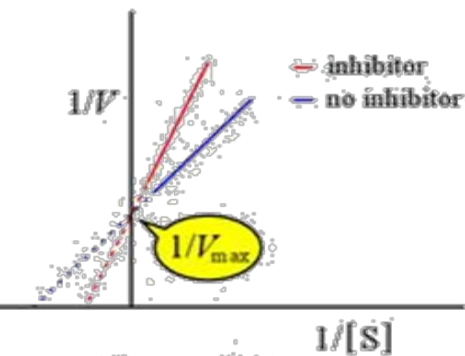


*active site*  
*substrate molecules product*  
*inhibitor يركب في الموقع النشط للإنزيم*  
*is not binding to enzyme alone*

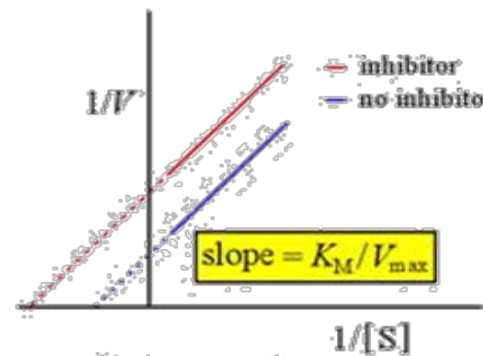




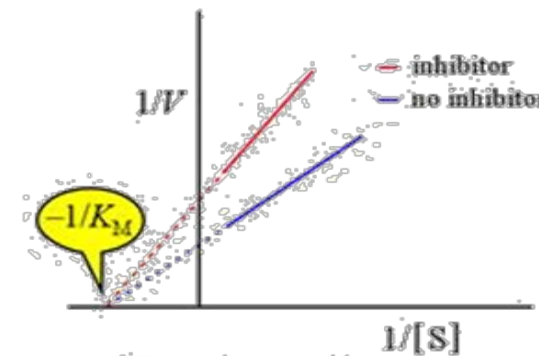
The Lineweaver-Burk plots for inhibition



**Competitive inhibition**  
 $K_M$  increased  
 $V_{max}$  unaffected



**Uncompetitive inhibition**  
 $K_M$  reduced  
 $V_{max}$  reduced



**Noncompetitive inhibition**  
 $K_M$  unaffected  
 $V_{max}$  reduced

انتزاعی

# Suicidal inhibition

inhibitor ہے بعد inhibition ہو گیا active

- Irreversible inhibition

the enzyme is self activation

اصول سے کہہ سکتے ہیں کہ بعض انٹوزیم کے انٹوزیم کے طور پر

active < inactive inhibitor

irreversibly inhibition added being converted from being inactive into active by the enzyme itself

Suicidal Inhibition

یہ اپنے ہی گروپ کو بند کر دیتا ہے functional group inhibiting this enzyme irreversibly

- Structural analog of the substrate is converted to more effective inhibitor with the help of enzyme to be inhibited.

- The new product irreversibly binds to the enzyme and inhibits further reaction.

- Such as ;

**Ornithine decarboxylase**: is irreversibly inhibited by

**difluormethyl ornithine**, as a result multiplication of

parasite is arrested .

use for suicidal inhibition of ornithine decarboxylase enzyme → is essential for cell division

Used against trypanosome in sleeping sickness

difluoromethyl ornithine to inhibit ornithine decarboxylase enzyme this will stop the multiplication of the parasite can be used as treatment

Suicidal inhibition of ornithine decarboxylase enzyme to stop the cell division as anti parasite treatment



# Allosteric inhibition

2- which type of inhibitor will be affected:  $V_{max}$  +  $K_m$  enzyme kinetics  $\rightarrow$   $V_{max}$  +  $K_m$   
 2- the application to this enzyme inhibitor

- Some enzymes have **other site** (allosteric site) similar but different from the active site which may or may not physically adjacent to the active site.

*means that the inhibitor is going to inhibit the enzyme by binding to another site and this word (Allosteric) is not applied to enzymes only but it also applied to group of proteins called Allosteric proteins like hemoglobin*

- This **site binds an effector** called the allosteric effector that may be an **activator** (positive modifier) or inhibitor (negative modifier):

*negative modifier*  $\rightarrow$  *Allosteric site*  $\rightarrow$  *positive modifier*  
*Some conformational changes*  $\rightarrow$  *negative modifier*  $\rightarrow$  *positive modifier*  
*improving the active site (making the active site suitable for binding the substrate to activate reaction)*

- The allosteric effector is usually a metabolite or a product resulting from the process of metabolism.

*and this will also cause some conformational changes in the active site making the active site unfit or unsuitable or unavailable for binding the substrate to the active site*

- Enzymes having these sites are called allosteric enzymes.

لو تكلمنا عن تركيب الهيموجلوبين كيانا IS -  
 it is consisted of 2 $\alpha$  and 2 $\beta$   
 chain and the center of each  
 one of the globin chains one  
 heme molecule and in the center  
 of heme molecule we have ferrous ion

How many molecules of O<sub>2</sub> can be carried out by hemoglobin

4  
 Are the 4 O<sub>2</sub> molecules binding to hemoglobin molecules one time or gradually  
 gradually (it is cooperatively) because  $\alpha$  and  $\beta$  globin chain are linked together  
 by bonds and the two dimers in hemoglobin molecules are linked  
 together by a group of bonds

what we called no O<sub>2</sub> binding; (تسمى الحالة التي لا يوجد فيها O<sub>2</sub> مرتبطة بالهيموجلوبين)  
 to the hemoglobin molecule (in which state is hemoglobin molecule?)  
 in a tense state (tight state)

hemoglobin molecule or O<sub>2</sub> step by step from tight (tense) state to relax state  
 cooperatively

why the O<sub>2</sub> molecule are not binding one time to hemoglobin  
 molecule  
 because the first O<sub>2</sub> molecule binding to hemoglobin molecule  
 will change the shape of hemoglobin molecule and this will help the second O<sub>2</sub> molecule  
 to bind to hemoglobin molecule

ferrous oxidation

ferrous iron  
 reduced form (ferrous)  
 oxidized form (ferric)

it will not carry O<sub>2</sub>  
 hemoglobin  
 med hemoglobin

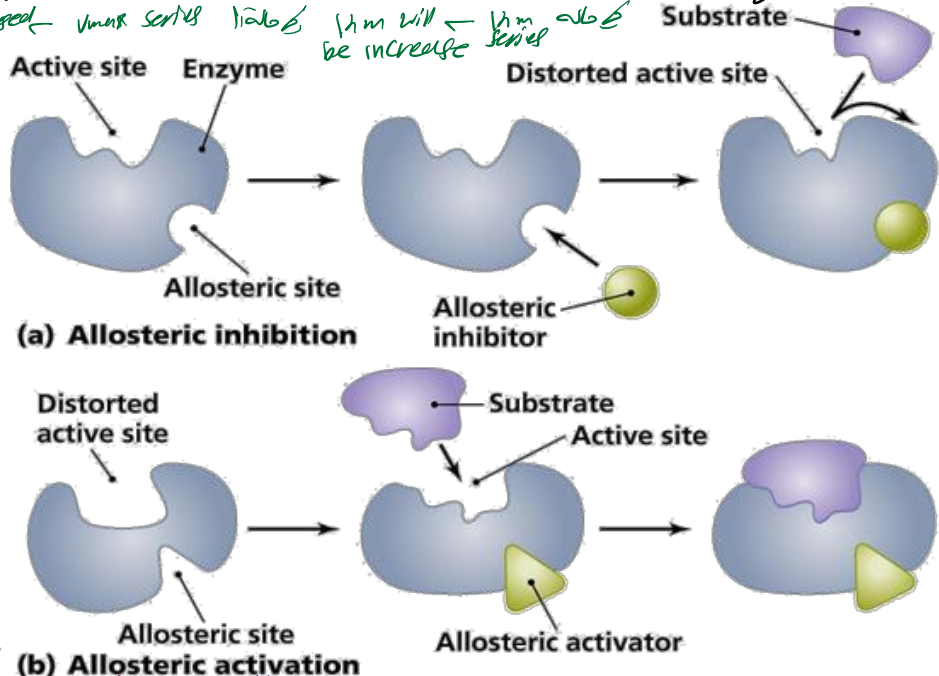
Cooperatively between the molecules in binding O<sub>2</sub> molecules to the heme molecules  
 one by one to avoid the oxidation of the ferrous  
 H ferrous ions  
 ferrous in the 4 heme molecules  
 med hemoglobin and the med hemoglobin is unable to carry O<sub>2</sub>

one by one  
 cooperatively between the 4 different heme molecules  
 we have an enzyme inside the erythrocyte  
 med hemoglobin reductase  
 what can be oxidized from the hemoglobin into med hemoglobin

- Inhibitor is not a substrate analogue. *and this type of inhibition is partially reversible if we increase the concentration*
- Partially reversible, when excess substrate is added.
- $K_m$  is usually increased (K series enzymes). *binding of inhibitor is decreasing the activity of enzyme*
- $V_{max}$  is reduced (V series enzymes). *activity with which concentration of the substrate*
- When the inhibitor binds the allosteric site, the configuration of the active site is changed so that the substrate can not bind properly. *the binding of inhibitor will affect the  $V_{max}$  (maximum activity of the enzyme)  $V_{max}$  will be decreased.  $V_{max}$  series  $K_m$  will be increase*
- Most allosteric enzymes possess quaternary structure. *Cooperativity of the polypeptide chain - one polypeptide chain*

*the inhibition of this enzyme or the activation of this enzyme is not taken place one time consisted of 4 polypeptide chains and the 4 polypeptide chains are linked together by group of bonds tertiary & quaternary structure*

*positive modifier or stimulator or activator it means that the binding of activator will taken place gradually*

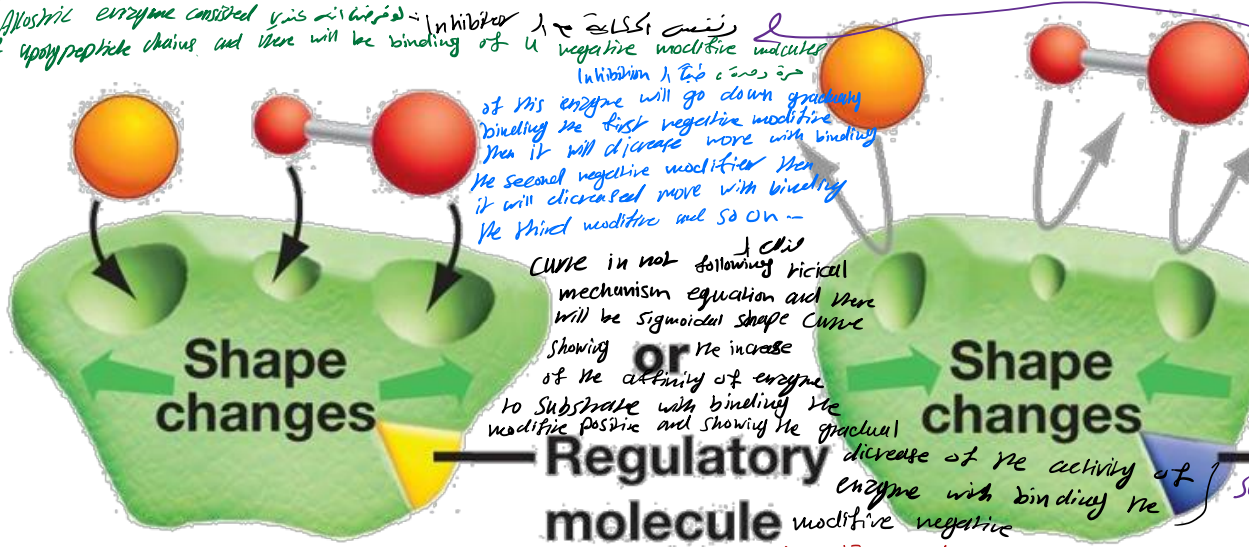


with breathing down the bonds between the poly peptide chains one by one (step by step) *حسب خطوة واحدة في كل مرة*

# (b) Allosteric regulation

*في الموديفر رصه دياتلبي رصه دياتلبي*  
 is going to increase the activity of the enzyme gradually  
*حسب خطوة واحدة في كل مرة*

*Allosteric enzyme considered as a dimer of two polypeptide chains and there will be binding of inhibitor*



*Allosteric*  
 is not hyperbolic dual is not a linear but it is sigmoidal (S) shaped curve  
 maximum activity *الحد الأقصى*

*Inhibitor* *مركب مثبط*  
 negative modifier *مركب مثبط*  
 of this enzyme will go down gradually  
 binding the first negative modifier  
 then it will decrease more with binding  
 the second negative modifier then  
 it will decrease more with binding  
 the third modifier and so on -

*Curve is not following Michaelis-Menten mechanism equation and there will be sigmoidal shape curve*  
 showing or the increase of the affinity of enzyme to substrate with binding the modifier positive and showing the gradual decrease of the activity of enzyme with binding the modifier negative

*breathing down of the bonds between the poly peptide chains there will be gradual increase in the affinity of the enzyme to its*

*Conformational changes in the active site of the enzyme*  
 the binding of modifier will cause

**Allosteric activation**  
 The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme.

**Allosteric deactivation**  
 The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

# Switching off

- When the inhibitor is present it fits into its site and there is a conformational change in the enzyme molecule.
- The enzyme's molecular shape changes.
- The active site of the substrate changes.
- The substrate cannot bind with the substrate and the reaction slows down.
- When the inhibitor concentration diminishes the enzyme's conformation changes back to its active form.

- This is not competitive inhibition but it is reversible

erythrocytic contain hemoglobin and responsible for carry O<sub>2</sub> -> 1- the cell should contain mitochondria  
 cannot reduce energy under aerobic condition -> 2- it should receive sufficient amount of O<sub>2</sub>  
 Condition because they are not contain mitochondria  
 will produce energy under anaerobic condition -> anaerobic condition  
 metabolic pathway  
 under aerobic condition  
 under anaerobic condition  
 Condition

**Example: Phosphofructokinase -1 (PFK-1)** -> the main enzyme of glycolysis  
 It catalyzes phosphorylation of fructose-6-phosphate into fructose 1, 6 biphosphate  
 Conformational changes  
 allosteric site in PFK-1  
 will produce energy under aerobic condition  
 under anaerobic condition to produce energy  
 glycolysis is producing energy in producing energy  
 under aerobic condition  
 under anaerobic condition  
 both conditions  
 which can be oxidized under

- It has an allosteric site for an ATP molecule (the inhibitor).

PFK-1 is one of allosteric enzymes which are having other sites for binding a modifier  
 more suitable for binding the substrate  
 allosteric site in PFK-1  
 lower form of energy (ADP)  
 switching on of the enzyme by modifier positive  
 2 molecules of inhibitor  
 & the highest form of energy (ATP)  
 switching off of the enzyme by modifier negative



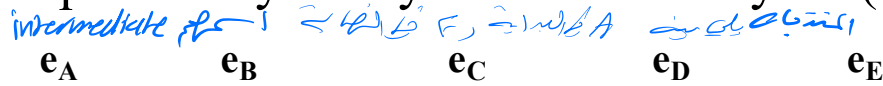
- When the level of ATP in the cell falls ( $\uparrow$  ratio of ADP to ATP) no ATP binds to the allosteric site of PFK-1, so, the enzyme's conformation changes and the active site accepts substrate molecules causing activation of glycolysis.
- The respiration pathway accelerates and the level of ATP in the cell increases ( $\uparrow$  ratio of ATP to ADP) in the cell, ATP molecules can fit into the allosteric site of PFK-1 molecules.
- The enzyme's conformation changes again and stops accepting substrate molecules in the active site
- Respiration slows down

تذكر الكلام :- انه صاندا لانتزاع الجسم منه توحسب به صا ATP و ADP  
 اذا زادت كمية الطاقة في الخلية بحدوث ATP يرتبط بالallostic site ولا ترتبط بالallostic site وتكون في حالة سكون وتكون في حالة سكون  
 اما لو قلت كمية الطاقة بحدوث ADP يرتبط بالallostic site وتكون في حالة سكون وتكون في حالة سكون  
 activation

# Feed back(end point) inhibition ← انحصار نقطة النهاية عبر تثبيط المتتالي

- Cell processes consist of series of pathways controlled by enzymes.

Each step is catalyzed by a different enzyme ( $e_A, e_B, e_C$  etc).



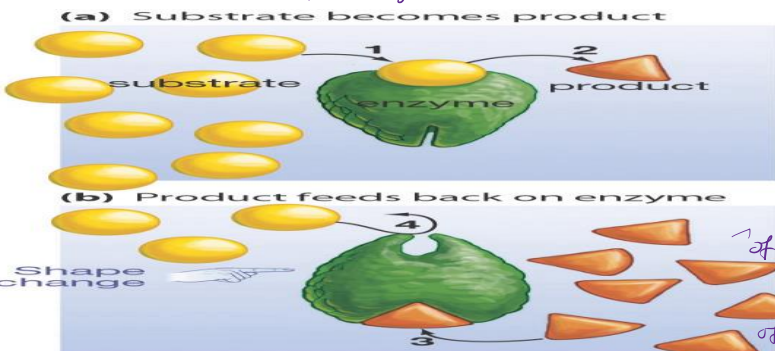
is going to inhibit the activity of the enzyme catalyzing the first reaction B or A or C or D or E intermediate, لا يمكن تثبيطه إلا بمنتجاته لا يمكن تثبيطه إلا بمنتجاته cannot be inhibited by the intermediate

- The first step (controlled by  $e_A$ ) is often controlled by the end product (F), therefore negative feedback is possible (end products are controlling their own rate of production, no build up of intermediates (B,C, D and E)).

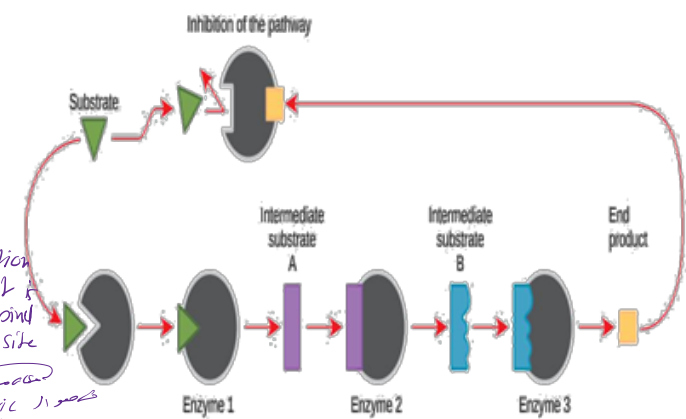
inhibition of (and product) ← body accumulates product and product inhibition of first enzyme of the pathway, body will accumulate product and product inhibition of first enzyme of the pathway

- Usually such end product inhibition can affect allosterically.

Accumulated product binds at a site other than the active site to bring about conformational changes, so as to inhibit the binding of the substrate and the reaction rate declines.



accumulation of the product is going to bind to a site of enzyme



(typical mechanism mental) ← انحصار نقطة النهاية عبر تثبيط المتتالي

Could be Km-series or Vmax-series

Feed back inhibition منع التغذية الراجعة

consisted of one polypeptide chain with quaternary structure تتكون من سلسلة بوليببتيد واحدة ببنية الرباعي

allosteric enzymes الإنزيمات البديلة  
one binding are binding to another site  
feed back inhibited منع التغذية الراجعة is binding to another site  
that causes conformational changes in the active site  
making the site unsuitable for binding the substrate

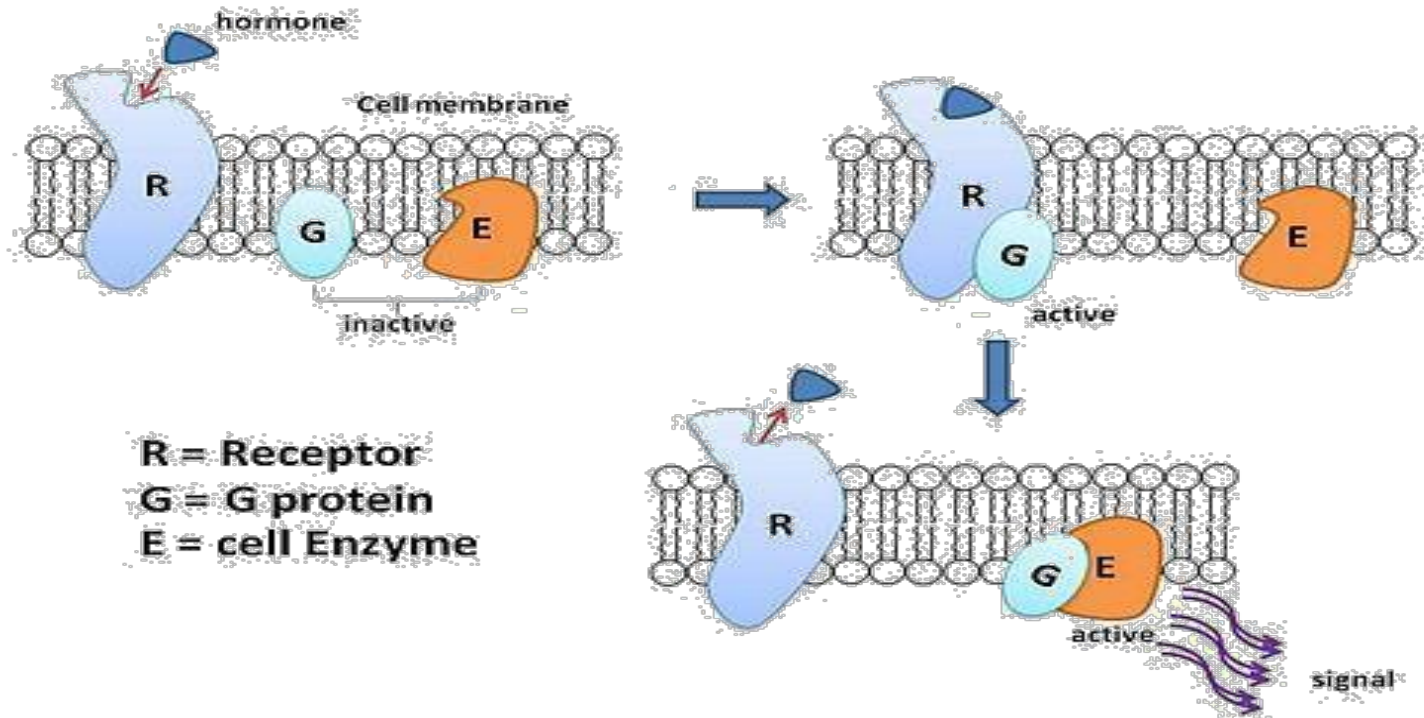
other site - موقع آخر  
allosteric inhibition التثبيط البديلي

Feed back inhibition التثبيط بالتغذية الراجعة

# Enzymology- An overview-4

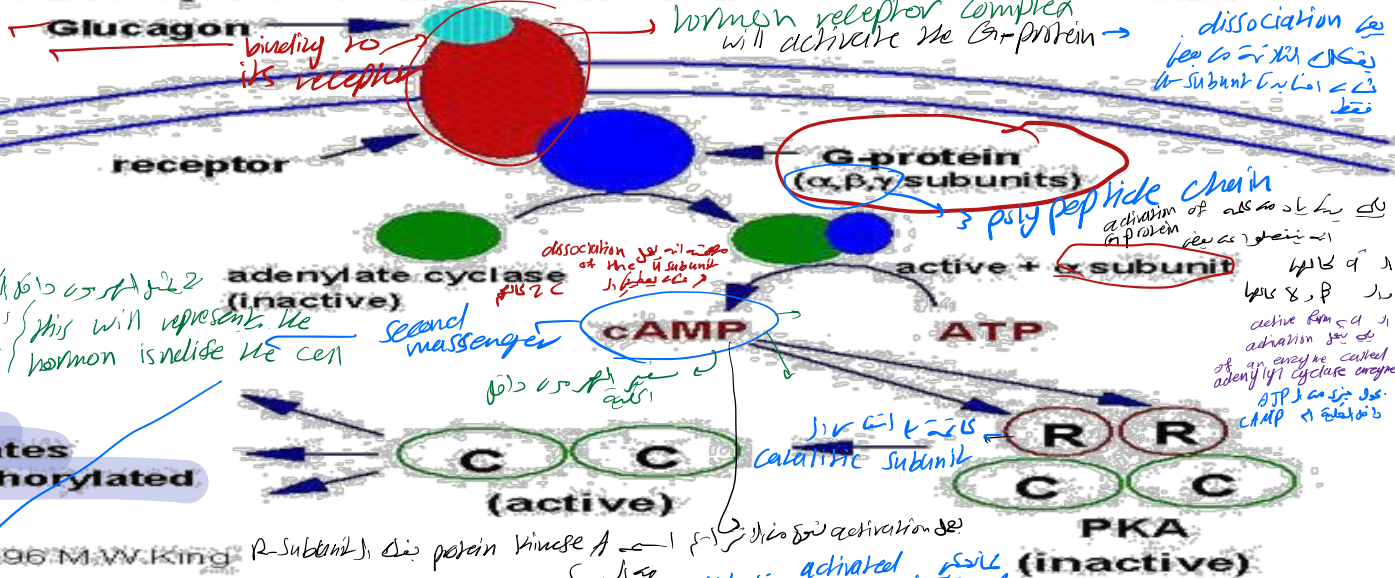


- Usually by the addition of or lysis of phosphate ( $\text{PO}_4$ ) groups to and from enzymes.
- Some enzymes are active when phosphorylated, while, others are inactive when phosphorylated.

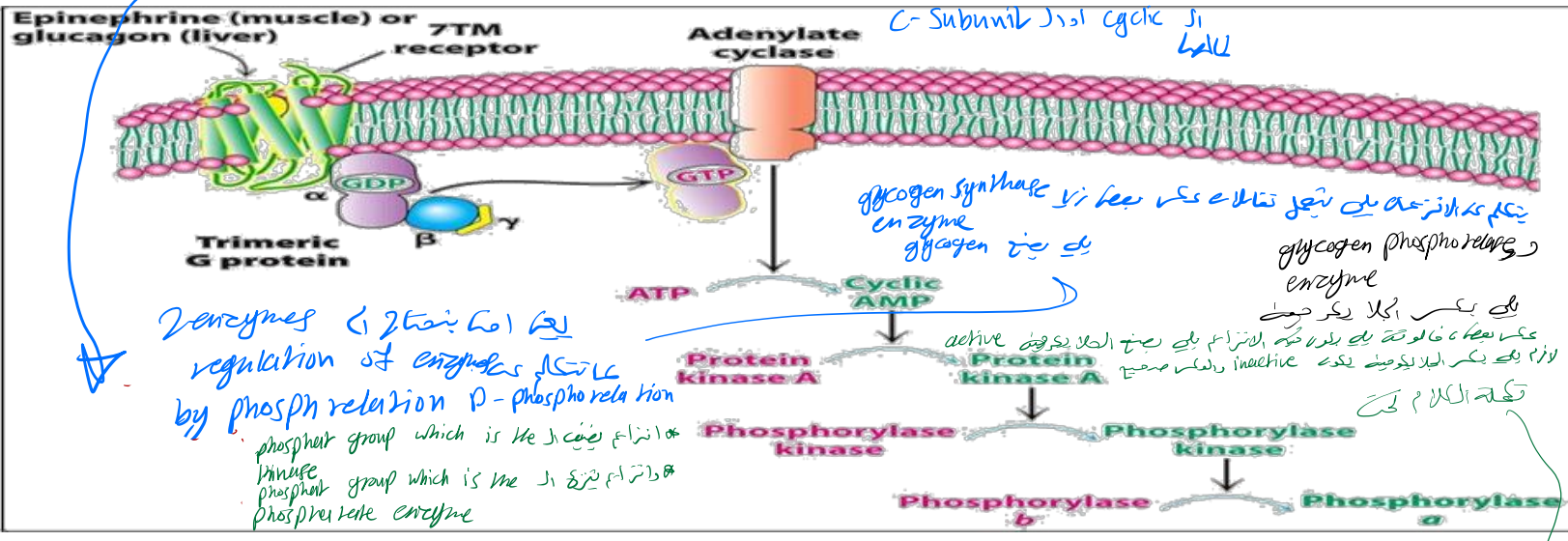


# Receptor-Mediated Activation of PKA

hydrophilic hormone



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enzymes regulation by phosphorelation

P-phosphorelation

phosphat group which is the phosphate enzyme

phosphat group which is the phosphate enzyme

glycogen synthase enzyme

glycogen

glycogen phosphorylase enzyme

active Protein kinase A

Phosphorylase kinase

Phosphorylase b

Phosphorylase a

phospholation of the two enzymes in the same time  
 activation after removal of the R-subunit  
 kinase enzyme

glycogen & phosphate group is glycogen synthase phosphate group is phosphorylase  
 inactive & active  
 glycogen phosphorylase → phosphate group to be active

for activating an enzyme phosphate group

glycogen synthase inhibition  
 kinase enzyme  
 active D-phosphorelated as glycogen synthase

inactive D-phosphorelated glycogen phosphorylase enzyme inactive phosphorelated

phosphatase enzymes active form for the removal of the phosphate group from the two enzymes in the same time

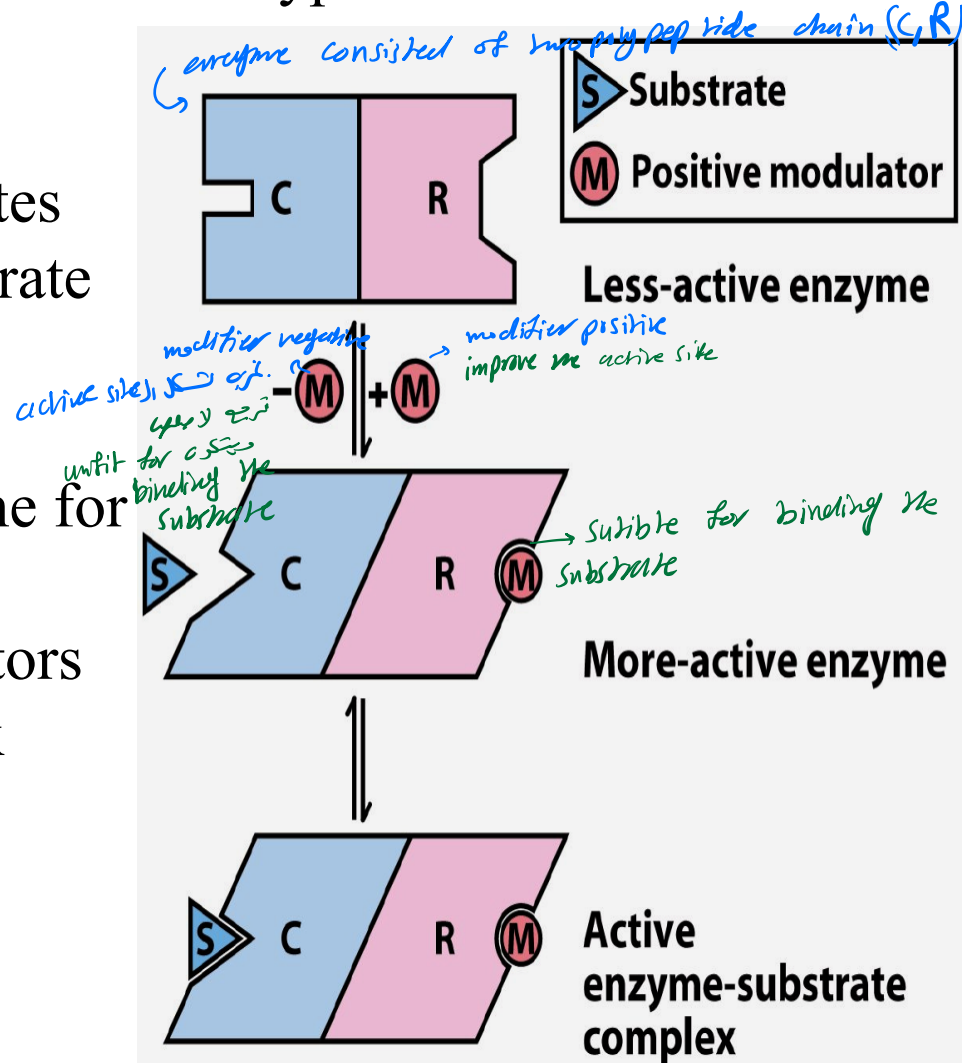
inactive & active active & inactive phosphate group

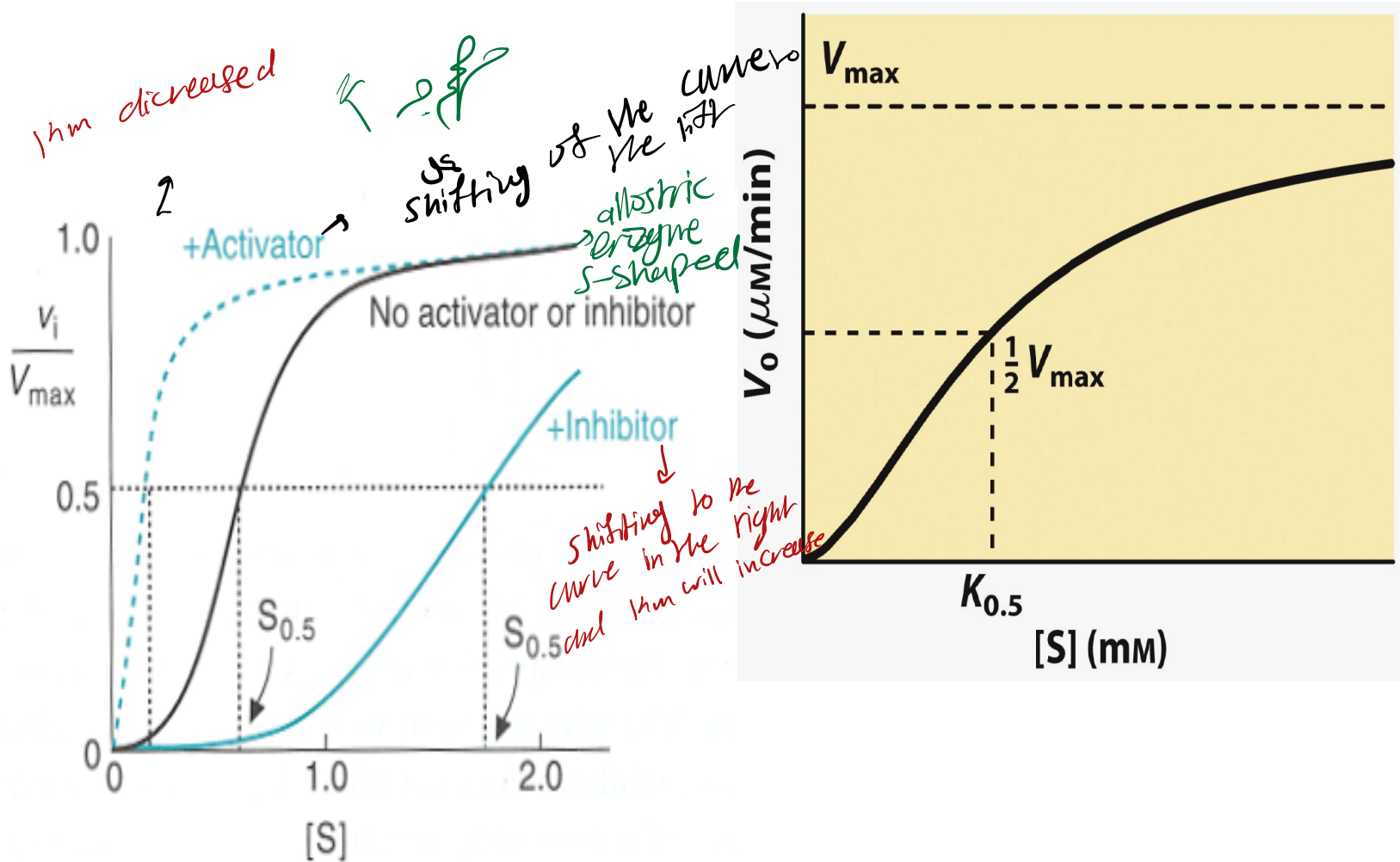
## **B- Allosteric regulation:**

- Allosteric regulation is the term used to describe cases where an enzyme is functioning at one site, then, affected by binding of a regulatory molecule at another site.
- Allosteric regulation may either inhibit or stimulate an enzyme activity by changing the enzyme either to its active or inactive forms.
- The binding of an allosteric activator stabilizes its active form, while binding the allosteric inhibitor stabilizes the inactive form of the enzyme.
- End products are often inhibitors.
- Often allosteric modulators do not resemble the substrate or the product of the enzyme catalyzing the reaction.
- Allosteric modulators bind non-covalently to the enzyme at a site rather than the substrate binding site.



- Allosteric enzymes usually have quaternary structure
- Allosteric enzymes do not exhibit typical Michaelis- Menton kinetics.
- Instead, the curve is sigmoidal, which indicates that the binding of substrate to the enzyme changes (e.g. increases) the affinity of the enzyme for substrate.
- Some allosteric modulators alters the  $K_m$ , the  $V_{max}$  remains constant.
- The modulators are not altered by the enzyme.





**Allosteric regulation gives sigmoidal curve**

**Effects of a positive (+) and a negative (-) modulator that alter the  $K_m$  without altering the maximum velocity  $V_{max}$**



# Proteolytic cleavage of proenzyme (zymogen)

Garrett & Grisham: Biochemistry, 2/e  
Figure 15.4

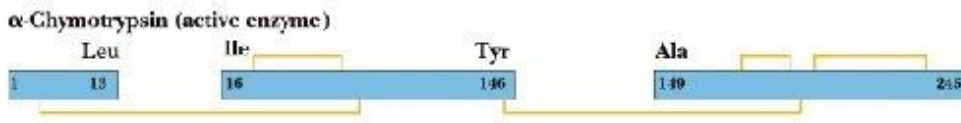


2nd peptide blocking activity of active site  
 1st amino acid blocking activity of active site  
 **$\alpha$ -Chymotrypsin (active enzyme)**

Cleavage at Arg<sup>15</sup> by trypsin



Self digestion at Leu<sup>13</sup>, Tyr<sup>146</sup>, and Asn<sup>148</sup> by  $\alpha$ -chymotrypsin



*Handwritten notes:*  
 chymotrypsin  
 the 4 amino acid removed  
 amino acid should be removed  
 2nd peptide blocking activity of active site  
 1st amino acid blocking activity of active site  
 but still inactive  
 activated portion of the chymotrypsin to activate amino acid  
 self activation of chymotrypsinogen  
 2nd peptide blocking activity of active site  
 1st amino acid blocking activity of active site  
 be converted into active form  
 activation of chymotrypsinogen

*Handwritten notes:*  
 still inactive  
 amino acid removed

*Handwritten notes:*  
 be converted in the active form

*Handwritten notes:*  
 be in the active form

*Handwritten notes:*  
 activation of chymotrypsinogen

# Enzyme/substrate Compartmentation:

- **Compartmentation ensures metabolic efficiency & simplifies regulation**
- Segregation of metabolic processes into distinct subcellular locations like the cytosol or specialized organelles (nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, etc.) is another form of regulation

Plasma membrane

Cytosol

Mitochondria

Nucleus

Endoplasmic reticulum  
(rough and smooth)

Lysosomes

Golgi apparatus

Peroxisomes

دنيا انما ضاقت الاتراحم له  
 ان كل شيء له انتم له  
 ان كل شيء له انتم له  
 side specific  
 Compartmentation →  
 في كل مكان

not all enzymes  
 can be found in the  
 same side of the  
 cell

metabolic  
 reaction are separated  
 to each others by the  
 membrane  
 في كل مكان

Amino acid transport systems, Na<sup>+</sup>-K<sup>+</sup> ATPase

Glycolysis, glycogenesis and glycogenolysis, hexose monophosphate pathway, fatty acid synthesis, purine and pyrimidine catabolism, aminoacyl-tRNA synthetases

Tricarboxylic acid cycle, electron transport and oxidative phosphorylation, fatty acid oxidation, urea synthesis

DNA and RNA synthesis

Protein synthesis, steroid synthesis, glycosylation, detoxification

Hydrolases

Glycosyl transferases, glucose-5-phosphatase, formation of plasma membrane and secretory vesicles

Catalase, D-amino acid oxidase, urate oxidase

# 4- Enzyme production (hormonal regulation):

- Enzyme synthesis (transcription and translation of enzymes genes) can be induced or decreased by hormonal activity that controls the genes.
- This mechanism of enzyme regulation is slower than other mechanisms (**long-term regulation**), i.e. covalent and allosteric modulation of enzyme activity.
- Causes changes in the concentration of certain "inducible enzymes" (are adaptive, i.e. synthesized as needed by the cell). (Constitutive enzymes synthesis is at a constant rate).
- Induction occurs usually by the action of hormones, (e.g. steroid and thyroxine) and is exerted by changes in the expression of gene encoding the enzymes.
- More or less enzyme can be synthesized by hormonal activation or inhibition of the genes.

دیر عرصہ کے انزائموں کو دہل

اس سے زیادہ صریح  
allosteric  
phosphoregulation  
co-precipitation

genetic level regulation  
دیر عرصہ کے انزائموں کو دہل

produced all the time in the low constant concentration

کہ اس سے زیادہ انزائموں کو دہل

induced enzyme  
ہاں اس کے انزائموں کو دہل  
انزائموں کو دہل

Example: *is inducing the enzyme of glycolysis*

- Insulin induces increased synthesis of enzymes: glucokinase, glycogen synthase and PFK-1

- Insulin decreases the synthesis of several key gluconeogenic enzymes (amino acid → glucose).

*the enzyme is not in the cell, its activity is inhibited*

*the enzyme is not existed in utilizing glucose*  
*inhibition*  
*induction of gluconeogenic enzyme*

### 5- Feed back inhibition v/s feed back regulation:

- It is the regulation of a metabolic pathway by using end product as an inhibitor within the pathway to keep cells from synthesizing more product than necessary.

*inhibition*  
*feed back inhibition in the metabolic pathway*

- Dietary cholesterol decreases hepatic synthesis of cholesterol, (feedback regulation not feedback inhibition).

*it doesn't mean that the enzyme will disappear*

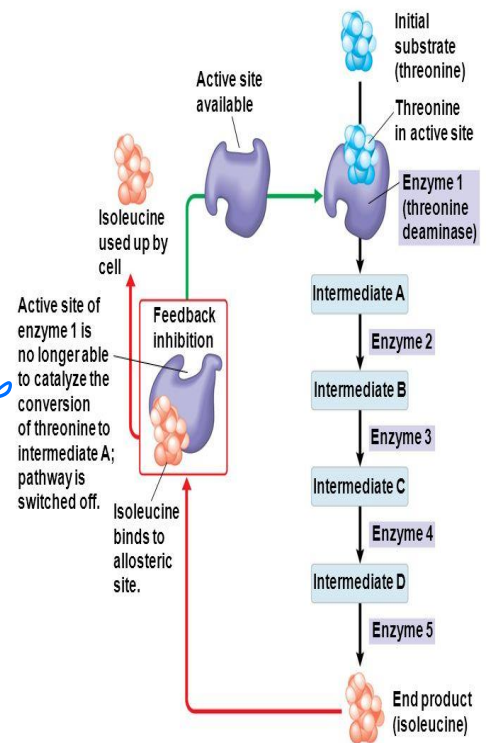
- HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, is affected, but cholesterol does not feedback-

inhibit its activity.

*inhibition in liver synthesis of cholesterol which is under competitive inhibition and it is under feed back regulation*  
*the cell is not going to produce this enzyme*  
*genetic level regulation*  
*feed back regulation*

- Regulation in response to dietary cholesterol involves the effect of cholesterol or a cholesterol metabolite on the expression of the gene that encodes HMG-CoA reductase (enzyme repression).

→ protein كواله انقرا  
 ↓ will inhibit the production of the enzyme



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	Level of Regulation	Control Mechanism	Example
DNA	5 level	change gene structure	phase variation porin synthesis
mRNA	Transcription	control rate of transcription	enzyme induction enzyme repression catabolite repression
Protein	Translation	modulate translation	ribosome components synthesis of toxin components
Modified Protein	Post-translational Modification	protein altered after synthesis	adenylation or phosphorylation of protein
Allosteric Protein	Protein Activity	modulation by the concentration of small molecules that are able to bind to their effector site	many enzymes in biosynthetic pathways many regulatory proteins involved in regulation of transcription

له بقت الپروتين والانتزاع  
 عارضه بصره adhesion تحركه  
 على دكانه  
 الانتزاع قس موجود