Enzyme Kinetics

- The study of reaction rates and how they change in response to changes in experimental parameters is known as **Kinetics**.
- Kinetics is that branch of enzymology that deals with the factors that affect the rate of enzyme-catalysed reactions.
Importance of Enzyme Kinetics

• Characteristic property and functions of E is the catalysis of chemical reaction.
• Catalytic function can be studied by measurement of the rate of the catalysed reaction.
  - Is essential for detailed study of an E.
  - Helps define the best condition for the action of E – so E limits can be precisely defined.
  - Act of various factors on E limits can be defined.
  - Help understanding of various biological phenomenon.
  - Help define how an E work in chemical terms and how it functions in the cell.
• If factors are analysed properly, it is possible to learn a great deal about the nature of the E catalysed reaction.
Factors affecting Enzyme Reaction Velocity

(i) Enzyme concentration.
(ii) Substrate concentration.
(iii) Temperature
(iv) pH.
(v) Activators.
(vi) Inhibitors
Effect of E concentration on rate of Enzymic reaction

The rate of E catalysed reaction is proportional to the Enzyme concentration (provided S is saturating E)

\[ \nu \propto [E]; \quad \nu = k[E] \]

As E increases rate of reaction increases in a linear manner. However, some deviations occur:
(a) upward curve
(b) downward curve
(a) **Upward Curve**
In the beginning the rate is low, but as E concentration is increased rate increases.

This is due to:
- Presence of **highly toxic impurity** in the reaction in the reaction mixture (not in E solution). So when E is in small amount it is inhibited, but as its concentration is increased, it overcomes the toxic impurity and \[ \therefore \] rate increases.
- Presence of a **dissociable activator or coenzyme** in the enzyme preparation. Binding of A \[ \text{E} + \text{A} \rightarrow \text{EA} \]
  \[ \uparrow \text{E} \rightarrow \uparrow \text{A} \text{ makes the E active: (e.g. some proteases)} \]
- Some E become active as they **aggregate at high concentration**.

(e.g. 6-phosphofructokinase)

\[ \text{F.6.P + ATP} \rightarrow \text{F.1.6-diP} \]
(b) **Downward Curve**

- This is more common.
- As E concentration is increased beyond a certain point, the rate decreases.

This may be due to the following:

- A **limitation** in the capacity of the method of estimation. This is not a true decrease, but occurs as the assay method cannot give higher reading. (e.g. in spectrophotometer the maximum O.D. is 2.0).
- The **coenzyme** may be limited and as the E remains as ApoE and ∴ loses activity.
- **Substrate** may be used up.
- Presence of a **reversible inhibitor** in the enzyme preparation.

\[ E + I \rightleftharpoons EI \]

As E concentration increases, I increases and inhibits E.
(ii) **Effect of Substrate Concentration on the rate of E catalysed reaction.**

- S most important factor in determining velocity of E reaction.

- At Low S concentration rate of reaction is low and \( r \alpha [S] \). A straight line is obtained. As S concentration is increased a mixed order reaction is obtained and the curve reaction is obtained.

- As S is increased further the rate does not change and becomes constant. This is because E active sites are all filled and E is saturated with S. At this point the velocity is equal to Maximum Velocity (Vmax).

- The S concentration at half Vmax (Vmax/2) is called Michaelis Constant (Km). This is a constant for an E and a specific substrate. It gives the affinity between E and S.
  - High Km indicates low affinity
  - Low Km indicates high affinity.
• The E which give **hyperbolic curve** with S obey Michaelis-Menten kinetics (see later).

• However, some E do not obey Michaelis-Menten kinetics and do not give a hyperbolic curve, but give a **Sigmoid curve**. These are **allosteric enzymes**. These are **regulatory enzymes** and have a **quarternary structure**.

![Graph showing hyperbolic and sigmoid curves](image)

- When [S] is plotted versus v, the saturation curve is **Sigmoidal**.
- This indicates **cooperative binding** of S to multiple sites. Binding of one Site affects binding at other.
• Allosteric E have **multiple binding sites:**
  • **Active sites:** binds S and converts to P.
  
  • **Modulatory site:** binds S and other modulatory molecules and this binding affects the activity of active site.

• Modulators may be:
  +ve Modulators → ↑ activity
  -ve Modulators → ↓ activity.
(iii) Effect of Temperature on E catalysed reactions:

- Optimum Temperature
- Low kinetic energy
- E is denature

- e.g. Human E: ~ 25-37°C
- DNA polymerase in Taq Polymerase active at upto 100°C.[from Thermus aquitus.]
• At very low temperature e.g. 0°C the rate of reaction may be almost zero.
• As temperature is increased rate of reaction increases. This occurs as the kinetic energy of the molecules increases.
• For every 10°C rise of temperature the rate is doubled. This is Q10 or Temperature Coefficient.
• But this occurs only up to a specific temperature which is known as Optimum temperature.
• Beyond this temperature, the rate decreases sharply. This occurs as the enzyme is denatured and the catalytic activity is lost.
• For most E, optimal temperature are at or slightly above those of the cell in which the E occurs. Some E in bacteria which survive in hot springs have high optimal temperature.
Effect of Temperature on Enzyme Catalysed Reactions

rate of reaction

temperature cent

4 37 95
(iv) **Effect of PH on E catalysed reaction.**

When E activity is measured at several pH values, *optimal activity* is generally observed between pH values of 5-9. However, some E such as pepsin have low pH optimum (! 2.0) which others have high pH optimum (e.g. Alkaline Phosphatase (pH ~ 9.5)).
The shape of pH activity curve is determined by the following:

(i) E is denatured at high or low pH.
(ii) Alteration in the charge state of the E or S or both.

• For E pH can affect activity by changing the structure or by changing the charge on a.a. which are functional in S binding or catalysis

\[ \text{e.g. } \text{Enz}^- + \text{SH}^+ \rightarrow \text{Enz.SH} \]

**At Low pH:** Enzyme is protonated and loses its negative charge,

\[ \text{Enz}^- + \text{SH}^+ \rightarrow \text{Enz-SH} \]

**At high pH** The substrate loses its proton and \( \because \) positive charge \( \text{SH}^+ \rightarrow \text{S}^- + \text{H}^+ \)

So \( \text{Enz}^- + \text{S}^- \rightarrow \text{No reaction} \)
Effect of pH on enzyme catalysed reactions

![Graph showing the effect of pH on enzyme catalysed reactions for Pepsin and Chymotrypsin.](image)

- **Pepsin**
- **Chymotrypsin**
**Effect of Inhibitors on rate of E catalysed reaction:**

- Inhibitors are substances that combine with E and decrease its activity.
- Presence of I decreases the rate of E catalysed reaction.
- Inhibitors may be:
  
  i. **Irreversible inhibitor**
      
      \[ E + I \rightarrow EI \]

  - This inhibitor cannot be removed by dialysis or other means:
  - Inhibition increases with time.

  **Examples of irreversible inhibitors**
  - CN inhibits xanthine oxidase.
  - Nerve gas inhibits cholinesterase.
  - Iodoacetamide, heavy metal ions (Hg^{++}), oxidising agents.

  ii. **Reversible inhibitors**
      
      \[ E + I \leftrightarrow EI \]

  - The reaction is reversible and the I can be removed by dialysis or other means. These are of three types:
    - Competitive
    - Non-competitive
    - Un-competitive
Examples of reversible inhibitors:
- Inhibition of succinate dehydrogenase by malonate.
- Inhibition of methanol dehydrogenase by ethanol.
- E may also undergo changes to conformation when pH is changed and this will affect the activity of the E.

(v) Effect of Activators on rate of E catalysed reactions.

- Some E require activators to increase the rate of reaction.
- Activators cause activation of E-catalysed reaction by either altering the velocity of the reaction or the equilibrium reached or both.
  e.g.:
  - Essential activators: Essential for the reaction to proceed. These are recognised as substrate that is not changed in the reaction e.g. metal ion such as Mg^{++} for kinases.
  - Non essential activators: Activator may act to promote a reaction which is capable of proceeding at a appreciable rate in the absence of activator.
Enzyme Kinetics
Derivation of Michaelis Menten Equation
Effect of Substrate on Enzyme Catalysed Reaction

Velocity

$V_{\text{max}}$

$v/2$

$1/2 V_{\text{max}}$

$[S]$
Michaelis-Menten Kinetics

- $E + S \leftrightarrow ES \leftrightarrow ES^* \leftrightarrow EP \leftrightarrow E + P$
- The Michaelis-Menten equation:
  \[ v_1 = \frac{V_{\text{max}} [S]}{K_m + [S]} \]
- Lineweaver-Burk equation:
  \[ \frac{1}{v} = \frac{K_m (1)}{V_{\text{max}} [S]} + \frac{1}{V_{\text{max}}} \]
Km of enzymes in physiological systems
Lineweaver and Burk Plot

Lineweaver-Burk equation:

\[
\frac{1}{v_0} = \left( \frac{K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{1}{V_{max}}
\]
Derivation of the Rate Equation
- By Equilibrium Theory
- By Steady State Theory
Enzyme Inhibitors
Enzyme Inhibitors

Substances that decrease the activity of enzymes
Enzyme inhibitors

Irreversible

E+I → EI

Reversible

E+I ↔ EI

Competitive

Noncompetitive

Uncompetitive
Inhibition of Enzyme Activity

- **Irreversible Inhibition**
  \[ E + I \rightarrow E - I \]  
  E does not regain activity.

- **Reversible Inhibition**
  \[ E + I \leftrightarrow E - I \]

- E & I bind by non-covalent bonds.
- E – I can be dissociated by:
  - dilution, dialysis

Types of reversible inhibitors:

(i) Competitive
(ii) Non-competitive
(iii) Uncompetitive
<table>
<thead>
<tr>
<th>Inhibitor Type</th>
<th>Binding Site on Enzyme</th>
<th>Kinetic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive Inhibitor</td>
<td>Specifically binds at the catalytic site, where it competes with substrate for binding in a dynamic equilibrium-like process. Inhibition is reversible by substrate.</td>
<td>$V_{\text{max}}$ is unchanged; $K_{m}$, as defined by $[S]$ required for 1/2 maximal activity, is increased.</td>
</tr>
<tr>
<td>Noncompetitive Inhibitor</td>
<td>Binds E or ES complex other than at the catalytic site. Substrate binding unaltered, but ESI complex cannot form products. Inhibition cannot be reversed by substrate.</td>
<td>$K_{m}$ appears unaltered; $V_{\text{max}}$ is decreased proportionately to inhibitor concentration.</td>
</tr>
<tr>
<td>Uncompetitive Inhibitor</td>
<td>Binds only to ES complexes at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor-binding site available. Inhibition cannot be reversed by substrate.</td>
<td>Apparent $V_{\text{max}}$ decreased; $K_{m}$, as defined by $[S]$ required for 1/2 maximal activity, is decreased.</td>
</tr>
</tbody>
</table>
Competitive

Un-competitive

Non-competitive
Competitive Inhibitor
(i) **Competitive**

- I binds with free E at active site
- I resembles S and competes with S to bind the active site.

\[
\begin{align*}
[S] & \quad I \\
E + I & \quad \leftrightarrow \quad EI \\
S & \quad \leftrightarrow \quad ES \\
E + S & \quad \leftrightarrow \quad ES \\
& \quad \leftrightarrow \quad EI \\
[SI] & \quad [EI]
\end{align*}
\]

- Inhibitor is not changed during reaction.

**Dissociation constant (Ki)**

\[
Ki = \frac{[E][I]}{[EI]}
\]

- Competitive I do not change Vmax so when S is increased it removes I from active site and the inhibition is reversed.
- Increase Km (decrease affinity of E for S).
E + S $\leftrightarrow$ ES $\stackrel{k+2}{\rightarrow}$ E + P

$+$

$\uparrow$

$\downarrow$

$[E_i] = [E_f] + [ES] + [EI]$

$v = \frac{V[S]}{[S] + Km (1 + [I])}$

$K_m \text{ app} = Km \left(1 + \frac{[I]}{K_i}\right)$

$1 = \frac{K_m}{V_m} \cdot \frac{1}{[S]} \left(1 + \frac{[I]}{K_i}\right) + 1$

$\frac{1}{v} = \frac{K_m}{V_m} \cdot \frac{1}{[S]} \frac{1}{K_i} \frac{1}{V_{max}}$
Effect of Inhibitors on Michaelis Menten curve
Competitive Inhibitor

(a) 
\[ E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_{\text{cat}}}{\rightarrow} E + P \]

\[ K_i \]

\[ EI \]

(b) 
\[ \frac{1}{v_0} \]

\[ \frac{1}{V_{\text{max}}} \]

\[ - \frac{1}{K_m} \]

\[ - \frac{1}{K_m^{\text{app}}} \]

\[ \frac{1}{[S]} \]

[I]

Control
(ii) Non-competitive Inhibition

• I binds to a site other than the active site.

• I can bind free E and ES complex.

• I does not resemble S.

• No competition between I and S.

• Increasing S does not decrease inhibition.

• Non-competitive I decreases $V_{max}$, but does not alter $K_m$. 
\[
\begin{align*}
E + S & \rightleftharpoons ES & \rightarrow & E + P \\
++ & & & +I \\
I & & & +I \\
\downarrow & & & \uparrow \\
\downarrow & & & \\
EI + S & \rightleftharpoons ESI \\
\end{align*}
\]

\[
V = \frac{V \ [S]}{K_m \left(1 + \frac{I}{K_i}\right) + [S] \left(1 + \frac{I}{K_i}\right)}
\]

\[
V_{\text{app}} = \frac{V}{\left(1 + \frac{I}{K_i}\right)}
\]

\[
\frac{1}{V} = \frac{K_m}{V} \left(1 + \frac{I}{K_i}\right) \frac{I}{[S]} + \frac{1}{V} \left(1 + \frac{[J]}{K_i}\right)
\]
Non-Competitive Inhibitor

(a)

\[ E + S \rightleftharpoons ES \rightarrow E + P \]

\[ E + S \rightleftharpoons EI + S \]

\[ K_i \uparrow \]

(b)

\[ \frac{1}{v_0} \]

\[ \frac{1}{[S]} \]

[1]

Control
(iii) **Uncompetitive Inhibition**

- The I does not bind free E, but binds E-S complex and forms an inactive E-S-I complex which cannot give normal product.

\[
ES + I \leftrightarrow ESI
\]

- I affects both Vmax and Km.
Uncompetitive Inhibitor

(a)

\[
E + S \underset{K_i}{\overset{+}{\rightleftharpoons}} ES \rightarrow E + P
\]

(b)

\[
\frac{1}{v_0} \quad \frac{1}{[S]} \quad [I]
\]

Control
$E + S \leftrightarrow ES \rightarrow E + P$

$$Ki = \frac{[ES] [I]}{[ESI]}$$

$$V = V[S] \frac{Km + [S]}{1 + \frac{I}{Ki}}$$

$$\frac{1}{V} = \frac{Km}{V} \frac{1}{[S]} + \frac{1}{V} \left(1 + \frac{I}{Ki}\right)$$

$$\frac{1}{V_{\text{app}}} = \frac{1}{V} \frac{1 + \frac{1}{Ki}}{\frac{1}{Km}}$$
(iv) **Mixed Inhibitors**

- Cause the reciprocal plot to intercept anywhere to the left of the \( l/v \) axis except on the \( l/[S] \) axis.
- Affect both apparent Km and Vmax.
- Vmax always decreases, but Km may increase or decrease.
Determination of Inhibitor Constant

- Lineweaver and Burk plot can be used to determine $K_i$ from the values of the appropriate intercept in presence and absence of inhibitor.

- A more accurate determination of $K_i$ can be obtained graphically from reciprocal plots made at a series of different inhibitor concentrations.

- $K_i$ can be obtained directly from the plot of $I/v$ Vs $[I]$ using different $[S]$ concentrations.

$$S_1 > S_2 \quad (1)$$

**Competitive I**

$S_1$ and $S_2$ are two different $S$ concentrations: $S_2 > S_1$
Non-competitive I

Un-competitive I
USES OF E INHIBITORS:

- As drugs (in pharmacology)
- Agricultural pesticides and insecticides.
- In study of Mechanism of E action.
- In diagnosis of disease e.g.
  
  e.g. In prostrate cancer in men $\rightarrow$ ↑ Acid phosphatase

L-tartrate inhibits competitively 95% of the acid phosphate from prostrate, but has lower inhibitory effect on acid phosphatase from other sources. Samples from suspected carcinoma patients can be assessed in presence and absence of L-tartrate.

Examples of I as drugs:

- Antiviral drugs
- Antibacterial drugs
- Anti tumor drugs

  e.g. sulfa drugs – Antibacterial drugs
This is an analog of p-amino benzoic acid

This is a competitive I of enzyme which forms folic acid from p-amino benzoic acid in bacteria

Dihyopteroate synthetase (needed for bacterial growth)

Need for purine and pyr. syn. H4 folate (Tetra hydro folate) Folic acid

Sulfanilamide treatment ↓ folate in bacteria → kills bacteria
(ii) Methotrexate: Structural analog of folic acid competes with dihydro-folate reductase.

Methotrexate is used for treatment of leukaemia.
(iii) 5’fluoro Uracil: Analog of thymine inhibits thymidylate synthetase.

\[ \downarrow \text{TMP} \rightarrow \downarrow \text{DNA synthesis} \]

\[ \rightarrow \downarrow \text{cell multiplication} \rightarrow \text{cell death} \]

used for treatment of many types of cancers (malignancy).
<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Use</th>
<th>Target Enzyme</th>
<th>Type of Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mevinolin</td>
<td>Hypercholesterolemia</td>
<td>HMG-CoA reductase</td>
<td>Competitive</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Cancer</td>
<td>Thymidylate synthase</td>
<td>Suicide</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Cancer</td>
<td>Dihydrofolate reductase</td>
<td>Competitive</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Gout</td>
<td>Xanthine oxidase</td>
<td>Suicide</td>
</tr>
<tr>
<td>Coumadin</td>
<td>Anticoagulant</td>
<td>γ-Glutamylcarboxylase</td>
<td>Competitive</td>
</tr>
<tr>
<td>Aspirin</td>
<td>anti-inflammatory</td>
<td>Cyclooxygenase</td>
<td>Suicide</td>
</tr>
<tr>
<td>Captopril</td>
<td>High blood pressure</td>
<td>Angiotensin-converting enzyme</td>
<td>Competitive</td>
</tr>
</tbody>
</table>
Enzymes in the Diagnosis
ENZYMES IN CLINICAL DIAGNOSIS:

In plasma:

• Functional E e.g. lipoprotein lipase, pro E of blood clotting.

• Non-Functional E:
  • No function in blood.
  • Normally their level is low in plasma as they are tissue E
  • Level ↑ due to tissue damage.
  • ↑ in plasma indicate: tissue destruction due to cellular necrosis, vigorous exercise, trauma can be used for diagnosis.

Examples:

   ↓
   Lipid → FA +Glyceral

   Starch ↑ Glucose
   ↑ in intestinal obstruction, acute pancreatitis, DM.
3. **Alkaline Phosphatase:**

\[ \text{H}_2\text{O} \quad \text{SPO}_4 \quad \text{S} + \text{PO}_4'' \]

↑ in Rickets, hyperparathyroidism, Pagets disease, osteoblastic sarcoma, obstructive jaundices and metastatic carcinoma.

4. **Acid Phosphatase:**

↑ in prostate carcinoma

5. **Transaminase:**

Aspartate Transaminase (SGOT) → ↑ Myocardial infarction

Alanine transaminase and ASP transaminase → ↑ acute hepatic diseases, sever trauma, muscle

6. **LDH:** ↑ in acute and chronic leukaemia carcinoma, hepatitis.
## Serum Enzymes Used in Clinical Diagnosis

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Major Diagnostic Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Liver and bone disease</td>
</tr>
<tr>
<td>Amylase</td>
<td>Acute pancreatitis</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Liver and heart disease</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>Viral hepatitis</td>
</tr>
<tr>
<td>Creatinine kinase</td>
<td>Muscle disorders and myocardial infarction</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Lipase</td>
<td>Acute pancreatitis</td>
</tr>
</tbody>
</table>
Serum Enzymes in Disease

• **Acid phosphatase**: a tumour marker in prostatic carcinoma.
• **Alanine aminotransferase (ALT)**: an indicator of hepatocellular damage.
• **Alkaline phosphatase**: increase in cholestatic liver disease and is a marker of osteoblast activity in bone disease.
• **Amylase**: an indicator of cell damage in acute pancreatitis.
• **Aspartate amino transferase (AST)**: an indicator of hepatocellular damage, or as a marker of muscle damage, such as a myocardial infarction (MI).
• **Creatine kinase**: a marker of muscle damage and acute MI.
• **γ-glutamyl transpeptidase**: a sensitive marker of liver cell damage.
• **Lactate dehydrogenase**: a marker of muscle damage.
ISOENZYMES

- Multiple forms of the same enzyme.
- Catalyse the same reaction. Act on the same S and give the same P.
- Differ in molecular weight or structure or charge. Can be separated by electrophoresis.
- Have different Km for the same S.
- Important in diagnosis of disease.

E.g. **Creatine phospho kinase (CPK)**

\[
\text{CPK} \quad \text{Creatine} + \text{ATP} \quad \rightarrow \quad \text{Creatine Phosphate} + \text{ADP}
\]

- Has 3 isoenzyme. Each isoenzyme has 2 subunits (polypeptides)
- Two types of polypeptides: M & B.
- Isoenzymes: MB : Mainly in Heart
  - BB : Mainly in Brain
  - MM : Mainly in Muscles
Lactate dehydrogenase

- LDH occurs in 5 closely related, but slightly different forms (isozymes)
- LDH 1 - Found in heart and red-blood cells
- LDH 2 - Found in heart and red-blood cells
- LDH 3 - Found in a variety of organs
- LDH 4 - Found in a variety of organs
- LDH 5 - Found in liver and skeletal muscle
**Lactate Dehydrogenase (LDH)**

Lactate $\leftrightarrow$ Pyruvate + NADH +H$^+$

- It is a tetramer. (4 subunits)
- Composed of 2 types of polypeptide chains (M & H).
- Has 5 isoenzymes, due to different combination of M & H chains.

\[
\begin{align*}
M_4 & \quad \text{LDH5} \quad \rightarrow \quad \text{In skeletal muscles and liver} \\
M_3H & \quad \text{LDH4} \quad \rightarrow \quad \text{In many tissues} \\
M_2H_2 & \quad \text{LDH3} \quad \rightarrow \quad \text{In lungs} \\
MH_3 & \quad \text{LDH2} \quad \rightarrow \quad \text{In Heart} \\
H_4 & \quad \text{LDH1} \quad \rightarrow \quad \text{In Heart muscles}
\end{align*}
\]

- These forms have different charge and can be separated on electrophoresis.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
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<tr>
<td>-</td>
<td></td>
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</tr>
</tbody>
</table>

- Useful in differential diagnosis.

e.g. ↑ LDH 1 and 2 .............. Myocardial infarction (MI)
Normally $\text{LDH}_1 = \frac{30\%}{10\%} : 3 : 1$

In MI $\text{LDH}_1 : 1 : 1$ Due to ↑↑↑ in $\text{LDH}_2$

↑ LDH2 > LDH1

- ↑↑↑ LDH5; (No change LDH2) – Liver disease (Viral Hepatitis)
- ↑↑↑ LDH5 & ↑↑ LDH2 – Infectious mononucleosis
- ↑↑↑ LDH3 – In lung disease (pumonary infection)
CPK isoenzymes

• Exists as **Three isoenzymes: MM, MB, BB**
• CK-1 (BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle.
• CK-3 (MM) is the predominant isozyme in muscle.
• CK-2(MB) accounts for about 35% of the CK activity in cardiac muscle, but less than 5% in skeletal muscle.
• Since most of the released CK after a myocardial infarction is MM, an increased RATIO of CK-MB to total CK may help in diagnosis of an acute MI, but an increase of total CK in itself may not.
Normal Heart Diseases

Brain Damage

CPK: ↑↑↑ in serum in Heart diseases, brain disease and muscle disease.

CPK isoenzymes help in different diagnosis:
- ↑↑ CPK BB: Brain injury, stroke.
- ↑↑ CPK MM: Muscle disease.
- ↑↑ CPK MB: Heart disease (Myocardial infarction.)
### Summary of Enzymes Analyses of Creatine Kinase (CK) and Lactate Dehydrogenase (LDH)

<table>
<thead>
<tr>
<th></th>
<th>Total CK (IU/L)</th>
<th>%MB</th>
<th>Total LDH (IU/L)</th>
<th>%LHD&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hours</td>
<td>242</td>
<td>5.6</td>
<td>188</td>
<td>15</td>
</tr>
<tr>
<td>8 hours</td>
<td>361</td>
<td>5.1</td>
<td>200</td>
<td>17</td>
</tr>
<tr>
<td>12 hours</td>
<td>193</td>
<td>3.2</td>
<td>287</td>
<td>42</td>
</tr>
<tr>
<td>24 hours</td>
<td>151</td>
<td>2.0</td>
<td>365</td>
<td>59</td>
</tr>
<tr>
<td>48 hours</td>
<td>147</td>
<td>1.7</td>
<td>348</td>
<td>56</td>
</tr>
<tr>
<td>60 hours</td>
<td>143</td>
<td>1.7</td>
<td>216</td>
<td>37</td>
</tr>
</tbody>
</table>
ZYMOGENS

Inactive Enzymes (pre enzymes).

Many E synthesised as zymogens, and are activated when needed. e.g.

**Zymogen**

- Trypsinogen
  - Enterokinase
  - Trypsin
  - Trypsin + Peptide
  - Autocatalysis
  - Chymotrypsinogen
  - Trypsin
  - Chymotrypsin + peptide
  - HCl
  - Pepsinogen
  - HCl
  - Pepsin + Peptide
  - Prothrombin
  - clotting factors
  - Thrombin + peptide

This provides protection to the body. As the active E may destroy body substances if activated in absence of S.

e.g. if thrombin is formed in the body, it will convert fibrinogen → Fibrin. This will form clot in blood: Stroke

Heart attach
COENZYMES

• Organic, heat stable molecules.
• Needed by some E for their function.
• Take part in enzyme catalysis.
• Many obtained as water soluble vitamins.

\[
\text{Apo E} + \text{CoE} \rightarrow \text{Holo E}
\]

(inactive) (Active)

• 2 Groups:
  • Those involved in H Transfer.
  • Those involved in transfer of groups other than H.

(∴ CoE serve as carriers of specific functional groups or atoms)
<table>
<thead>
<tr>
<th>CoE</th>
<th>Group Transfer</th>
<th>Precursor in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <strong>H transfer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• NAD&lt;sup&gt;+&lt;/sup&gt;, NADP&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Hydrogen ion</td>
<td>Nicotinic acid (Niacin)</td>
</tr>
<tr>
<td>• FAD, FMN</td>
<td>Electron / H&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Riboflavin (vit. B2)</td>
</tr>
<tr>
<td>• Lipoic acid</td>
<td>Electrons</td>
<td>Lipoic acid</td>
</tr>
<tr>
<td>CoE</td>
<td>Group Transfer</td>
<td>Precursor in dial</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>2. Groups Other than $H^+$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Thiamine – pyrophosphate (TPP)</td>
<td>CO$_2$ removal</td>
<td>Thiamine (vitamin B1)</td>
</tr>
<tr>
<td>· Coenzyme A</td>
<td>Acyl Group</td>
<td>Pantothenic acid</td>
</tr>
<tr>
<td>· Pyridoxal Phosphate</td>
<td>Amino Group</td>
<td>Pyridoxine (vitamin B6)</td>
</tr>
<tr>
<td>· 5’ deoxyadenosyl cabalamin</td>
<td>H$^+$ atom and alkyl group</td>
<td>Vit. B12</td>
</tr>
<tr>
<td>· Biocytin</td>
<td>CO$_2$ addition</td>
<td>Biotin</td>
</tr>
<tr>
<td>· Tetra hydro-folate</td>
<td>One-C-units</td>
<td>Folic acid</td>
</tr>
<tr>
<td>· ATP</td>
<td>Many functions</td>
<td>Not derived from vitamin</td>
</tr>
<tr>
<td>· Cytidine di phosphate</td>
<td>Phosphoryl choline diacyl glycerol</td>
<td></td>
</tr>
<tr>
<td>· Uridine di p</td>
<td>Monosaccharides</td>
<td>“</td>
</tr>
<tr>
<td>· Phosphoadenosine phosphosulphate (PAPS)</td>
<td>Sulfate</td>
<td>“</td>
</tr>
<tr>
<td>· S. Adenosyl methionine</td>
<td>Methyl</td>
<td>“</td>
</tr>
<tr>
<td>Metals as cofactors for Enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fe</strong>&lt;sup&gt;++&lt;/sup&gt; or <strong>Fe</strong>&lt;sup&gt;+++&lt;/sup&gt;</td>
<td>Cytochrome oxidase, catalase, peroxidase</td>
<td></td>
</tr>
<tr>
<td><strong>Cu</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Cytochrome oxidase</td>
<td></td>
</tr>
<tr>
<td><strong>Zn</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Carbonic anhydrase, Alcohol dehydrogenase</td>
<td></td>
</tr>
<tr>
<td><strong>Mg</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Hexokinase, (all kinases), G-6-phosphatase</td>
<td></td>
</tr>
<tr>
<td><strong>Mn</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Arginase, Ribonucleotide reductase</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong>&lt;sup&gt;+ &lt;/sup&gt;</td>
<td>Pyruvate kinase</td>
<td></td>
</tr>
<tr>
<td><strong>Ni</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Urease</td>
<td></td>
</tr>
<tr>
<td><strong>Mo</strong>&lt;sup&gt;++&lt;/sup&gt;</td>
<td>Dinitrogenase</td>
<td></td>
</tr>
<tr>
<td><strong>Se</strong></td>
<td>Glutathione peroxidase</td>
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</table>
Metals as Cofactors

- Metalloenzymes
- Apo E + Metal $\leftrightarrow$ Holo E (active)
- Prosthetic group: if the metal is linked strongly to the E molecule.
- Some metals present in active site and play a role in the catalysis mechanism.
Allosteric Enzymes

• Control [regulatory] enzymes
• Have quaternary structure
• Have active site and modulatory site
  – Active site binds substrate to give product
  – Modulatory site binds +ve or – ve modulator to increase or decrease the activity of the active site
• Catalyse an irreversible reaction
• Inhibited by end product
  – A ➔ B ↔ C ↔ D ➔ E ↔ F ➔ G ↔ H
  – Feedback inhibition
• Activated by substrate and other positive modulators
• Do not obey Michaelis Menten Kinetics
• Can be activated by substrate (homotropic) or other molecules (heterotropic).
• Also inhibited by end product (Feed-back inhibition).
• Do not obey Michaelis – Menten kinetic.
• Give sigmoid curve with $S$

\[ V_{max} \]

\[ v \]

\[ K_{0.5} \]

\[ \frac{1}{2} V_{max} \]

Cooperative effect
The sigmoid, or S-shaped, increase in reaction rate produced by most allosteric enzymes as substrate concentration increases.
Feedback Inhibition

Threonine

Enzyme 1 Threonine Deaminase

Intermediate A

Enzyme 2

Intermediate B

Enzyme 3

Intermediate C

Enzyme 4

Intermediate D

Enzyme 5

Isoleucine

High levels of isoleucine Allosteric Inhibitor

Substrate (Threonine)

Enzyme (Threonine deaminase)

Low levels of isoleucine
Positive and negative modulator
Activation of PFK by ADP

- +70 μM ADP
- +20 μM ADP
- No ADP

$\nu_0$

[Fructose 6-phosphate] (mM)
Structure of PFK
Activation of Pyruvate dehydrogenase

ATP → Pyruvate dehydrogenase kinase → ADP

Pyruvate dehydrogenase → ATP

Pyruvate dehydrogenase phosphatase → Pi

H₂O → Pyruvate dehydrogenase
## Enzyme Purification

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Vol. (ml)</th>
<th>Conc. u/ml</th>
<th>Total U</th>
<th>Protein (mg/ml)</th>
<th>Specific Activity</th>
<th>Yield (%)</th>
<th>Purification</th>
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<tbody>
<tr>
<td>Extract</td>
<td>7000</td>
<td>2.85</td>
<td>19,950</td>
<td>35.5</td>
<td>0.08</td>
<td>100</td>
<td>1.0</td>
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<tr>
<td>Chloroform supernatant</td>
<td>5800</td>
<td>3.6</td>
<td>20,880</td>
<td>19.2</td>
<td>0.187</td>
<td>105</td>
<td>2.34</td>
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<tr>
<td>37.5-55 (NH₄)₂SO₄</td>
<td>1500</td>
<td>11.25</td>
<td>16,875</td>
<td>21.4</td>
<td>0.525</td>
<td>84.4</td>
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<tr>
<td>DEAE – Cellulose</td>
<td>2380</td>
<td>3.82</td>
<td>9090</td>
<td>1.0</td>
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<td>45.5</td>
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<tr>
<td>Affinity column</td>
<td>450</td>
<td>15.05</td>
<td>6772</td>
<td>0.9</td>
<td>16.7</td>
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<td>209</td>
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<tr>
<td>Gel filtration</td>
<td>52</td>
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