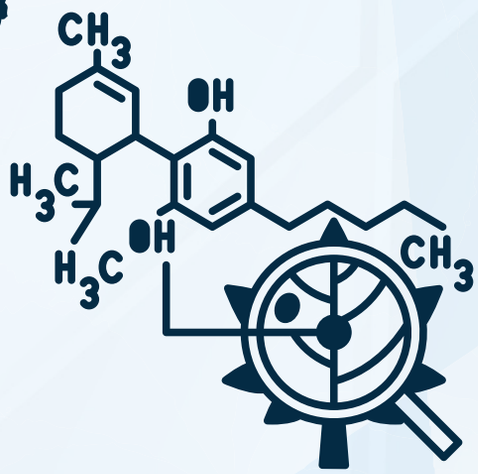
Doctor 2023
Medicine – MU



Biochemistry Sheet

Enzymology 2

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Enzymology- An Overview- 2

Factors affecting Enzyme activity:

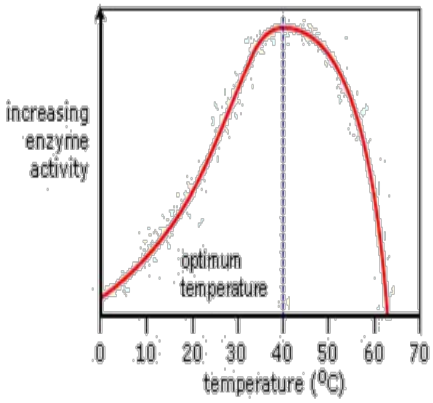
- **Numerous factors affect the reaction rate:**

1. Temperature:

- **The optimal temperature of enzymes in our bodies is 37 degrees Celsius.**
- **The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature**
- **Most animal enzymes rapidly become denatured (breaking bonds that stabilize the tertiary or secondary forms of proteins) at temperatures above 40 degrees Celsius.**
- **The optimal temperatures of the enzymes in higher organisms rarely exceed 50 degrees Celsius.**
- **The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 degrees Celsius increase in temperature (i.e. there will be increase in the enzymatic activity for each 10 degrees Celsius).**
- **The optimal temperature of the plants is very high.**

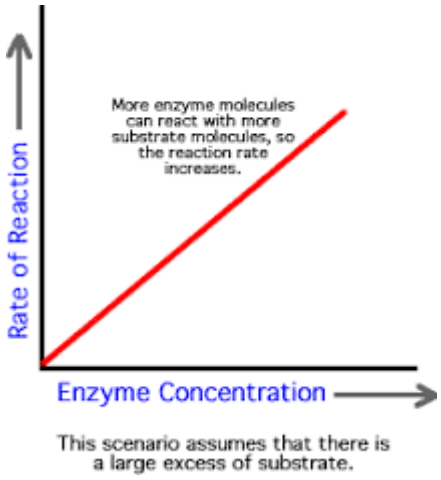
Effect of Temperature:

- **For mammals and other homeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.**



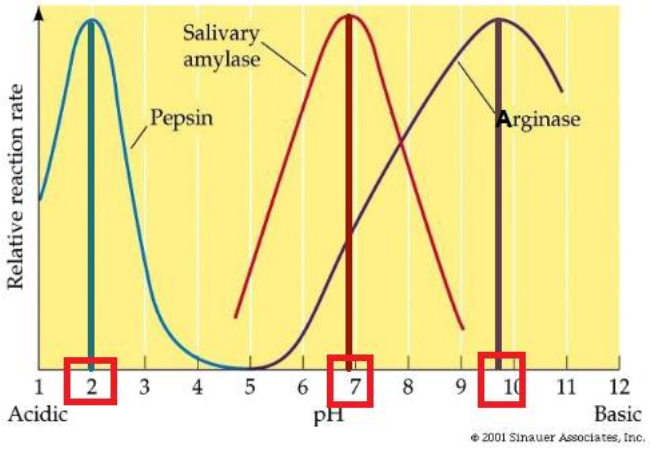
2. Effect of enzyme concentration: (1 factor)

- **As the amount of enzyme is increased, the rate of reaction increases.**
- **If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.**
- **Reaction rate therefore increases then it levels off (i.e. when there is no more substrate, there will be no enzymatic activity).**



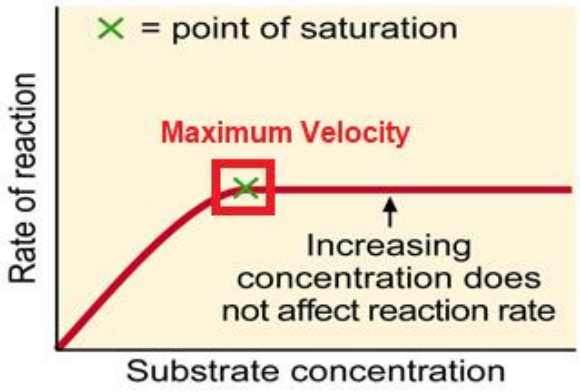
3. *Effect of pH on enzyme activity:*

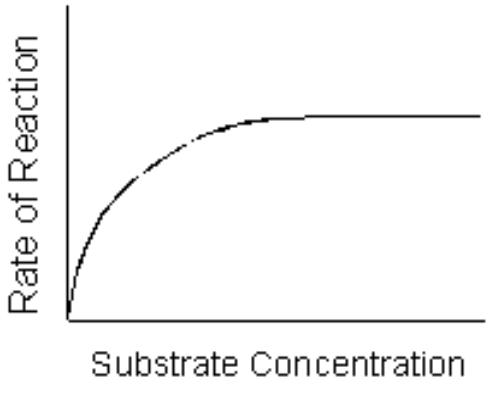
- **The rate of almost all enzyme catalyzed reactions exhibits a significant dependence on hydrogen ion concentration.**
- **Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9 (in human body the optimal pH ranges between 7.35 and 7.45).**
- **The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrate or both.**
- **Except for Pepsin, acid phosphatase and alkaline phosphates, most enzymes have optimum pH between 5 to 9.**
- **The essential bond of any enzyme is peptide bond -as enzyme is a protein eventually-. However, the bonds responsible for maintaining the 3-dimensional (3D) form are: electrostatic bonds, disulfide bonds, hydrogen bonds and hydrophobic bonds.**
- **Reaction rate therefore increases then it levels off (i.e. when there is no more substrate, there will be no enzymatic activity).**



4. *Effect of substrate concentration:*

- **<u>At low concentration,</u> the active sites on most of the enzyme molecules are not filled because there is not much substrate.**
- **<u>At higher concentrations,</u> 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 (i.e. all the active sites were filled with substrates).**
- **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.**





- **Enzyme Kinetics:** (maximum activity and enzymatic equilibrium constant)
- **It is the study of the chemical reactions that are catalyzed by enzymes.**
- **In enzyme kinetics, the reaction rate is measured and how get changes in response to changes in experimental parameters such as: substrate concentration [S], enzyme concentration etc.**
- **This is the oldest approach to understanding enzyme mechanisms and remains the most important.**
- **The initial rate (or initial velocity), designated Vi (for one reaction), when [S] is much greater than the concentration of enzyme [E] can be measured by Michaelis- Menten kinetics. It is one of the simplest and best- known models of enzyme kinetics.**
- **Note# Michaelis- Menten equation, the rate equation for a one- substrate enzyme-catalyzed reaction.**

Michaelis- Menten Kinetics:

- **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 km (which are set by the particular equation). Moreover, there is other constants like K_1: rate constant of forward formation of enzyme- substrate complex, K_2: rate constant for the conversion of the enzyme-substrate complex into products, K^{-1}: dissociation rate constant; for the reverse reaction (enzyme- substrate complex dissociates into enzyme and substrate) and K^{-2}: rate constant for the reverse reaction of product converting back into the enzyme-substrate complex (very rare to happen).**
- **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 (km).**

Michaelis- Menten equation:

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

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

1. **When [S] is much less than Km, the term km + [S] is essentially equal to km. Since Vmax and Km are both constants, their ratio is a constant (k). In other words, when [S] is considerably below Km, Vmax is proportionate to K [S]. The initial reaction velocity therefore is directly proportionate to [S].**

$$V_i = \frac{V_{max}}{K_m} = K$$

   - **Note that at the beginning of the reaction there is only one reaction (forward).**

2. **When [S] is much greater than Km,** the term Km + [S] is essentially equal to [S]. Replacing Km + [S] with [S] reduces equation to:

$$V_i = \frac{V_{max} \cdot [S]}{[S]} = V_{max}$$

Thus, when [S] greatly exceeds Km, the reaction velocity is maximal (V max) and unaffected by further increases in substrate concentration (steady state).

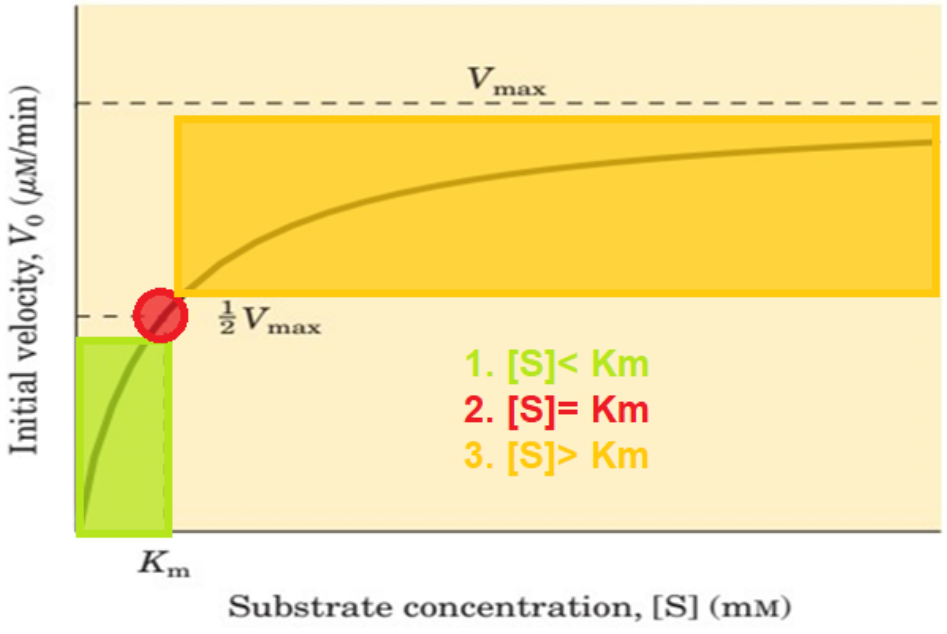
3. **When [S] = Km** → ($K_1 + K_2 + K^{-1} + K^{-2}$), equation states that when [S] equals Km, the initial velocity is half- maximal.

$$V_i = \frac{V_{max} \cdot [S]}{\{K_m + [S]\}} = V_i = \frac{V_{max} \cdot [S]}{\{[S] + [S]\}} = V_i = \frac{V_{max} \cdot \cancel{[S]}}{2\cancel{[S]}}$$

$$V_i = \frac{V_{max}}{2} = \frac{1}{2} V_{max}$$

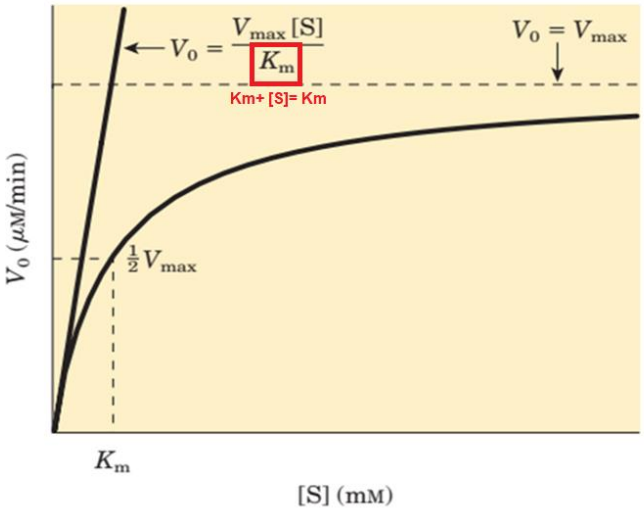
What about Km value? Equation also reveals that Km is a constant and may be determined experimentally from the substrate concentration at which the initial velocity is half- maximal.

Plot of substrate concentration versus reaction velocity:



Graphical Representation of Michaelis- Menten equation:

- The equation describes the kinetic behavior of all enzyme that that Michaelis Menten kinetics.
- This equation practically determines the value of Km and Vmax and, also describe the analysis of inhibitor action.
- But double- reciprocal plot is more convenient procedure, to determine an approximate value of Km.



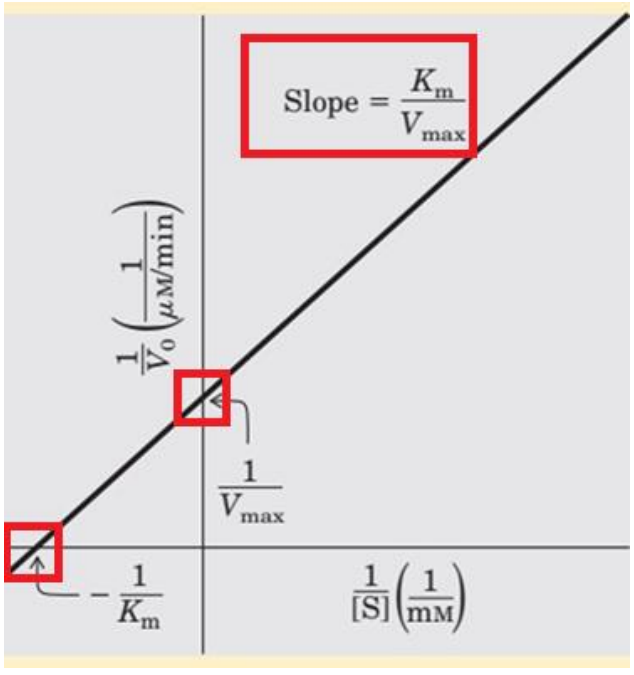
Lineweaver- Burk Plot:

- A linear form of the Michaelis- Menten Equation is used to determine Km & Vmax:

$$V_i = \frac{V_{max}\cdot[S]}{K_m+[S]}\text{, Invert: } \frac{1}{V_i} = \frac{K_m+[S]}{V_{max}\cdot[S]}\text{, Factor: } \frac{1}{V_i} = \frac{K_m}{V_{max}\cdot[S]} + \frac{[S]}{V_{max}\cdot[S]}$$

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

- Lineweaver- Burk plot has the great advantage of allowing a more accurate determination of Vmax and Km.
- The double- reciprocal plot is very useful to determine the mechanism of enzymatic reaction.
- This line has a slope of Km/Vmax (Slope= $\frac{y}{X} = \frac{\frac{1}{V_{max}}}{\frac{1}{K_m}} = \frac{K_m}{V_{max}}$), an intercept of 1/Vmax on the 1/V0 y- axis, and an intercept of -1/Km on the 1/[S] x-axis. (i.e. x- axis is 1/[S] and its intercept= -1/Km while y- axis is 1/V0 and its intercept is 1/Vmax).



Km and its significance:

- **The Michaelis constant K_m is the substrate concentration at which V_i is half the maximal velocity (Vmax/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. (in other words, each enzyme has its own Km value at a specific temperature, time and pH combination).**
- **K_m determines the affinity of an enzyme for its substrate, lesser the Km for is the affinity and vice versa, it is inversely proportionate to the affinity, in other words:**
  - **If the enzyme requires more substrate to reach Vmax (low affinity/ high Km)**
  - **If the enzyme requires less substrate to reach Vmax (high affinity/ low Km)**
- **K_m value helps in determining the true substrate for the enzyme (if we know the enzyme and its Km we can determine what the substrate is).**

يَطلُع عليك فجر اليوم، بفرصة جديدة،
لتُحَسّن نفسك التي لا تُرضيكَ، لتُعالج
الخطأ، وتهدم الذَّنب، وتقترب!
كلّ فجرٍ لا يُشرق بقلبك أولاً؛ لم يشرق.
– أ. قصي.