ENZYMES

1st YEAR MBBS
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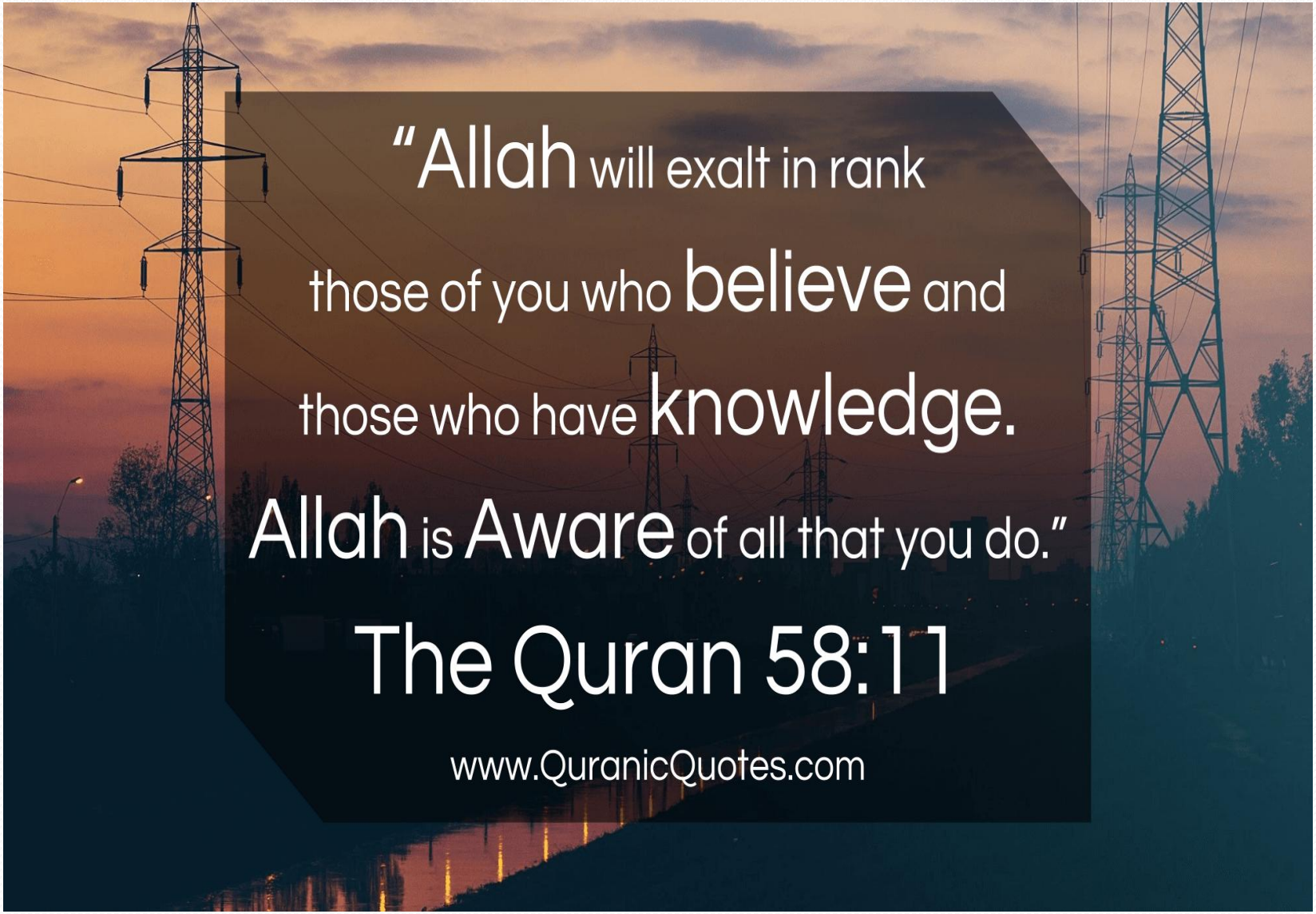
KGMC


بسم الله الرحمن الرحيم


"Allah will exalt in rank
those of you who believe and
those who have knowledge.
Allah is Aware of all that you do."
The Quran 58:11
www.QuranicQuotes.com


رَّبِّ زِدْنِي عِلْمًا
My Lord, increase me in knowledge
(SURAH TAHA, 20:114)

Objectives

- Factors affecting enzyme activity
- Enzyme kinetics

    Michealis Menton equation

    Line weaver burk equation

FACTORS AFFECTING ENZYME ACTIVITY

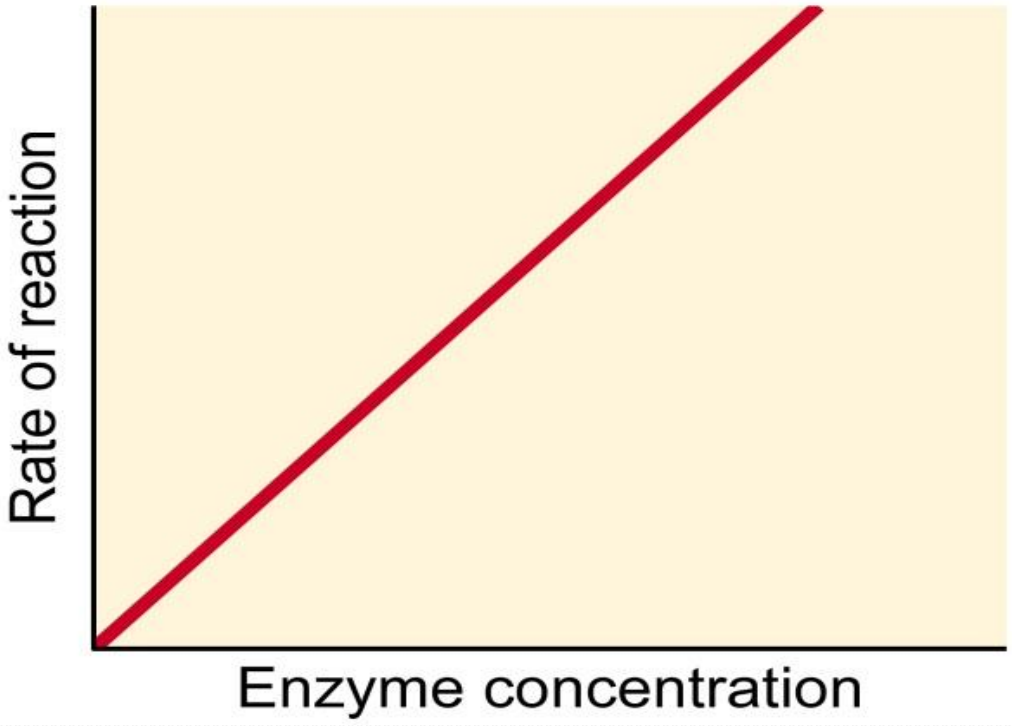
- There are certain factors that affect the activity of enzymes.These factors are as follows:
- Concentration of enzymes
- Effect of product of reaction
- Effect of temperature
- Effect of PH
- Effect of time

- Effect of Rdiations
- Effect of activators and coenzymes
- Effect of modulators and inhibitors
- Substrate concentration

Concentration of enzymes

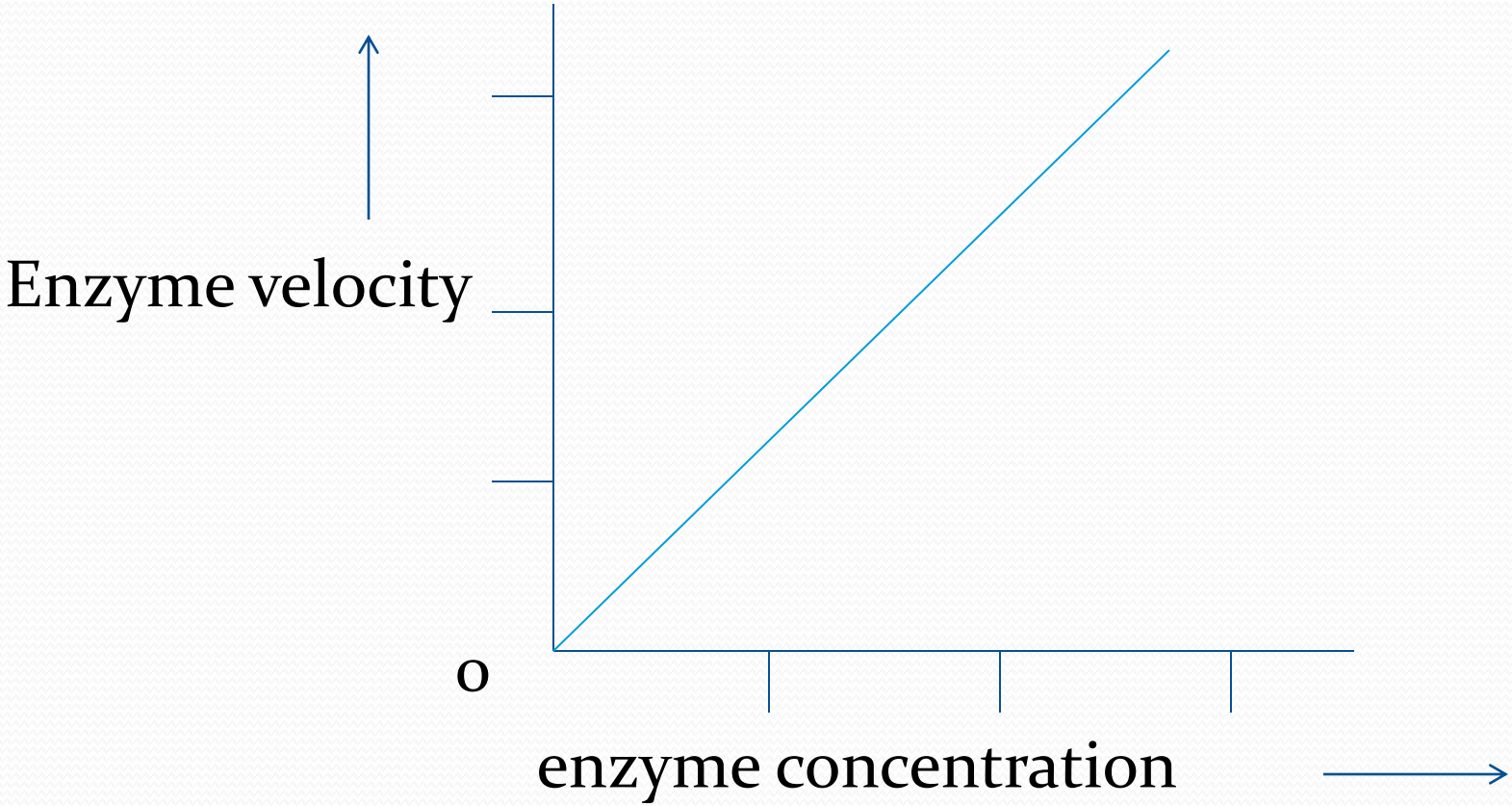
- The rate of enzyme catalyzed reaction is directly proportional to the amount of enzyme present.
- Greater the concentration of enzyme,speedy will be the rate of that reaction

- Thus when enzyme concentration is plotted against enzyme activity, a straight line will be obtained.



Factors affecting enzyme activity

- Concenteration of enzymes



the velocity of reaction is directly proportional to enzyme concentration.

Effects of product concentration

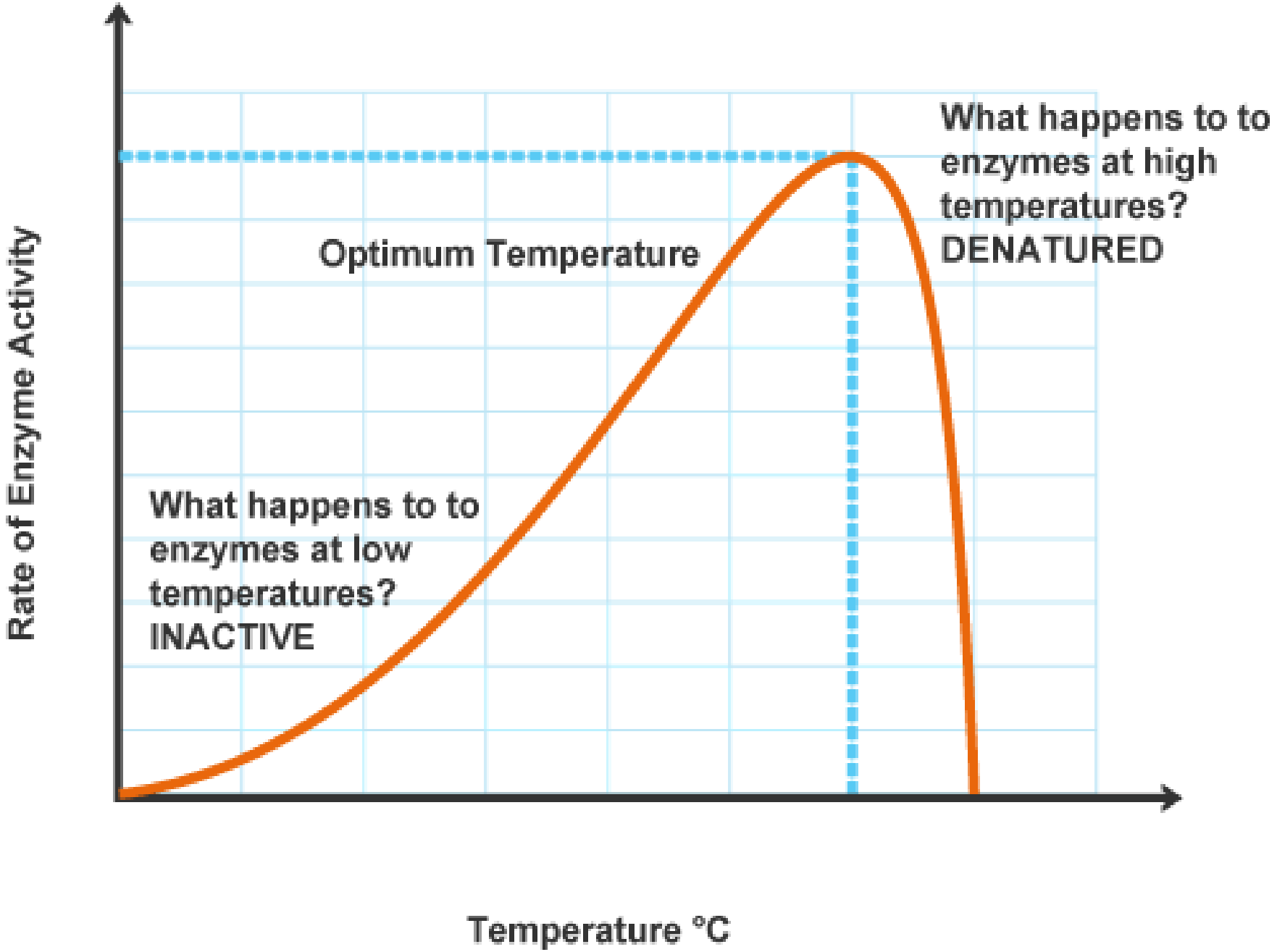
The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes the product combines with the active site and forms a loose complex,thus inhibits the enzyme activity.

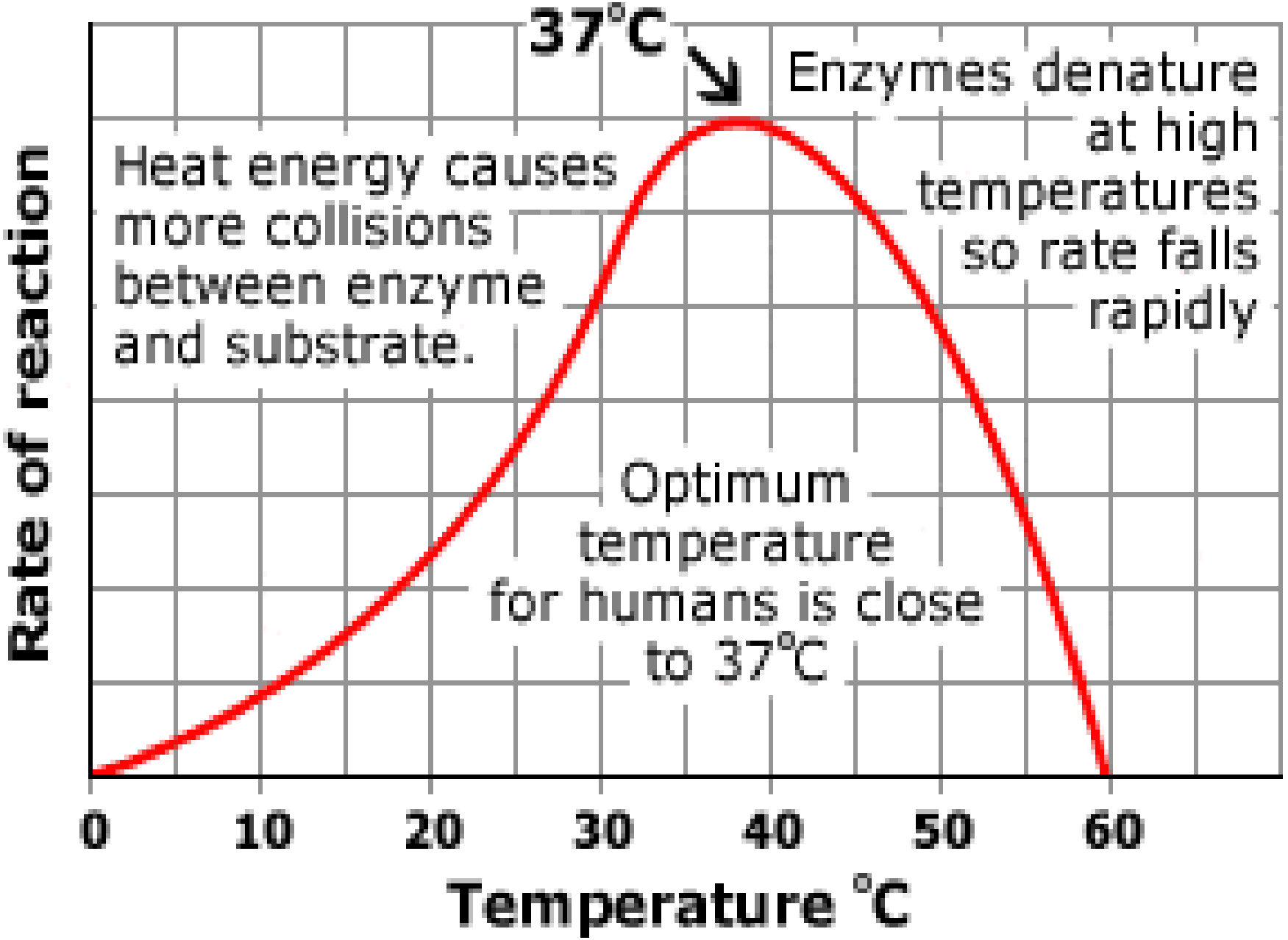
In the living system this type of inhibition is prevented by quick removal of products formed. This high conc.of products may also result in a reversal of the rxn favoring reformation of the substrate

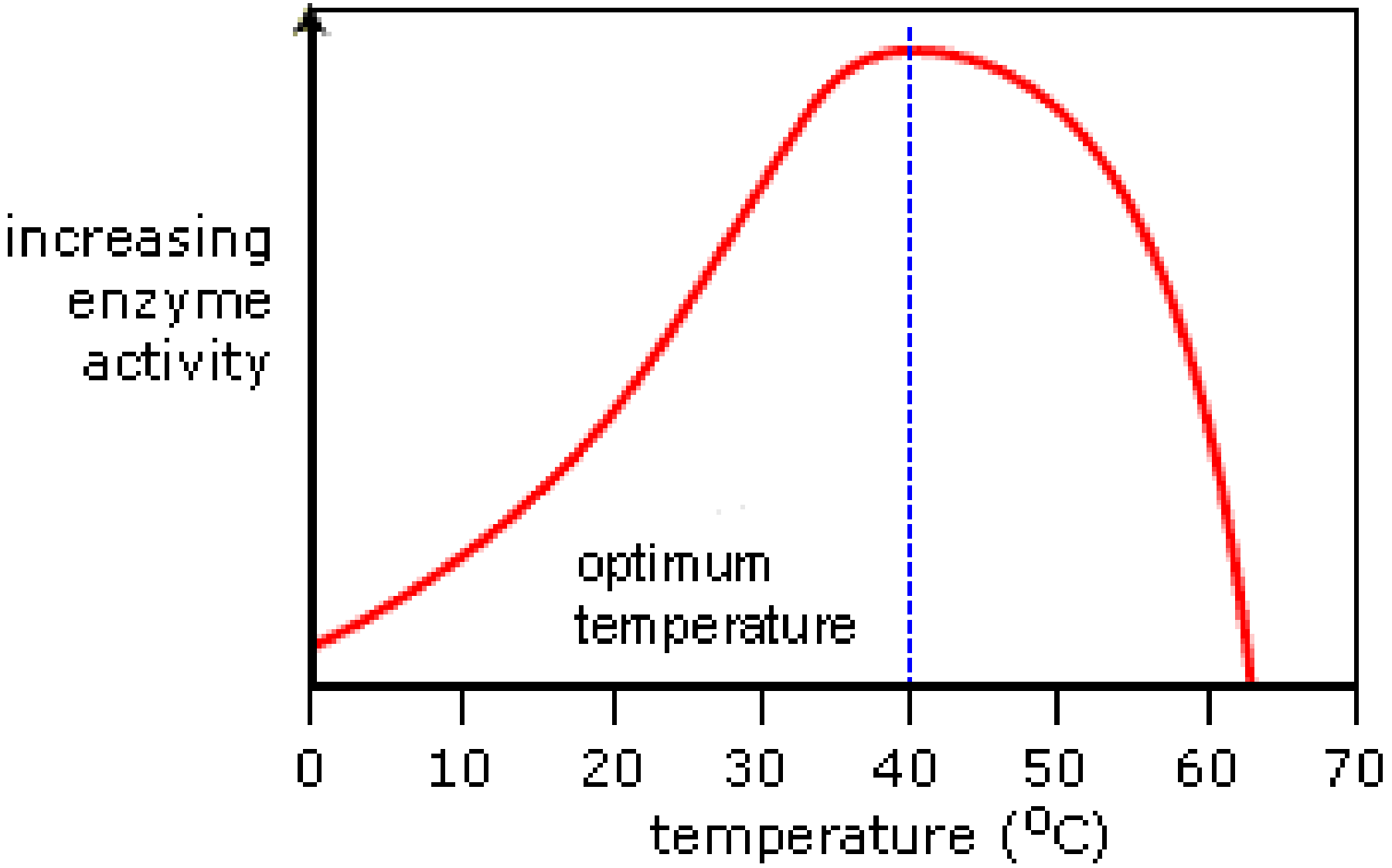
EFFECT OF TEMPERATURE

- Each enzyme is most effective at a certain , specific temperature , which is called the OPTIMUM TEMPERATURE
- The OPTIMUM TEMPERATURE IS THAT TEMPERATURE AT WHICH THE ACTIVITY OF ENZYME IS MAXIMUM.

- Activity of enzymes will decrease if temperature of rxn is above or below the optimum temp.
- However increase in temperature also causes denaturation of enzymes.
- In humans the optimum temperature is within the range of 35-40◦C.

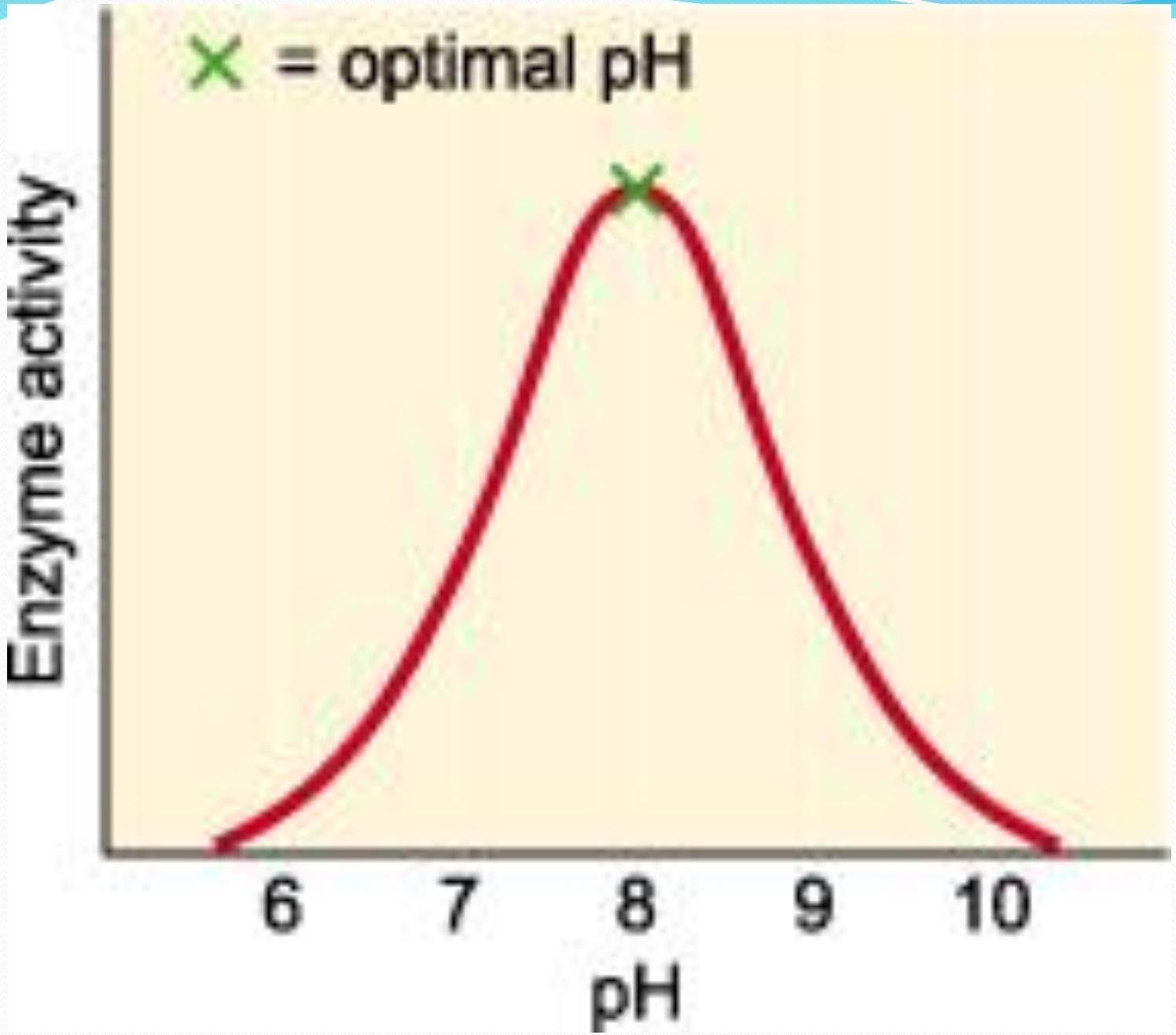

Rate of Enzyme Activity
What happens to to enzymes at high temperatures? DENATURED
Optimum Temperature
What happens to to enzymes at low temperatures? INACTIVE
Temperature °C


37°C
Enzymes denature at high temperatures so rate falls rapidly
Heat energy causes more collisions between enzyme and substrate.
Optimum temperature for humans is close to 37°C
Rate of reaction
0
10
20
30
40
50
60
Temperature °C

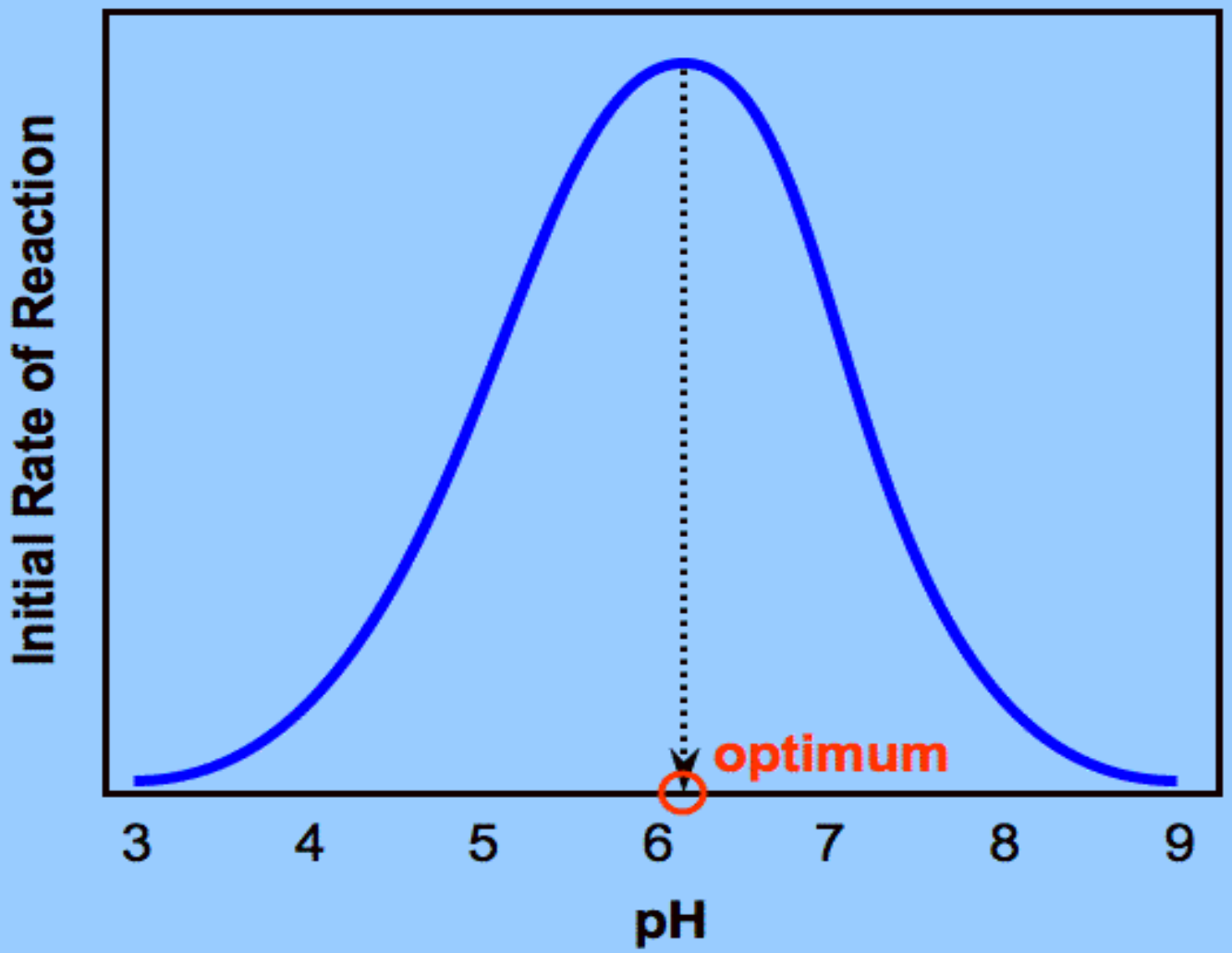

increasing enzyme activity
optimum temperature
0
10
20
30
40
50
60
70
temperature (°C)

EFFECT OF pH

- The rate of enzymatic reactions depend upon pH of the medium . The enzymatic activity is at its maximum at a particular pH which is called the OPTIMUM pH
- The optimum pH of most of enzymes is between 4-9
- The rate of almost all enzyme catalyzed rxns exhibits a significant dependence on hydrogen ion concentration.


X = optimal pH
Enzyme activity
6
7
8
9
10
pH

pH influences the rate of an enzyme-catalyzed reaction



- Hydrogen ions in the medium may alter the ionisation of active site or substrates. Ionisation is a requirement for ES complex formation.
- Gain or loss of critically charged groups adversely affects substrate binding & thus will retard or abolish catalysis.

- Extreme pH levels will produce **denaturation**
- At a very low or high pH the H-bonds may be inactivated in the protein structure destroying its 3D structure.
- The structure of the enzyme is changed

  The active site is distorted and the substrate molecules will no longer fit in it

- At pH values slightly different from the enzyme's optimum value, small changes in the charges of the enzyme and it's substrate molecules will occur
- This change in ionisation will affect the binding of the substrate with the active site.

EFFECT OF TIME

- The time required for the completion of enzymatic reaction increases with decrease in temperature from its optimum temerature.
- However with optimum temperature and pH time required for enzymatic reaction is less.
- Time is inversely proportional to activity of enzme.

Effect of Radiation

Exposure of enzyme to ultraviolet,beta, alpha, gamma and X-rays inactivate certain enzymes due to formation of peroxides.

EFFECT OF ACTIVATORS & COENZYMES

- The activity of certain enzymes is greatly dependent upon coenzymes and metal ion activators.

Some enzymes require organic metallic cations like
Mg^{+2} ,$Zinc^{+2}$, Mn^{+2} Na^{+}, K^{+}
These function as activators of enzyme velocity by
combining with substrate,
formation of ES-metal complex,
direct participation in reaction ,
bringing conformational changes in enzymes.
Two types
Metal activated eg ATPase (Mg^{+2},Ca^{+2}).
Metalloenzymes eg carbonic anhydrase, alkaline phosphatase.

EFFECT OF MODULATORS AND INHIBITORS

- Whenever the active site is not available for substrate binding the rate of rxn will decrease.
- The substance which stop or modify the enzymatic rxn are called INHIBITORS & MODULATORS.
- Their presence in the surrounding medium can adversely affect the rate of enzyme reactions.

SUBSTRATE CONCENTRATION

- For a known quantity of enzyme, the rate reaction is directly proportional to the concentration of substrate.
- However this will be the case for upto a certain concentration after which increasing the concentration of substrate will not further increase the velocity of the reaction

- For a typical enzyme as substrate concentration increases,the initial velocity “vi” also increases until it reaches to maximum value, the “Vmax”.
- (Vmax is the maximum velocity which is efficient enough to change substrate into a product).
- When further increase in substrate concentration does not further increase the “vi” the enzyme is said to be **SATURATED** with substrate

At any given point only substrate molecules that are combined with the enzyme as an ES complex can be transformed into a product.

- Therefore even when the substrate is present in excess only a fraction of the enzymes will form E-S complex (points A & B in fig)

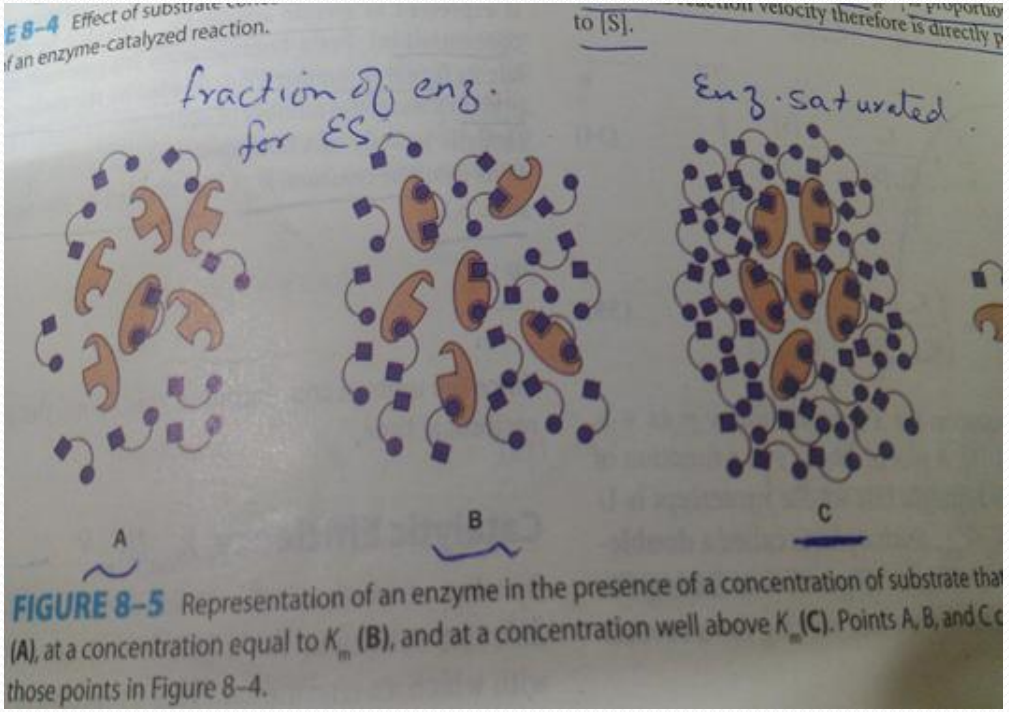
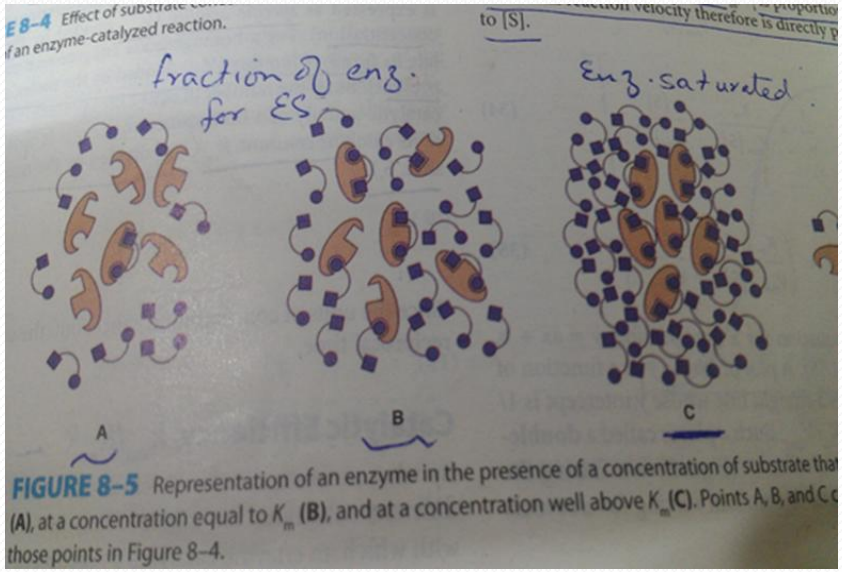


FIGURE 8–5 Representation of an enzyme in the presence of a concentration of substrate tha (A), at a concentration equal to K_m (B), and at a concentration well above K_m (C). Points A, B, and C c those points in Figure 8–4.

- At points A or B increasing or decreasing (S) therefore will increase or decrease the number of E-S complexes with a corresponding change in vi.
- At point C essentially all the enzymes are present as the E-S complex.Since no free enzyme remains available to form further E-S, therefore further increase in (S) can not increase the rate of the reaction.



- Under these saturating conditions, vi will solely depend on and thus is limited by the rapidity with which product dissociates from the enzyme so that it may combine with more substrates.

as the ES complex. Since no

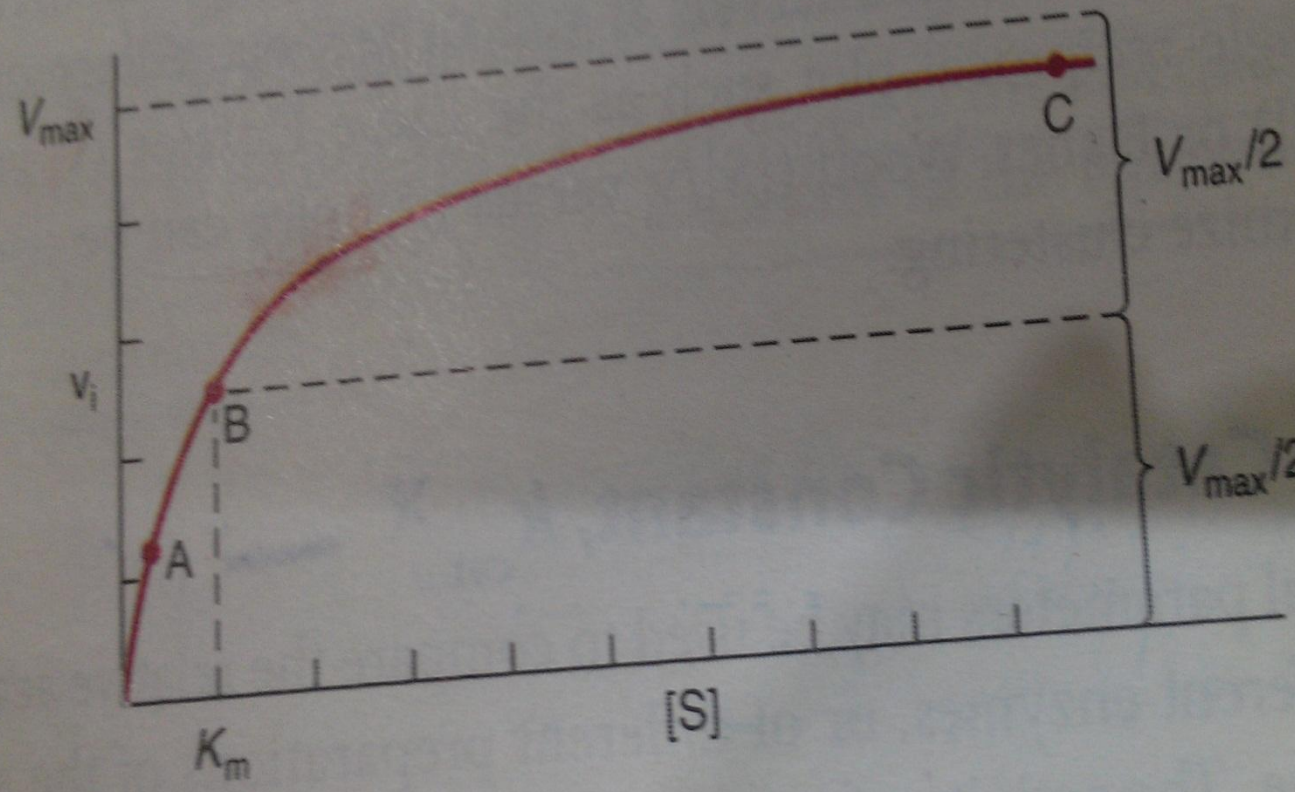


FIGURE 8–4 Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction.

1.

& 8–5)

$K_m + [$

when

are

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The

to

Enzyme kinetics

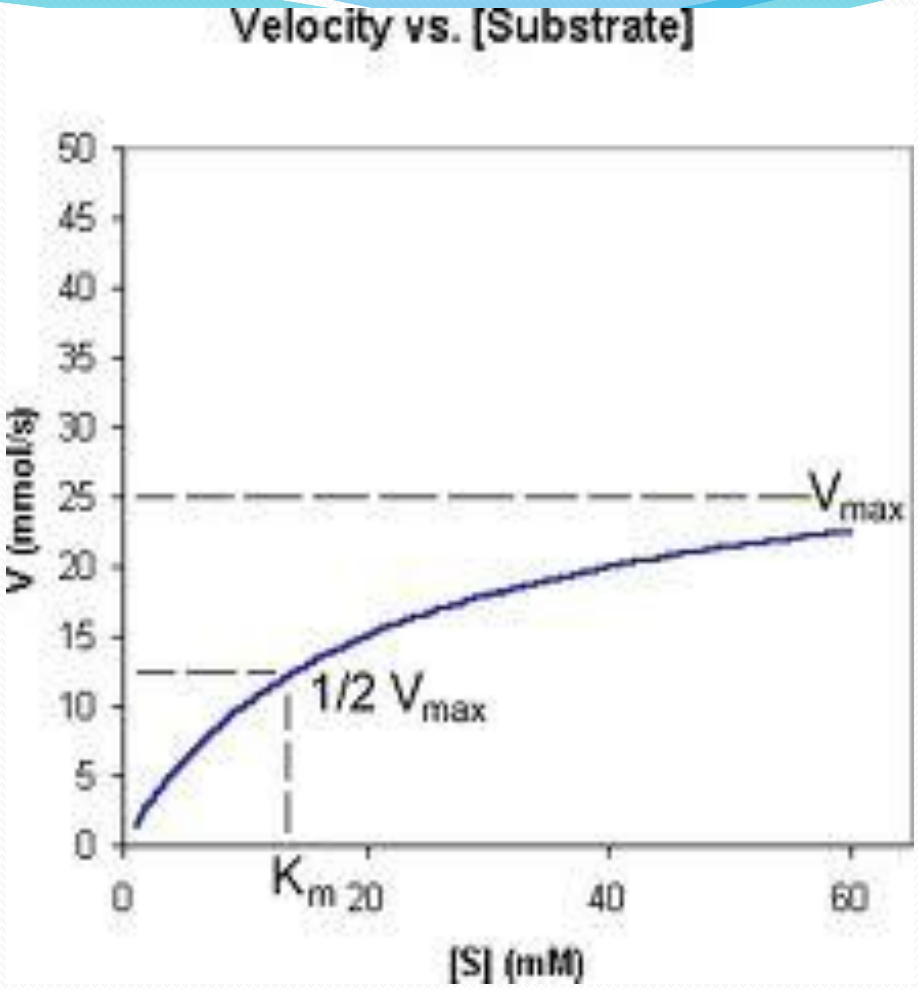
- Two types
- Zero order: when the substrate concentration is much greater than K_m, the rate of reaction is independent of substrate concentration.
- First order: when the velocity of reaction is almost proportional to the substrate concentration.

MICHAELIS MENTEN EQUATION

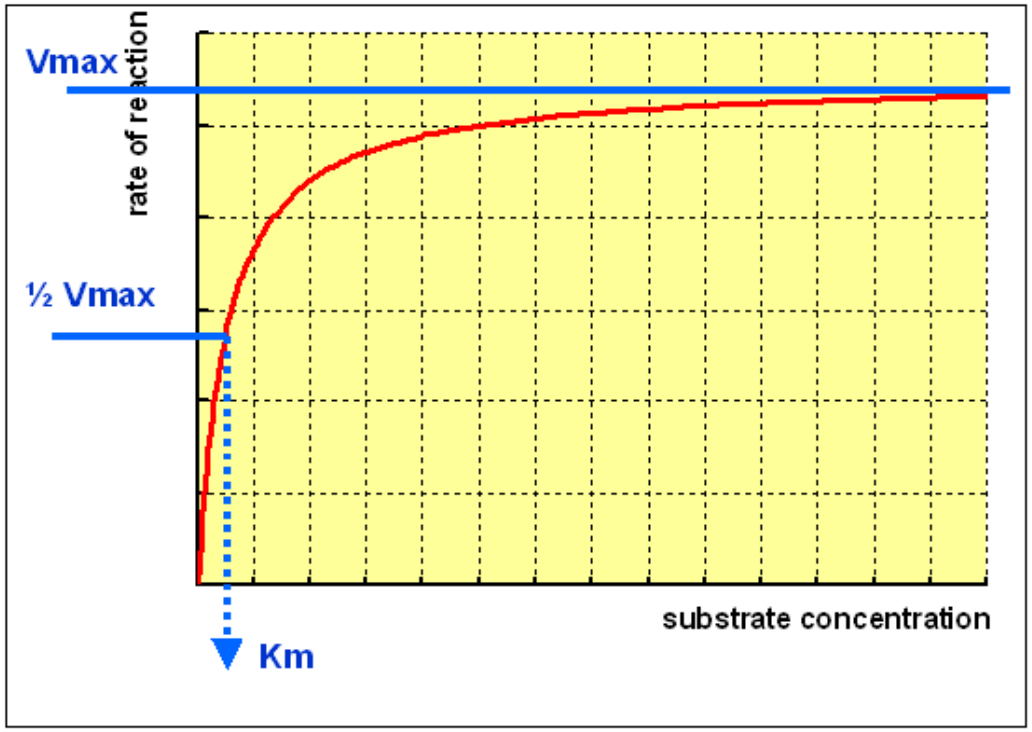
- The Michaelis Menten equation mathemetically shows the relationship b/w initial rxn velocity the vi and the substrate concentration [S]

$$vi = \frac{Vmax\,[S]}{Km + [S]}$$

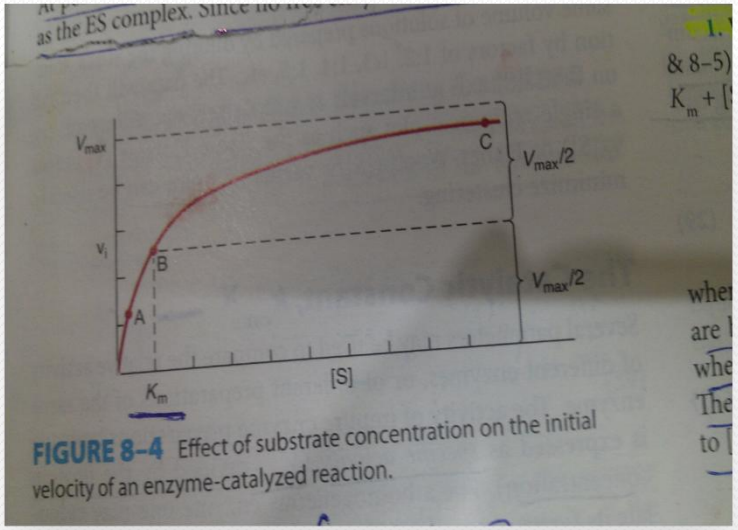
- The Michaelis constant Km is the substrate concentration at which vi is half the maximal velocity (Vmax / 2) attainable at a particular concentration of enzyme.
- Km can also be defined as affinity of enzyme for its substrate.
- Km thus has dimensions of substrate concentration.



- The Michaelis Menten equation is the basic eq of enzyme kinetics,when it is plotted it gives a hyperbolic curve.



- The dependence of initial reaction velocity on [S] & Km maybe illustrated by evaluating the M.M eq under three conditions…..
- When [S] is much less than Km….point A in figure



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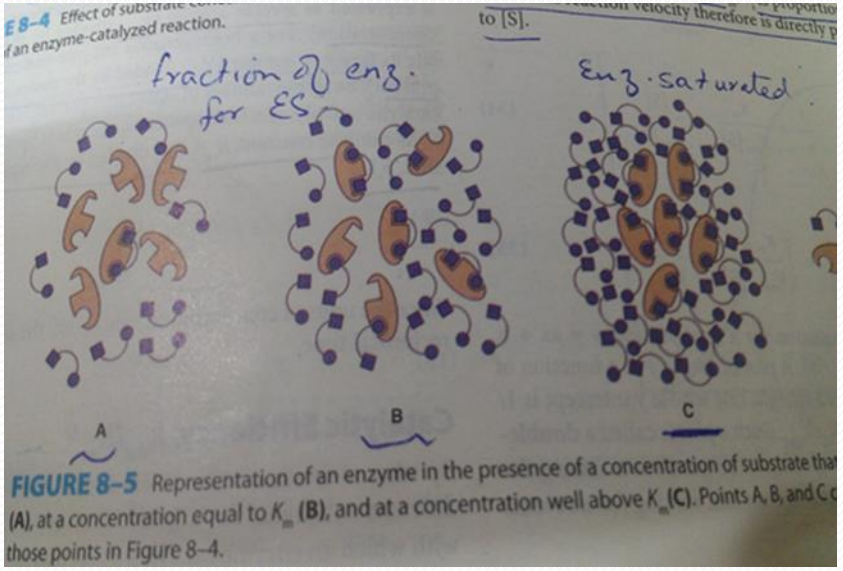


FIGURE 8–5 Representation of an enzyme in the presence of a concentration of substrate (A), at a concentration equal to K_m (B), and at a concentration well above K_m (C). Points A, B, and C those points in Figure 8–4.

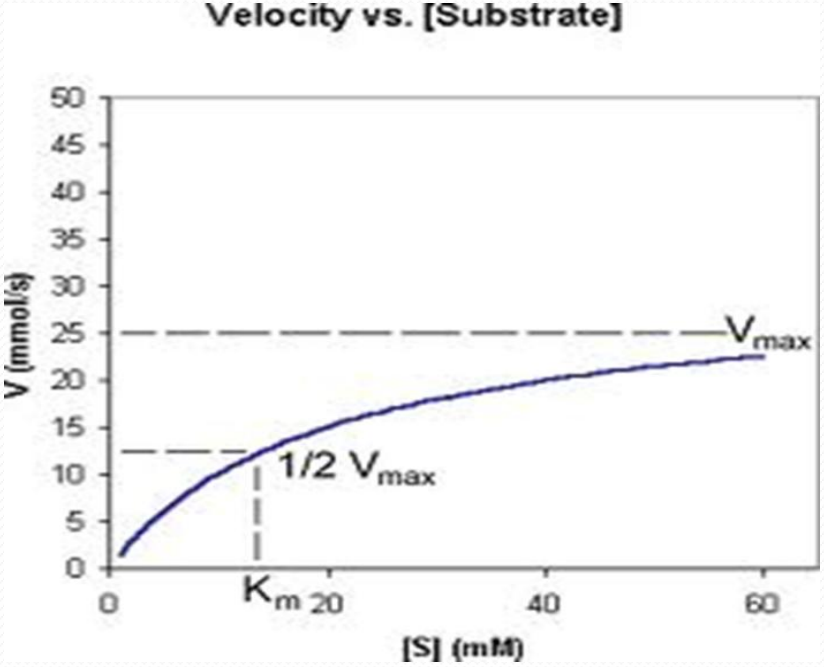
- The term Km + [S] is essentially equal to Km.
- Therefore replacing Km + [S] with Km reduces the equation

$$vi = \frac{Vmax\,[S]}{Km + [S]}$$

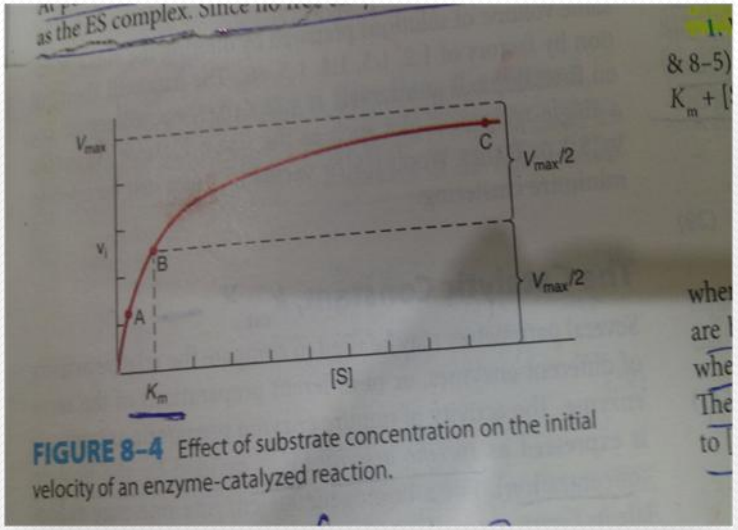
- To

- $vi = \frac{Vmax\,[S]}{Km} = \frac{Vmax}{Km}\,[S]$

- Since Vmax and Km are both constants their ratio is constt. In other words when [S] is considerably below Km , vi is approximate ly k[S].
- T initial velocity therefore is directly proportinate to [S].
- vi = [S]



- When [S] is much greater than Km……point C



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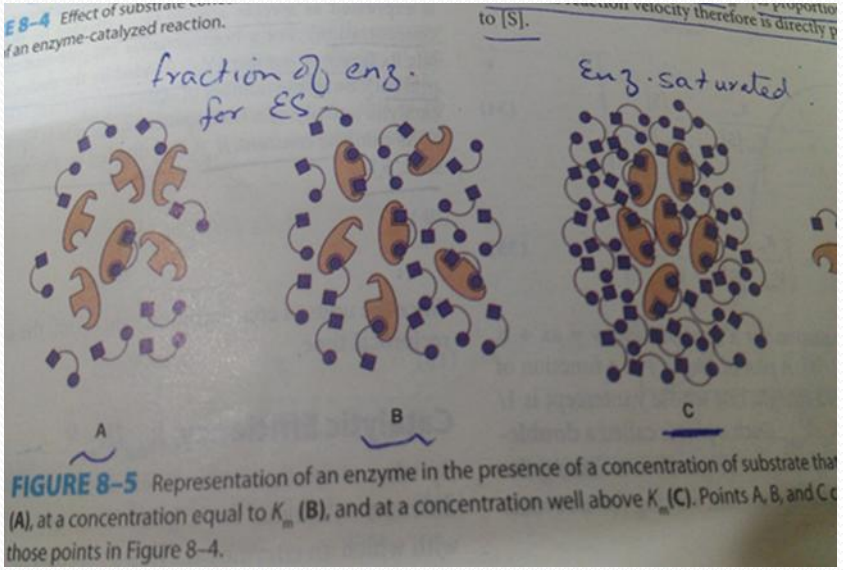
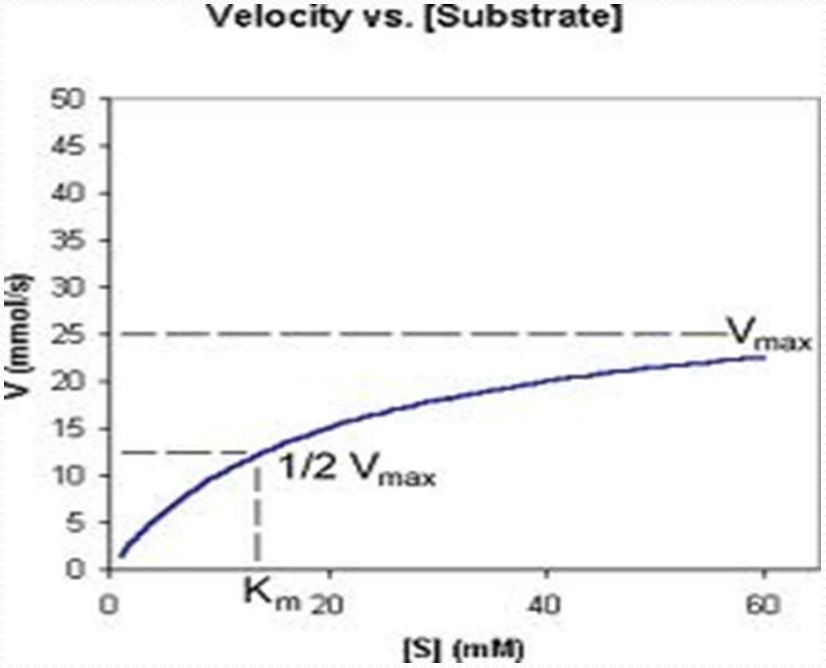


FIGURE 8–5 Representation of an enzyme in the presence of a concentration of substrate that (A), at a concentration equal to K_m (B), and at a concentration well above K_m (C). Points A, B, and C those points in Figure 8–4.

- The term Km + [S] is essentially equal to [S].
- Replacing Km + [S] with [S] reduces equation

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

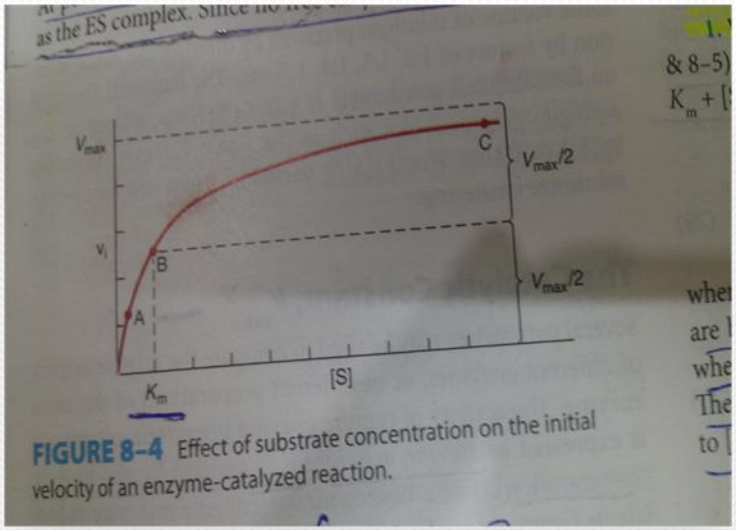
- $v_i = \frac{V_{max}[S]}{[S]} = V_{max}$



- Thus when [S] greatly exceeds Km the reaction velocity is maximal [Vmax] and unaffected by further increase in substrate conc.

- When [S] is equal to Km…….point B

$$v_i = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}[S]}{2[S]} = \frac{V_{max}}{2}$$



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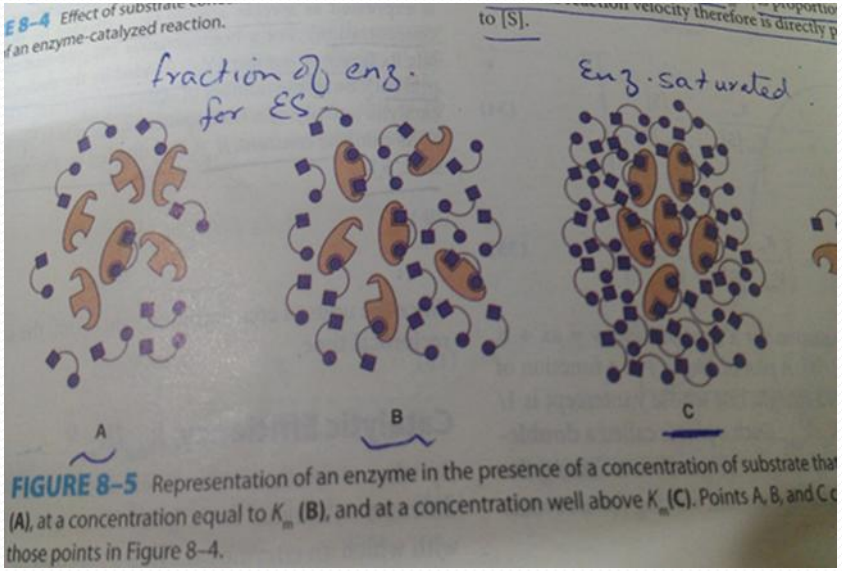

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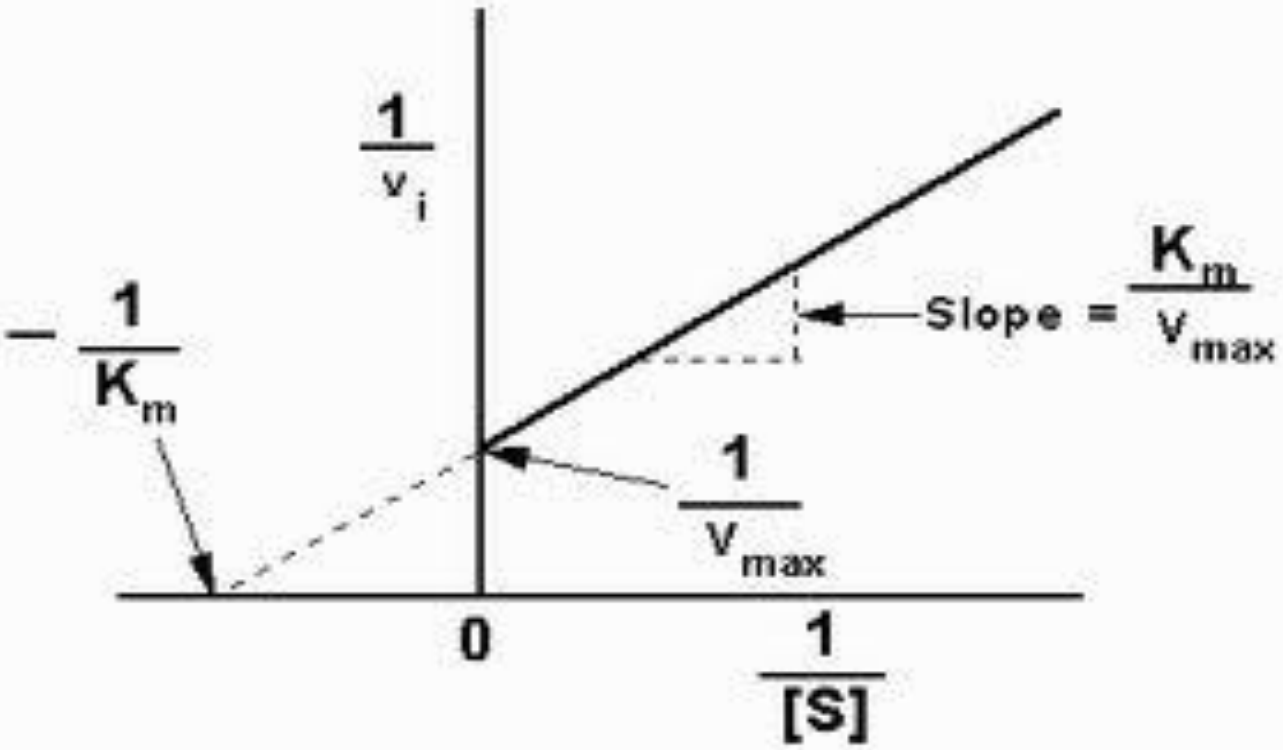
LINEWEAVER-BURK PLOT

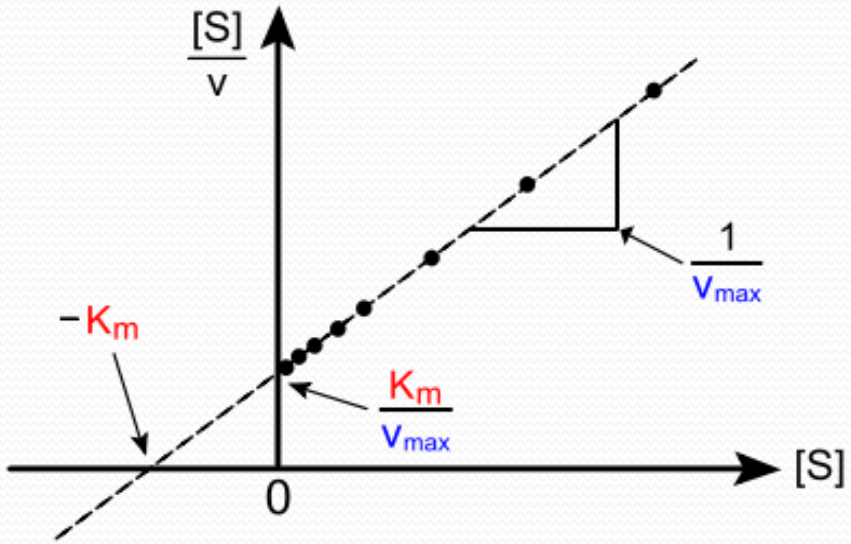
- The direct measurement of the numeric value of Vmax and therefore the calculation of Km requires impractically high concentration of substrate to achieve the saturating conditions.
- A linear form of M.M eq circumvents this difficulty and permits Vmax & Km to be extrapolated from initial velocity data obtained at less than saturating concentrations of substrate.

It was proposed to plot 1/vi versus 1/[S], because this gives a straight line.by taking the reciprocal of M.M eq
such plot could be obtained.



- Such equation is called Lineweaver-Burk equation or Double Reciprocal equation.

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$



Michaelis-Menten equation $\Rightarrow$ $$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

invert

$$\boxed{\frac{1}{V_o} = \frac{K_m + [S]}{V_{max}[S]}}$$

factor

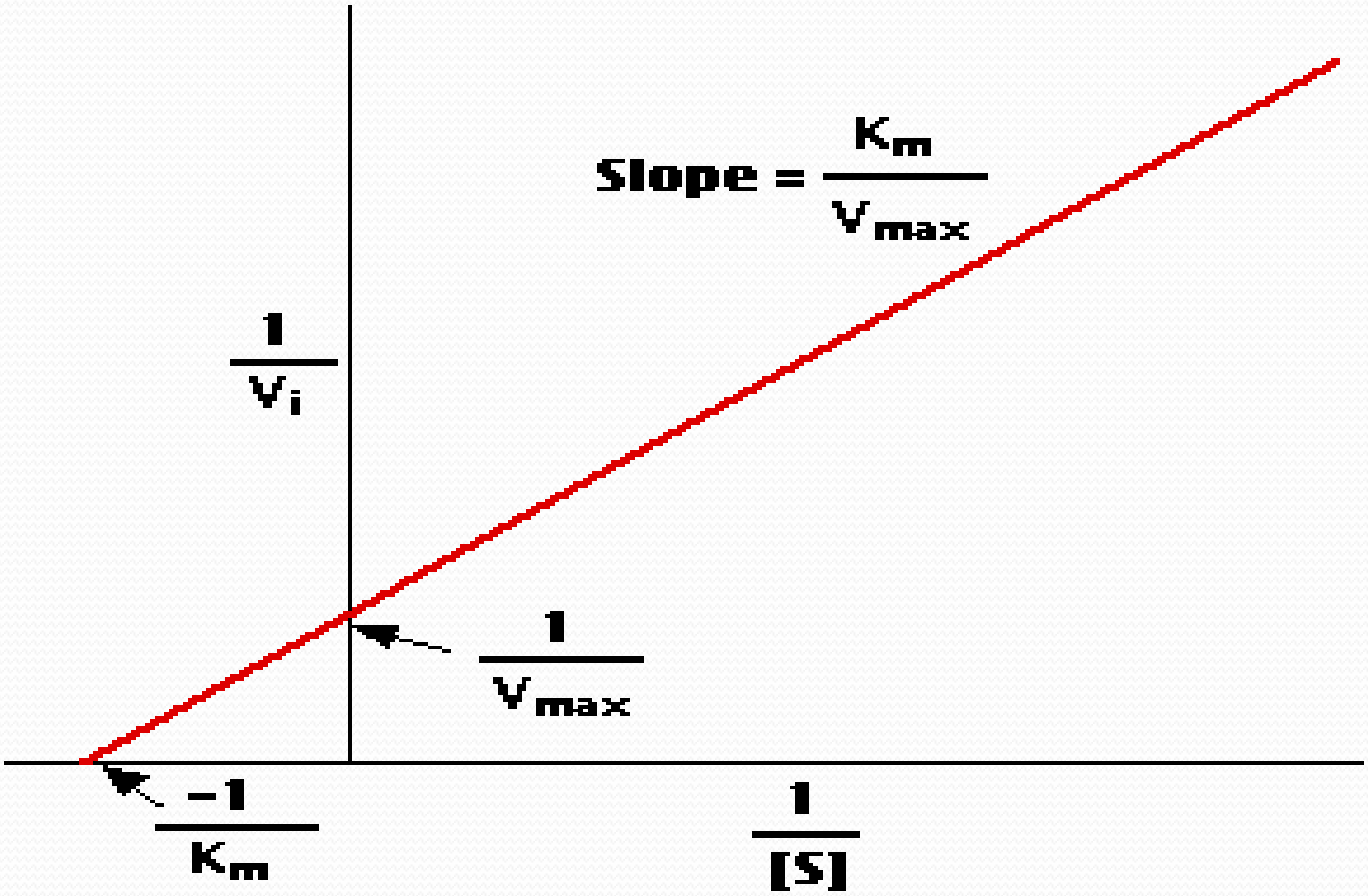
$$\boxed{\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}}$$

and simplify

Double-Reciprocal or Lineweaver-Burk equation $\Rightarrow$

$$\boxed{\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}}$$

- This plot helps in calculating Vmax & Km
- It also helps to determine the mechanism of action of enzyme inhibition.




That's all Folks!