Clinical Enzymology

11-23/10/2011

1. **Lecture 1**: The Biochemical Role of Enzymes / Introduction

2. **Lecture 2**: Kinetics of Enzyme-Catalyzed Reactions / Regulation of enzymes by substrate and product concentration.

3. **Lecture 3**: Kinetics of Enzyme-Catalyzed Reactions / Enzyme Inhibitors kinetics of Enzyme-Catalyzed Reactions

4. **Lecture 4**: Plasma Enzymes in Diagnosis

5. **Lecture 5**: Clinically Important Enzymes and Diagnostic Applications

6. **Lecture 6**: Enzymes assays

**Aim and objective of the above six lectures is to understand:**

1. The biochemical role of enzymes in the metabolic activity of the human body.
2. Kinetics of enzymes inhibition and the role of toxins and drugs
3. The disorder of enzyme activity in disease
4. Diagnostic application of enzymes

**References:**

1. "Biochemistry" by Lubert Stryer  
   *(textbook)*

2. "Textbook of Biochemistry with Clinical Correlations" by T.M.Devlin  
   *(additional reading)*

3. "Lippincott's Illustrated Reviews in Biochemistry" by P.C.Champe, R.A.Harvey and D.R.Ferrier  
   *(additional reading)*

   *(additional reading)*

5. "Clinical Laboratory Science Review" By Robert R. Harr  
   *(additional reading)*
The Biochemical Role of Enzymes

Introduction

11/10/2010
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(Ph.D., Post Doctorate)
Enzyme-Catalyzed Reactions

A. Enzymes are catalysts that increase the rate or velocity, \( v \), of many physiologic reactions.
   a. In the absence of enzymes, most reactions in the body would proceed so slowly that life would be impossible.
   b. Enzymes can couple reactions that would not occur spontaneously to an energy-releasing reaction, such as ATP hydrolysis, that makes the overall reaction favorable.
   c. Another of the most important properties of enzymes as catalysts is that they are not changed during the reactions they catalyze, which allows a single enzyme to catalyze a reaction many times.

B. Enzymes specifically bind the reactants in order to catalyze biologic reactions.
   a. During the reaction, the reactants or substrates are acted on by the enzyme to yield the products.
   b. Each substrate binds at its binding site on the enzyme, which may contain, be near to, or be the same as the active site harboring the amino acid side chains that participate directly in the reaction.
   c. Enzymes exhibit selectivity or specificity, a preference for catalyzing reactions with substrates having structures that interact properly with the catalytic residues of the active site.

C. A deficiency in enzyme activity can cause disease.
   a. Inherited absence or mutations in enzymes involved in critical metabolic pathways—eg, the urea cycle or glycogen metabolism—are referred to as inborn errors of metabolism. If not detected soon after birth, these conditions can lead to serious metabolic derangements in infants and even death.
   b. An enzyme deficiency can produce a deficiency of the product of the reaction it catalyzes, which may inhibit other reactions that depend on availability of that product.
   c. Accumulation of the substrate or metabolic byproducts of the substrate due to an enzyme deficiency can have profound physiologic consequences.
   d. Most inborn errors of metabolism manifest after birth because the exchange of metabolites between mother and fetus provides for fetal metabolic needs in utero.
   e. Therapeutic strategies for enzyme deficiency diseases include dietary modification and potential gene therapy or direct enzyme replacement (Table).
Table: Example of enzyme replacement therapy for inherited disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme deficiency</th>
<th>Normal Function of the Enzyme</th>
<th>Major Symptoms Or Findings on Examination</th>
<th>Physiologic Consequences and Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pompe Disease</td>
<td>Acid α-1,4-glucosidase</td>
<td>Hydrolysis of glycogen</td>
<td>Weakness, fatigue, failure to thrive, lethargy</td>
<td>Glycogen accumulation in several organs, including heart and skeletal muscle Congestive heart failure</td>
</tr>
<tr>
<td>Gaucher Disease</td>
<td>Gluco-Cerebrosidase</td>
<td>Hydrolysis of the glycollipid, glucocerebroside, a product of degradation of RBCs and WBCs</td>
<td>Easy bruising, fatigue, anemia, reduced platelet count</td>
<td>Accumulation of glucocerebroside in several organs, reduced lung and brain function, pain in upper trunk region, seizures, convulsions</td>
</tr>
<tr>
<td>Fabry Disease</td>
<td>α-Galactosidase A</td>
<td>Hydrolysis of the lipid, globotriaosylceramide</td>
<td>Severe fatigue, painful paresthesias (numbness and tingling) of the feet and arms, purplish skin lesions on abdomen and buttocks</td>
<td>Accumulation of globotriaosylcer-amide in endothelial cells of the blood vessels, altered cellular structure of heart and glomeruli, renal failure</td>
</tr>
</tbody>
</table>
**Enzyme Classification**

1. Enzymes can be made of either protein or RNA.
2. Most enzymes are proteins, which are grouped according to the six types of reactions they catalyze.

The top-level classification is

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>Trivial Names and examples</th>
<th>Type of reaction catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidoreductases</td>
<td>Dehydrogenases</td>
<td>Addition or subtraction of electrons</td>
</tr>
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<td></td>
<td></td>
<td>Reductases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidases</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Transferases</td>
<td>Kinases-Phosphotransferases</td>
<td>Transfer of small groups: amino, acyl, phosphoryl, one-carbon, sugar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aminotransferases</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hydrolases</td>
<td>Glycosidases</td>
<td>Add water across bonds to cleave them</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peptidases</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Lyases</td>
<td>Decarboxylase</td>
<td>Add the elements of water, ammonia, or carbon dioxide across a double bond (or the reverse reaction)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dehydratases</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydratases</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Isomerases</td>
<td>Mutases</td>
<td>Structural rearrangements</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epimerases</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ligases</td>
<td>Synthases</td>
<td>Join molecules together</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Synthetases</td>
<td></td>
</tr>
</tbody>
</table>

3. Several important physiologic catalysts are made of RNA, and these RNA-based enzymes or ribozymes are of two general types:
   a. RNA molecules that undergo **self-splicing**, in which an internal portion of the RNA molecule is removed while the parts on either side of this **intron** are reconnected.
b. Other RNA molecules that do not undergo self-splicing can act on other molecules as substrates are true catalysts.
   i. Ribonuclease P cleaves transfer RNA precursors to their mature forms.
   ii. The 23S ribosomal RNA is responsible for the peptidyl transferase activity of the bacterial ribosome.

4. Isozymes are protein-based enzymes that catalyze the same reaction but differ in amino acid composition.
   a. Because of their structural differences, isozymes may often be distinguished by separation in an electric field (electrophoresis) or by reactivity with selective antibodies.
   b. Several clinical uses have been made of isozymes selectively expressed by different tissues.

Example:

**Diagnosis of Heart Attack and Muscle Damage**

1. The enzyme creating kinase (CK) is formed of two subunits that can either be of the brain (B) type or the muscle (M) type, and different combinations of these types lead to isozymes that predominate in the brain (BB), skeletal muscle (MM), and heart muscle (MB).

2. Within 3-4 hours of a heart attack, damaged myocardial cells release CK of the MB type, which can be detected in serum by a monoclonal antibody and is useful to confirm the diagnosis.

3. Skeletal muscle myopathy often leads to release of CK of the MM type. Rhabdomyolysis is one of the major side effects of treatment with the cholesterol-lowering drugs the statins.
   o Inflammation of the muscle (myositis) leads to cell death.
   o The condition is characterized by muscle pain, weakness, elevated CK MM, and myoglobinuria.
Mechanisms of Enzyme Catalysis

A. Enzymes use a variety of strategies to catalyze reactions, and individual enzymes often use more than one strategy.

B. Substrate binding by an enzyme helps catalyze the reaction by bringing the reactants into **proximity** with the optimal **orientation** for reaction.

C. Amino acid side chains within active sites of many enzymes assist in catalysis by acting as **acids** or **bases** in reaction with the substrate.
   1. In the mechanism of the pancreatic hydrolase **ribonuclease**, a specialized histidine within the active site acts as a **general acid or proton donor** to begin cleavage of the phosphodiester linkage of the substrate RNA.
   2. The digestive enzyme **chymotrypsin** has a serine in its active site that acts as a **general base or proton acceptor** during hydrolysis of peptide bonds in protein substrates (Figure).

D. The binding of polysaccharide substrates that have six or more sugar groups to lysozyme, the enzyme in tears and saliva that cleaves such molecules, induces strain in the sugar nearest the active site making the nearby bond more susceptible to hydrolysis.

Figure from (Nelson & Cox, *Lehninger Principles of Biochemistry*, 3rd ed., 2000):

Figure from Garrett & Grisham, *Biochemistry*, 2nd ed., 1999) **Lysozyme mechanism**

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E. **In covalent catalysis**, the enzyme becomes covalently coupled to the substrate as an intermediate in the reaction mechanism before release of the products.

1. The active site serine of chymotrypsin attacks the protein substrate, which is cleaved and a portion of it becomes temporarily connected through the serine by an acyl linkage to the enzyme.

2. The acyl-enzyme intermediate reacts further by transfer of the polypeptide segment to water, completing cleavage (or **hydrolysis**) of the protein substrate.

Examples:

**Snake Venom Enzymes: Hydrolases that Produce Toxic Effects**

- Snake venoms are composed of a toxic mixture of enzymes that can kill or immobilize prey.
- **Neurotoxic** venoms of cobras, mambas, and coral snakes inhibit the enzyme acetylcholinesterase.
  - This hydrolase normally breaks down the neurotransmitter acetylcholine within nerve synapses.
  - The resultant elevation of acetylcholine causes a transient period of contraction followed by prolonged depolarization in the postsynaptic muscle cell, which induces relaxation and then paralysis of the victim.
- **Hemotoxic** venoms of rattlesnakes and cottonmouths contain as their principal toxin phosphodiesterase, an enzyme that catalyzes hydrolysis of phosphodiester bonds in ATP and other substrates.
  - One consequence of this activity is altered metabolism of endothelial cells, which leads to cardiac effects and rapid decrease in blood pressure.
  - These venoms induce circulatory shock and potentially death.

**Enzymes as Therapeutic Agents**

- The catalytic efficiency and exquisite specificity of enzymes have been exploited for use as therapeutic agents in certain diseases.
- Patients with **cystic fibrosis** use aerosol inhaler sprays of the DNA-hydrolyzing enzyme deoxyribonuclease to help reduce the viscosity of mucous secretions, which contain large amounts of DNA arising from destruction of WBCs as they fight lung infections.
- Patients who have had a **heart attack** or **stroke** are frequently treated by intravenous administration of tissue plasminogen activator (tPA) or streptokinase, enzymes that break down fibrin clots that clog blood vessels.
Allosteric Regulation of Enzymes

A. Key enzymes that catalyze rate-limiting steps of metabolic pathways or that are responsible for major cellular processes must be regulated to maintain homeostasis of individual cells and the organism overall.

B. Allosteric regulation refers to binding of a molecule to a site on the enzyme other than the active site and induces a subsequent change in shape of the enzyme causing an increase or decrease in its activity.

C. Many allosteric enzymes have multiple subunits whose interaction accounts for their unusual kinetic properties.

i. Enzymes that are subject to allosteric regulation by either positive or negative effectors exhibit cooperativity.

2. In the presence of positive cooperativity, a plot of $v$ versus $[S]$ shows sigmoidal kinetics, ie, is S-shaped (Figure).

   a. This kinetic behavior signifies that the enzyme's affinity for the substrate increases as a function of substrate loading.
   b. This is analogous to $O_2$ binding by hemoglobin, in which $O_2$ loading to one subunit facilitates $O_2$ binding to the next subunit, and so on.

D. Feedback inhibition occurs when the end product of a metabolic pathway accumulates, binds to and inhibits a critical enzyme upstream in the pathway, either as a competitive inhibitor or an allosteric effector.

Figure: Relationship between $V_i$ and $[S]$ for a reaction catalyzed by an allosteric enzyme, showing the effects of positive and negative effectors.
I - REGULATION OF ENZYMES BY SUBSTRATE AND PRODUCT CONCENTRATION
II - ENZYME INHIBITORS
REGULATION OF ENZYMES BY SUBSTRATE AND PRODUCT CONCENTRATION

The velocity of all enzymes is dependent on the concentration of substrate.

The hypothesis of enzyme kinetics assumes the rapid, reversible formation of a complex between an enzyme and its substrate (the substance upon which it acts to form a product).

It also assumes that the rate of formation of the product, P, is proportional to the concentration of the complex.

The velocity of such a reaction is greatest when all the sites at which catalytic activity can take place on the enzyme molecules (active sites) are filled with substrate; i.e., when the substrate concentration is very high.

\[ E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} E + P \]

\[ k_{-1} \]

E= enzyme,

S= substrate,

ES= enzyme-substrate complex, P= product

k₁, k₋₁ and k₂ are rate constants

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The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

\[
V_{\text{max}}[S]
\]

\[
v_0 = \frac{V_{\text{max}}}{K_m + [S]}
\]

\[V_{\text{max}}\text{=}\text{maximum velocity}, \ v_0 = \text{initial velocity}, \ K_m = \frac{(k_1 + k_2)}{k_1}\]

To determine the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is achieved. This is the maximum velocity \(V_{\text{max}}\) of the enzyme. In this state, all enzyme active sites are saturated with substrate.

The Michaelis-Menten constant \((K_m)\) is the substrate concentration required for an enzyme to reach one half its maximum velocity. Each enzyme has a characteristic \(K_m\) for a given substrate.

The key features of the plot are marked by points A, B and C. At high substrate concentrations the rate represented by point C the rate of the reaction is almost equal to \(V_{\text{max}}\), and the difference in rate at nearby concentrations of substrate is almost negligible.
If the Michaelis-Menten plot is extrapolated to infinitely high substrate concentrations, the extrapolated rate is equal to $V_{\text{max}}$. When the reaction rate becomes independent of substrate concentration, or nearly so, the rate is said to be zero order. (Note that the reaction is zero order only with respect to this substrate. If the reaction has two substrates, it may or may not be zero order with respect to the second substrate). At lower substrate concentrations, such as at points A and B, the lower reaction velocities indicate that at any moment only a portion of the enzyme molecules are bound to the substrate. In fact, at the substrate concentration denoted by point B, exactly half the enzyme molecules are in an ES complex at any instant and the rate is exactly one half of $V_{\text{max}}$. 


Inhibition of Enzyme Catalyzed Reactions

To avoid dealing with curvilinear plots of enzyme catalyzed reactions, biochemists Lineweaver and Burk introduced an analysis of enzyme kinetics based on the following rearrangement of the Michaelis-Menten equation:

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

Plots of \(1/v\) versus \(1/[S]\) yield straight lines having a slope of \(K_m/V_{max}\) and an intercept on the ordinate at \(1/V_{max}\).

The Lineweaver-Burk transformation of the Michaelis-Menten equation is useful in the analysis of enzyme inhibition. Since most clinical drug therapy is based on inhibiting the activity of enzymes, analysis of enzyme reactions using the tools described above has been fundamental to the modern design of pharmaceuticals.

Many Drugs Acts As Enzyme Inhibitors

- Many drugs, including antibiotics and antiviral agents, operate by inhibiting critical enzyme-catalyzed reactions or serve as alternative dead-end substrates of such reactions.
- The antibiotic activity of penicillin is due to its ability to inhibit transpeptidases responsible for crosslink formation in construction of bacterial cell walls, leading to lysis of the weakened cells.
Sulfanilamides (sulfa drugs) are antibiotics that serve as structural analogs of para-aminobenzoic acid (PABA), a substrate in the formation of folic acid by many bacteria. Substitution of the sulfanilamide compound in place of PABA in the reaction prevents formation of the critical coenzyme folic acid.

Inhibitors of the HIV protease are useful in antiviral therapy strategies because this enzyme is absolutely required for processing of proteins needed for synthesis of the viral coat.

The use of methotrexate in cancer chemotherapy to semi-selectively inhibit DNA synthesis of malignant cells, the use of aspirin to inhibit the synthesis of prostaglandins which are at least partly responsible for the aches and pains of arthritis.

Examples of irreversible (suicidal) inhibitors

Organophosphorous Pesticides: Suicide Inhibitors of Acetylcholinesterase

- Organophosphates form stable phosphoesters with the active site serine of acetylcholinesterase, the enzyme responsible for hydrolysis and inactivation of acetylcholine at cholinergic synapses.

- Irreversible inhibition of the enzyme leads to accumulation of acetylcholine at these synapses and consequent neurologic impairment.

- Poisoning by pesticides that contain organophosphate compounds produces a variety of symptoms, including nausea, blurred vision, fatigue, muscle weakness and, potentially, death caused by paralysis of respiratory muscles.
In addition, many poisons, such as cyanide, carbon monoxide and polychlorinated biphenols (PCBs), produce their life-threatening effects by means of enzyme inhibition.

Enzyme inhibitors fall into two broad classes:

1. Those causing irreversible inactivation of enzymes: Inhibitors of the first class usually cause an inactivating, covalent modification of enzyme structure. Cyanide is a classic example of an irreversible enzyme inhibitor: by covalently binding mitochondrial cytochrome oxidase, it inhibits all the reactions associated with electron transport. The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme, thus decreasing the maximum possible concentration of ES complex. Since the limiting enzyme reaction rate is often $k_2[ES]$, it is clear that under these circumstances the reduction of enzyme concentration will lead to decreased reaction rates. Irreversible inhibitors are usually considered to be poisons and are generally unsuitable for therapeutic purposes.

2. Those whose inhibitory effects can be reversed. Reversible inhibitors can be divided into two main categories; competitive inhibitors and noncompetitive inhibitors, with other three categories, rarely encountered, partially completive inhibitors uncompetitive inhibitors and mixed inhibitors.
<table>
<thead>
<tr>
<th>Inhibitor Type</th>
<th>Binding Site on Enzyme</th>
<th>Kinetic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Competitive Inhibitor</strong></td>
<td>Specifically 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><strong>Noncompetitive Inhibitor</strong></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><strong>Partially competitive Inhibitor</strong></td>
<td>similar to that of non-competitive, except that the EIS-complex has catalytic activity, which may be lower or even higher (partially competitive activation) than that of the enzyme-substrate (ES) complex.</td>
<td>lower $V_{\text{max}}$, but an unaffected $K_{m}$ value</td>
</tr>
<tr>
<td><strong>Uncompetitive Inhibitor</strong></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>
<tr>
<td><strong>Mixed inhibition</strong></td>
<td>Inhibitor bind to both the enzyme and the ES complex. It has the properties of both competitive and uncompetitive inhibition.</td>
<td>Both a decrease in $V_{\text{max}}$ and an increase in the $K_{m}$ value are seen in mixed inhibition.</td>
</tr>
</tbody>
</table>
The feature of all the reversible inhibitors is that when the inhibitor concentration drops, enzyme activity is regenerated. Usually these inhibitors bind to enzymes by non-covalent forces and the inhibitor maintains a reversible equilibrium with the enzyme. The equilibrium constant for the dissociation of enzyme inhibitor complexes is known as $K_I$:

$$K_I = \frac{[E][I]}{[E--I--complex]}$$

The effects of $K_I$ are best observed in Lineweaver-Burk plots. Probably the best known reversible inhibitors are competitive inhibitors, which always bind at the catalytic or active site of the enzyme. Most drugs that alter enzyme activity are of this type.

Competitive inhibitors are especially attractive as clinical modulators of enzyme activity because they offer two routes for the reversal of enzyme inhibition:

1. First, as with all kinds of reversible inhibitors, a decreasing concentration of the inhibitor reverses the equilibrium regenerating active free enzyme.

2. Second, since substrate and competitive inhibitors both bind at the same site they compete with one another for binding. Raising the concentration of substrate (S), while holding the concentration of inhibitor constant, provides the second route for reversal of competitive inhibition. The greater the proportion of substrate, the greater the proportion of enzyme present in competent ES complexes.
High concentrations of substrate can displace virtually all competitive inhibitor bound to active sites. Thus, it is apparent that $V_{\text{max}}$ should be unchanged by competitive inhibitors. This characteristic of competitive inhibitors is reflected in the identical vertical-axis intercepts of Lineweaver-Burk plots, with and without inhibitor. panel B.

**Panel A**

- $1/V$ vs. $1/[S]$ for uninhibited enzyme

**Panel B**

- $1/V$ vs. $1/[S]$ for uninhibited enzyme with competitive inhibitor

**Panel C**

- $1/V$ vs. $1/[S]$ for uninhibited enzyme with noncompetitive inhibitor

**Panel D**

- $1/V$ vs. $1/[S]$ for uninhibited enzyme with uncompetitive inhibitor

**Lineweaver-Burk Plots of Inhibited Enzymes**

Analogously, panel C illustrates that noncompetitive inhibitors appear to have no effect on the intercept at the x-axis implying that noncompetitive inhibitors have no effect on the $K_m$ of the enzymes they inhibit.

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Since noncompetitive inhibitors do not interfere in the equilibration of enzyme, substrate and ES complexes, the $K_m$'s of Michaelis-Menten type enzymes are not expected to be affected by noncompetitive inhibitors, as demonstrated by x-axis intercepts in panel C. However, because complexes that contain inhibitor (ESI) are incapable of progressing to reaction products, the effect of a noncompetitive inhibitor is to reduce the concentration of ES complexes that can advance to product. Since $V_{\text{max}} = k_2[E_{\text{total}}]$, and the concentration of competent $E_{\text{total}}$ is diminished by the amount of ESI formed, noncompetitive inhibitors are expected to decrease $V_{\text{max}}$, as illustrated by the y-axis intercepts in panel C.

A corresponding analysis of uncompetitive inhibition leads to the expectation that these inhibitors should change the apparent values of $K_m$ as well as $V_{\text{max}}$. Changing both constants leads to double reciprocal plots, in which intercepts on the x and y axes are proportionately changed; this leads to the production of parallel lines in inhibited and uninhibited reactions.
Clinical Problems

A Polish man and his friend who is of Japanese descent are sharing conversation over drinks at a party. After the Polish man finishes his second bottle of beer, he notices that his friend, despite having drunk only half his drink, appears flushed in the face. His friend then complains of dizziness and headache and asks to be driven home.

1. The marked difference in tolerance to alcohol illustrated by these men is most likely due to a gene encoding which of the following enzymes?
   A. Alcohol dehydrogenase
   B. Acetate dehydrogenase
   C. Alcohol reductase
   D. Aldehyde dehydrogenase
   E. Aldehyde aminotransferase

Comment: The answer is D. Many Asians lack a low-\(K_m\), form of acetaldehyde dehydrogenase, which is responsible for detoxifying acetaldehyde generated by oxidation of ethanol in the liver. Acetaldehyde accumulation in the blood of such individuals leads to the facial flushing and neurologic effects exhibited by the man of Japanese descent.
An alcoholic has consumed antifreeze as a substitute for ethanol. Ethyleneglycol, an ingredient in antifreeze, is also a substrate for the enzyme alcohol dehydrogenase (ADH), which normally converts ethanol to acetaldehyde. Ethylene glycol, however, is converted by ADH to a highly toxic product. Ethanol is administered as a treatment in this case of poisoning.

2. Why is ethanol an effective treatment for ethylene glycol poisoning?

A. ADH exhibits a much higher affinity (K$_m$ for ethanol than for ethylene glycol
B. Ethanol is an allosteric effector of ADH
C. Ethanol combines with the toxic product formed by the reaction of ADH with ethylene glycol and renders it harmless
D. Acetaldehyde is of therapeutic value.
E. Ethanol induces another enzyme that is capable of metabolizing ethylene glycol

Comment: The answer is: 1-A: Aldehyde dehydrogenase (ADH), which exhibits a broad substrate specificity for alcohols, has a much higher affinity for ethanol [i.e., a lower Km] than for ethylene glycol. Saturating ADH with ethanol by administration of therapeutic levels prevents it from converting ethylene glycol to the toxic aldehyde, and allows ethylene glycol to eventually be excreted unmetabolized.
Ethylene Glycol is a competitive inhibitor of alcohol dehydrogenase (ADH) activity. Ethylene glycol is converted by ADH, to oxalic acid, calcium oxalate crystals in kidney.

- In Non-Asians, ethanol is administered as antidote to ethylene glycol ingestion
- In Asians, ethanol administration is not efficacious due to low, non-inducible ADH activity.

Interesting story in Japanese press several years ago Drunk Japanese businessman was spared from death due to ethylene glycol poisoning siphoning antifreeze from a friend's car habitual alcohol user, had higher steady-state ADH levels.
3. If one compares Lineweaver-Burk plots for the reactions of ADH with ethanol and ethylene glycol, which of the following would be observed?

A. They exhibit identical slopes.

B. They exhibit identical y-intercepts.

C. They exhibit identical x-intercepts.

D. Only the plot for the reaction of ethanol is linear

E. Only the plot for the reaction of ethylene glycol is linear

Comment: The answer is 2-B: If ADH obeys Michaelis-Menten kinetics, then the Lineweaver-Burk plot, which is a linear transform of the Michaelis-Menten equation, will be linear for both substrates. ADH exhibits different Km values for ethanol and ethylene glycol. Therefore, the x-intercept (1/ K_m and the slope (K_m/V_max) are different. If only the affinity for the alternative substrate is different, then the V_max will be the same as will the y-intercept (1/V_max).
4. Blood was taken from this patient and analyzed for the serum levels of certain enzymes. Which one of the following enzymes will most likely be present at elevated levels?

(A) Amylase

(B) Creatine kinase

(C) Alanine aminotransferase

(D) Acid phosphatase

(E) Lactate dehydrogenase

Comment: the answer is 3-C: Chronic alcoholics are likely to exhibit signs of liver damage. Alanine aminotransferase is present in the cytosol of liver cells, and its release into the serum is diagnostic of hepatocellular damage. Lactate dehydrogenase and creatine kinase isozymes are analyzed to diagnose heart attacks. Amylase levels are elevated in patients with acute pancreatitis, and elevated acid phosphatase levels may be diagnostic of prostate cancer.
5. A noncompetitive enzyme inhibitor

A. Decreases $V_{\text{max}}$ and increases $K_m$.

B. Decreases $V_{\text{max}}$ and has no effect on $K_m$.

C. Has no effect on $V_{\text{max}}$ or $K_m$.

D. Has no effect on $V_{\text{m:K}}$ and increases $K_m$.

E. Has no effect on $V_{\text{max}}$ and decreases $K_m$.

Comment: The answer is B. A noncompetitive inhibitor binds to the enzyme at a site other than the substrate binding site, so it has little measurable effect on the enzyme’s affinity for substrate, as represented by the $K_m$. However, the inhibitor has the effect of decreasing the availability of active enzyme capable of catalyzing the reaction, which manifests itself as a decrease in $V_{\text{max}}$. 
6. A 47-year-old man is evaluated for a 12-hour history of nausea, vomiting and, more recent, difficulty breathing. His past medical history is unremarkable, and he takes no medications. However, he is a farmer who has had similar episodes in the past after working with agricultural chemicals in his fields. Just yesterday he reports applying diazinon, an organophosphate insecticide, to his sugar beet field. After consultation with the poison center, you conclude that this patient’s condition is most likely due to inhibition of which of the following enzymes?

A. Acetate dehydrogenase
B. Alanine aminotransferase
C. Streptokinase
D. Acetylcholinesterase
E. Creatine kinase

Comment: The answer is D. Organophosphates react with the active site serine residue of hydrolases such as acetylcholinesterase and form a stable phosphoester modification of that serine that inactivates the enzyme toward substrate. Inhibition of acetylcholinesterase causes overstimulation of the end organs regulated by those nerves. The symptoms manifested by this patient reflect such neurologic effects resulting from the inhalation or skin absorption of the pesticide diazinon.
7. Accidental ingestion of ethylene glycol, an ingredient of automotive antifreeze, is fairly common among children because of the liquid’s pleasant color and sweet taste. Ethylene glycol itself is not very toxic, but it is metabolized by alcohol dehydrogenase to the toxic compounds glycolic acid, glyoxylic acid, and oxalic acid, which can produce acidosis and lead to renal failure and death. Treatment for suspected ethylene glycol poisoning is hemodialysis to remove the toxic metabolites and administration of a substance that reduces the metabolism of ethylene glycol by displacing it from the enzyme.

Which of the following compounds would be best suited for this therapy?

A. Acetic acid
B. Ethanol
C. Aspirin
D. Acetaldehyde
E. Glucose

Comment: The answer is B. The therapeutic rationale for ethylene glycol poisoning is to compete for the attention of alcohol dehydrogenase by providing a preferred substrate, ethanol, so that the enzyme is unavailable to catalyze oxidation of ethylene glycol to toxic metabolites. Ethanol will displace ethylene glycol by mass action for a limited time, during which hemodialysis is used to remove ethylene glycol and its toxic metabolites from the patient's bloodstream.
8. Glucose taken up by liver cells is rapidly phosphorylated to glucose 6-phosphate with ATP serving as the phosphate donor in the initial step of metabolism and assimilation of the sugar. Two enzymes, which may be considered isozymes, are capable of catalyzing this reaction in the liver cell. Hexokinase has a low $K_m$ of -0.05 mM for glucose, whereas glucokinase exhibits sigmoidal kinetics with an approximate $K_m$ of ~5 mM. After a large meal, the glucose concentration in the hepatic portal vein may approximate 5 mM.

After such a large meal, which of the following scenarios describes the relative activity levels for these two enzymes?

<table>
<thead>
<tr>
<th>Hexokinase</th>
<th>Glucokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Not active</td>
<td>Not active</td>
</tr>
<tr>
<td>B. $v \approx \frac{1}{2}V_{max}$</td>
<td>Not active</td>
</tr>
<tr>
<td>C. $v \approx V_{max}$</td>
<td>Not active</td>
</tr>
<tr>
<td>D. $v \approx V_{max}$</td>
<td>$v \approx \frac{1}{2}V_{max}$</td>
</tr>
<tr>
<td>E. $v \approx V_{max}$</td>
<td>$v \approx V_{max}$</td>
</tr>
</tbody>
</table>

Comment: The answer is D. This problem provides a practical illustration of the use of the Michaelis-Menten equation. The high concentration of glucose in the hepatic portal vein after a meal would promote a high rate of glucose uptake into liver cells, necessitating rapid phosphorylation of the sugar. The glucose concentration far exceeds the $K_m$ of hexokinase, ie, $[S] > K_m$, meaning that the enzyme will be nearly saturated with substrate and $v \approx V_{max}$. However, the $[S] = K_m$ for glucokinase, which will be active in catalyzing the phosphorylation reaction and $v \approx \frac{1}{2}V_{max}$.
Plasma Enzymes in Diagnosis

[Prof. Dr. H.D.El-Yassin]

18 October [2011]
Plasma Enzymes in Diagnosis

Most enzymes are present in cells at much higher concentrations than in plasma. Some occur predominantly in cells of certain tissues, where they may be located in different cellular compartments such as the cytoplasm or the mitochondria. 'Normal' plasma enzyme levels reflect the balance between the rate of synthesis and release into plasma during cell turnover, and the rate of clearance from the circulation.

Plasma contains several enzymes, some of which are functional in the plasma, while others are merely present in plasma due to leakage from tissues. Lipoprotein lipase, psuedocholinesterase and enzymes concerned in the coagulation of blood and the dissolution of the blood clot are enzymes that serve a function in the plasma. Though many other enzymes have no function in the plasma, they are still useful as diagnostic tools. Measurement of their levels in plasma offers valuable information about diseases involving the tissue of their origin.

It is easier to measure enzyme activity in body fluids, by monitoring changes in either substrate or product concentrations, than to measure enzyme protein concentration directly.

The enzyme activity in plasma may be:

- Increased due to proliferation of cells, an increase in the rate of cell turnover or damage or in enzyme synthesis (induction), or to reduced clearance from plasma
- Lower than normal, very occasionally due to reduced synthesis or congenital deficiency.

Changes in plasma enzyme activities may sometimes help to detect and localize tissue cell damage or proliferation, or to monitor treatment and progress of disease.
Assessment of Cell Damage and Proliferation

Plasma enzyme levels depend on:

1. The rate of release from damaged cells which, in turn, depends on the rate which damage is occurring:
2. The extent of cell damage.

In the absence of cell damage the rate release depends on:

1. The rate of cell proliferation:
2. The degree of induction of enzyme synthesis

These factors are balanced by:

• The rate of enzyme clearance from circulation.

Acute cell damage, for example in viral hepatitis, may cause very high plasma enzyme activities that fall as the condition resolves. By contrast, the liver may be much more extensively involved in advanced cirrhosis but the rate of cell damage is often low and consequently plasma enzyme activities may be only slightly raised or be within the reference range. In very severe liver disease plasma enzyme activities may even fall terminally, when the number of hepatocytes is grossly reduced.

It is not known how most enzymes are removed from, or their action inhibited in the circulation. Relatively small peptides, such as α-amylase, can be cleared by the kidneys: most enzymes are large proteins and are probably catabolized by plasma proteases before being taken up by the reticuloendothelial system. In health each enzyme has a fairly constant and characteristic biological half-life; knowledge of this half-life may be of help in assessing the time since the onset of an acute illness. After a myocardial infarction, for example, plasma levels of creatine kinase and aspartate transaminase fall to normal before those of lactate dehydrogenas, which has a longer half-life. The half-life may be lengthened if there is circulatory impairment.

Renal glomerular impairment may delay the rate of fall of those plasma enzymes cleared through the kidneys. For example plasma amylase activity may be high due to renal glomerular impairment, rather pancreatic damage.
**Localization of Damage**

Most of the enzymes commonly measured to assess tissue damage are present in nearly all cells, although their relative concentrations in certain tissues may differ. Measurement of the plasma activity of an enzyme known to be in high concentration within cells of a particular tissue may indicate an abnormality of those cells, but the results will rarely enable a specific diagnosis to be made. For example if there is circulatory failure after a cardiac arrest very high plasma levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance: the raised plasma levels of 'cardiac' enzymes do not necessarily mean that a myocardial infarct caused the arrest.

The diagnostic precision of plasma enzyme analysis may be improved by

1. **Estimation of more than one enzyme**. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For instance, although both alanine and aspartate transaminases are abundant in the liver, the concentration of aspartate transaminase is much greater than that of alanine transaminase in heart muscle.

2. **Isoenzyme determination**. Some enzymes exist in more than one form: these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues such identification will give more information than the measurement of plasma total enzyme activity: for example, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.

3. **Serial enzyme estimations**. The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance.

The distribution of enzymes within cells may differ. Alanine transaminase and lactate dehydrogenase are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas aspartate transaminase occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities.
Non-specific Causes of Raised Plasma Enzyme Activities

Before attributing a change in plasma enzyme activity to a specific disease process it is important to exclude the presence of factitious or nonspecific causes.

Slight rises in plasma aspartate transaminase activities are common, non-specific findings in many illnesses. Moderate exercise, or a large intramuscular injection, may lead to a rise in plasma creatine kinase activity; isoenzyme determination may identify skeletal muscle as the tissue of origin.

Some drugs may induce synthesis of the microsomal enzyme, gamma-glutamyltransferase, and so increase its plasma activity in the absence of disease.

Plasma enzyme activities may be raised if the rate of clearance from the circulation is reduced. In the absence of disease this may occur if for example the plasma enzyme forms:-

- macromolecules (aggregates), such as in macroamylasaemia.
- complexes with immunoglobulins. as occasionally occur with lactate dehydrogenase, alkaline phosphatase, creatine kinase.

Factors Affecting Results of Plasma Enzyme Assays

1. Analytical factors affecting results. The total concentration of all plasma enzyme proteins is less than 1 g/L. Results of enzyme assays are not usually expressed as concentrations, but as activities. Changes in concentration may give rise to proportional changes in catalytic activity, but the results of such measurements depend on many analytical factors. These include the concentrations of the substrate and product, the pH and temperature at which the reaction is carried out. The type of buffer, and the presence of activators or inhibitors. Because the definition of ‘international units’ does not take these factors into account, results from different laboratories, apparently expressed in the same units, may not be directly comparable. Therefore, plasma enzyme activities must be interpreted in relation to the reference ranges from the issuing laboratory.
2. Physiological factors affecting enzyme activities include for example:
   a. Age: plasma aspartate transaminase activity is moderately higher during the neonatal period than in adults: plasma alkaline phosphatase activity of bony origin is higher in children than in adults and peaks during the pubertal bone growth spurt before falling to adult levels.
   b. Sex: plasma γ-glutamyltransferase activity is higher in men than in women.
   c. Physiological conditions:

      Plasma alkaline phosphatase activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme: several enzymes, such as the transaminases and creatine kinase rise moderately in plasma during and immediately after labour or strenuous exercise.

Plasma enzyme activities must be interpreted in relation to the sex and age-matched reference ranges of the issuing laboratory.
Genetic basis for enzyme synthesis:

Since all enzymes are proteins, their synthesis follows the general pattern of protein synthesis and is regulated by genes. For every enzyme, there is said to be one gene (one gene, one enzyme hypothesis); where an enzyme is a complex protein containing more than one protein subunit more than one gene may be concerned in its synthesis. Genetic mutation results in abnormal DNA code and synthesis of an abnormal enzyme protein. Since the abnormal enzyme cannot serve the normal function, a metabolic abnormality occurs and this is transmitted to the progeny. Such transmittable abnormalities of metabolism due to abnormal enzyme molecules are known as: 'Molecular diseases' or 'Inborn Errors of Metabolism'. Phenylketonuria, alkaptonuria, pentosuria, glycogen storage disease, galactosemia and cystinuria are but a few of several known molecular diseases.

1. Phenylketonuria: is an autosomal recessive disorder caused by an abnormality of the phenylalanine hydroxylase. Because phenyl alanine can not be converted to tyrosine it accumulates in plasma and is secreted in the urine with its metabolites, such as phenylpyruvic acid; the disease acquired its name from the detection of the latter "phenylketone" in the urine. (see diagram below)
2. **Alkaptonuria**: is an autosomal recessive disorder due to the deficiency of homogentisic acid oxidase. Homogentisic acid accumulates in tissue and blood and is passed in the urine. Oxidation and polymerization of homogentisic acid produces the pigment alkapton in much the same way as polymerization of DOPA produce melanin. Deposition of alkapton in cartilages, with consequent darkening is called ochronosis and result in visible darkening of the cartilages of the ears. Conversion of homogentisic acid to alkapton is accelerated in alkaline conditions and most obvious abnormality in alkaptonuria is darkening of the urine as it becomes more alkaline on standing. The condition is compatible with normal life span despite the tendency for patient to develop arthritis in later life. (refer to previous diagram)

3. **glycogen storage disease**: a deficiency of one of the enzymes involved in the glycogenesis or glycogenolysis results in the accumulation of normal or abnormal glycogen with hepatomegaly: in von Gierk’s disease, the least rare glycogen storage disorder, there is a deficiency of glucose-6-phosphate. Fasting hypoglycemia occurs because the enzyme is essential for the conversion of glucose-6-phosphate to glucose.

4. **galactosaemia** an autosomal recessive disorder due to a deficiency of galactose-1-phosphate uridylytransferase, may cause cirrhosis of the liver if untreated.

5. **cystinuria**: is an autosomal recessive inherited abnormality of tubular reabsorption, with excessive urinary excretion of the dibasic amino acids: cystine, arginine and lysine.
Clinically Important Enzymes and Diagnostic Applications

Prof. Dr. H.D.El-Yassin
20 October 2011
## Clinically Important Enzymes and Diagnostic Applications

*Distribution and application of clinically important enzymes*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Principle Sources of Enzyme in blood</th>
<th>Clinical applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase</td>
<td>Liver</td>
<td>Hepatic parenchymal diseases</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Liver, bone, intestinal mucosa, placenta</td>
<td>Bone diseases, hepatobiliary diseases</td>
</tr>
<tr>
<td>Amylase</td>
<td>Salivary glands, pancreae</td>
<td>Pancreatic diseases</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>Liver, skeletal muscle, heart erythrocytes</td>
<td>Hepatic parenchymal disease, muscle disease</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>Liver</td>
<td>Organophosphorus insecticide poisoning, hepatic parenchymal disease</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Skeletal muscle, heart</td>
<td>Muscle diseases(M.I.)</td>
</tr>
<tr>
<td>γ-glutamyl transferase</td>
<td>Liver</td>
<td>Hepatobiliary diseases, marker of alcohol abuse</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Heart, liver, skeletal muscle, erythrocytes, platelets, lymph nodes</td>
<td>Hemolysis, hepatic parenchymal diseases, tumor marker</td>
</tr>
<tr>
<td>Lipase</td>
<td>Pancreas</td>
<td>Pancreatic diseases</td>
</tr>
<tr>
<td>5’-nucleotidase</td>
<td>Liver</td>
<td>Hepatobiliary diseases</td>
</tr>
<tr>
<td>Trypsin</td>
<td>pancreas</td>
<td>Pancreatic diseases</td>
</tr>
</tbody>
</table>
Pancreatic enzymes:

Acute pancreatitis is an inflammatory process in which pancreatic enzymes are activated and cause autodigestion of the gland. It is a result of anatomical changes that arise from two events.

1. The first is the autodigestion of the acinar cells by inappropriate activation of the pancreatic enzymes (especially trypsinogen) within the cell.
2. The second is the cellular injury response that is mediated by proinflammatory cytokines. The mechanisms by which the digestive enzymes become activated within the acinar cell are unclear. However, such inappropriate activation of pancreatic enzymes leads to destruction of the acinar cell and surrounding fat deposits, and it weakens the elastic fibers of the blood vessels, resulting in leakage. There are some enzymes that are synthesized and stored as the active enzymes in the zymogen granules. These include α-amylase, carboxyl ester lipase, lipase, colipase, RNase, and DNase.

1. **α-Amylase: (EC3.2.1.1; 1,4-α-D-glucan glucohydrolase; AML)** is an enzyme of the hydrolyase class that catalyzes the hydrolysis of 1,4-α-glycosidic linkages in polysaccharides. AMYs are calcium metaloenzymes, with the calcium absolutely required for functional integrity.

AMYs normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass the glomeruli of the kidneys and AMY is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY) origin.

**Clinical Significance**

Normal values of amylase: 28-100 U/L = 0.48-1.7 µkat/L

**CAUSES OF RAISED PLASMA AMYLASE ACTIVITY**

- **Marked increase (five to 10 times the upper reference limit):**
  - acute pancreatitis:
  - severe glomerular impairment:
  - perforated peptic ulcer especially if there is perforation into the lesser sac.

- **Moderate increase (up to five times the upper reference limit):**
  - other acute abdominal disorders:
    - perforated peptic ulcer:
    - acute cholecystitis:
intestinal obstruction:
abdominal trauma:
salivary gland disorders:
mumps:
salivary calculi:
Sjögren's syndrome:
severe glomerular dysfunction (may be markedly raised);
myocardial infarction (occasionally):
acute alcoholic intoxication:
diabetic ketoacidosis (may be markedly raised);
macroamylasaemia.

Pancreatic pseudocyst. If the plasma amylase activity fails to fall after an attack of acute pancreatitis there may be leakage of pancreatic fluid into the lesser sac (a pancreatic pseudocyst). Urinary amylase levels are high, differentiating it from macroamylasaemia. This is one of the few indications for estimating urinary amylase activity, which is inappropriately low relative to the plasma activity if there is glomerular impairment or macroamylasaemia.

Macroamylasaemia. In some patients a high plasma amylase activity is due to a low renal excretion of the enzyme, despite normal glomerular function. The condition is symptomless; it is thought that either the enzyme is bound to a high molecular weight plasma component such as protein, or that the amylase molecules form large polymers that cannot pass through the glomerular membrane. This harmless condition may be confused with other causes of hyperamylasaemia.
2. **Lipase: (EC 3.1.1.3; triacylglycerol acylhydrolase; LPS)** is a single–chain glycoprotein with molecular weight of 48 kDa. For full catalytic activity and greatest specificity the presence of bile salts and a cofactor called colipase, which is secreted by the pancreas, is required. LPS is a small molecule and is filtered through the glomerulus. It is totally reabsorbed by the renal tubules, and it is not normally detected in urine.

**Clinical Significance**

Normal values: 40-200 U/L

Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.

Note: serum amylase is increased in mumps, pancreatic disease or due to some other cause, whereas lipase is increased only in pancreatitis. Therefore, the determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

3. **Trypsin: (EC 3.4.21.4; no systemic name; TRY)** is a serine proteinase that hydrolyze the peptide bonds formed by the carboxyl groups of lysine arginine with other amino acids.

**Clinical Significance**

Normal values of trypsin: 25±5.3 µg/L

Increased in pancreatic disease. But as there is no distinct role of trypsin estimation in the routine management of patients with acute pancreatitis, this test is therefore considered of limited clinical value.

4. **Chymotrypsin (EC 3.4.21.1; no systemic name; CHY)**

5. **Elastas-1 (EC 3.4.21.36; no systemic name; E1)**
Liver enzymes:

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various heptobiliy disorders.

There are three types of enzymes:

1. Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage = markers of hepatocellular damage.
2. Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes) = markers of cholestasis.
3. Enzymes which are synthesized in the hepatocyte = indicates disturbances in the hepatocellular synthesis.

1. Markers of hepatocellular damage.

1. Aminotransaminases

The transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid: they need the cofactor, pyridoxal phosphate for optimal activity. They are widely distributed in the body.

The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus AST catalyzes the reaction:

\[
\begin{align*}
\text{COO}^\ominus & \quad + \quad \text{COO}^\ominus \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{L-Aspartate} & \quad \text{2-Oxoglutarate}
\end{align*}
\]

\[\xrightarrow{\text{AST, P-5'-P}} \]

\[
\begin{align*}
\text{COO}^\ominus & \quad + \quad \text{COO}^\ominus \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{Oxaloacetate} & \quad \text{L-Glutamate}
\end{align*}
\]
ALT catalyzes the analogous reaction:

\[
\text{L-Alanine} + \text{2-Oxoglutarate} \rightarrow \text{Pyruvate} + \text{L-Glutamate}
\]

The reactions are reversible, but the equilibrium of AST and ALT reactions favor formation of aspartate and alanine respectively.

- **Location:**
  - AST present in cytosol and mitochondria
  - ALT located in cytosol of liver
- In the liver, the concentration of ALT per unit weight of the tissue is more than AST.
- These enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
- The highest activities of ALT are found in hepatocytes and muscle cells.
- Again the hepatocytes have very high activity of ALT.
- Therefore elevations in serum ALT are considered to be relatively specific for liver disease.
- AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.
- In liver disease, the ALT level is increased markedly compared to AST.
- In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST
Table: Aminotransferase activities in human tissues, relative to serum as unity

<table>
<thead>
<tr>
<th></th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>7800</td>
<td>450</td>
</tr>
<tr>
<td>Liver</td>
<td>7100</td>
<td>2850</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5000</td>
<td>300</td>
</tr>
<tr>
<td>Kidneys</td>
<td>4500</td>
<td>1200</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1400</td>
<td>130</td>
</tr>
<tr>
<td>Spleen</td>
<td>700</td>
<td>80</td>
</tr>
<tr>
<td>Lungs</td>
<td>500</td>
<td>45</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Serum</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>


a. Aspartate Transaminase (EC 2.6.1.1; L-aspartate:2-oxoglutarate aminotransferase; AST)

Clinical Significance

Normal values of AST:  male:  <35 U/L = <0.60 µkat/L
                       Female: <31 U/L = <0.53 µkat/L

AST (glutamate oxaloacetate transaminase. GOT) is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. Half-life = 17 hours.

CAUSES OF RAISED PLASMA AST ACTIVITIES

• Artefactual.

    Due to in vitro release from erythrocytes if there is haemolysis or if separation of plasma from cells is delayed.

• Physiological.

    During the neonatal period (about 1.5 times the upper adult reference limit).
• Marked increase (10 to 100 times the upper adult reference limit):

  Circulatory failure with 'shock' and hypoxia:
  Myocardial infarction
  Acute viral or toxic hepatitis.

• Moderate increase-

  Cirrhosis (may be normal, but may rise to twice the upper adult reference limit):
  Infectious mononucleosis (due to liver involvement):
  Cholestatic jaundice (up to 10 times the upper adult reference limit):
  Malignant infiltration of the liver (may be normal, but may rise to twice the upper reference limit):
  Skeletal muscle disease:
  After trauma or surgery (especially after cardiac surgery):
  Severe haemolytic episodes (of erythrocyte origin).

b. Alanine Transaminase (EC 2.6.1.2; L-alanine:2-oxoglutarate aminotransferase; ALT)

Clinical Significance

Normal values of ALT: male: <45 U/L = <0.77 µkat/L
                        Female: <34 U/L = <0.58 µkat/L

ALT (glutamate pyruvate transaminase, GPT) is present in high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Half-life = 47 hours

In liver damage, both enzymes are increased but ALT increases more. In myocardial infarction AST is increased with little or no increase in ALT.
CAUSES OF RAISED PLASMA ALT ACTIVITIES

• Marked increase (10 to 100 times the upper limit of the adult reference range circulatory failure with ‘shock’ and hypoxia:
  Acute viral or toxic hepatitis.

• Moderate increase:
  Cirrhosis (may be normal or up to twice the upper adult reference limit): infectious mononucleosis (due to liver involvement):
  Liver congestion secondary to congestive cardiac failure:
  cholestatic jaundice (up to 10 times the upper reference limit in adults); surgery or extensive trauma and skeletal muscle disease (much less affected than AST)

2. Markers of cholestasis

I. Alkaline phosphatase (EC 3.1.3.1; orthophosphoric-monoester phosphohydrolase [alkaline optimum]; ALP). Half-life= 10 days

Clinical Significance
The alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but are in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. The exact metabolic function of ALP is unknown but it is probably important for calcification of bone. In adults plasma ALP is derived mainly from bone and liver in approximately equal proportions: the proportion due to the bone fraction is increased when there is increased osteoblastic activity that may be physiological.

Causes of raised Plasma ALP activity

• Physiological: There is a gradual increase in the proportion of liver ALP with age: in the elderly the plasma bone isoenzyme activity may increase slightly.

• Bone disease
  rickets and osteomalacia

  secondary hyperparathyroidism.

• Liver disease.
• Malignancy.
  bone or liver involvement or direct tumor production.
POSSIBLE CAUSES OF LOW PLASMA ALP ACTIVITY

• Arrested bone growth

• Hypophosphatasia: an autosomal recessive disorder, associated with rickets or osteomalacia.

Table: reference intervals for alkaline phosphatase activities in serum

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Reference Interval (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>4–15</td>
<td>54-369</td>
</tr>
<tr>
<td>Males</td>
<td>20-50</td>
<td>53-128</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
<td>56-119</td>
</tr>
<tr>
<td>Females</td>
<td>20-50</td>
<td>42-98</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
<td>53-141</td>
</tr>
</tbody>
</table>

ISOENZYMES OF ALKALINE PHOSPHATASE

Bone disease with increased osteoblastic activity, or liver disease with involvement of the biliary tracts, are the commonest causes of an increased total alkaline phosphatase activity.

Rarely the cause is not apparent and further tests may be helpful. The isoenzymes originating from cells of bone, liver, intestine and placenta may be separated by electrophoresis, but interpretation may be difficult if the total activity is only marginally raised.

Assays for ALP isoenzymes are needed when:

I. The source of an elevated ALP in serum is not obvious and should be clarified.
II. The main clinical question is concerned with detecting the presence of liver or bone involvement
III. In the case of metabolic bone disorders, to ascertain any modifications in the activity of osteoblastes to monitor the disease activity and the effect of appropriate therapies.

2. Gamma-glutamyl-transferase (EC 2.3.2.21; γ-glutamyl-peptide: amino acid γ-glutamyltransferase; GGT): catalyzes the transfer of the γ-glutamyl group from peptides and compounds that contain it to an acceptor

Gamma-glutamyltransferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

Clinical Significance

Normal values for GGT

<table>
<thead>
<tr>
<th></th>
<th>Male:</th>
<th>Female:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;55 U/L = &lt;0.94 µkat/L</td>
<td>&lt;38 U/L = &lt;0.65 µkat/L</td>
</tr>
</tbody>
</table>
Causes of raised plasma GGT activity

• Induction of enzyme synthesis, without cell damage, by drugs or alcohol.

• Hepatocellular damage, such as that due to infectious hepatitis:

A patient should never be labeled an alcoholic because of a high plasma GGT activity alone.

Other enzymes

1. Cholinesterase (EC 3.1.1.7, acetylcholine acetylhydrolase), which is called true cholinesterase or choline esterase I. found in:
   a. erythrocytes
   b. lung and spleen
   c. nerve endings
   d. the gray matter of the brain.

It is responsible for the prompt hydrolysis of acetylcholine released at the nerve endings to mediate transmission of the neural impulse across the synapse. The degradation of acetylcholine is required for the depolarization of the nerve so that it is repolarized in the next conduction event.

The second cholinesterase is acylcholine acylhydrolase (EC 3.1.1.8, CHE)

It is also called: (1)pseudocholinesterase, (2)serum cholinesterase, (3)butyryl choline esterase, (4) choline esterase II

Although it is found in the:

1. liver
2. pancreas
3. heart
4. white matter of the brain
5. serum

Its biological role is unknown

Clinical Significance

Normal values for CHE: 4.9-11.9 U/mL

Measurements of CHE activity in serum are used:

1. as a test of liver function
2. as an indicator of possible insecticide poisoning
Causes of decreased plasma cholinesterase activity

- Hepatic parenchymal disease (reduced synthesis).
- Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.
- Inherited abnormal cholinesterase variants, with low biological activity.

Causes of increased plasma cholinesterase activity

- Recovery from liver damage (actively growing hepatocytes)
- Nephrotic syndrome

2. Glutamate dehydrogenase (EC 1.4.1.3; L-glutamate: NAD(P)^+ oxidoreductase, deaminating; GLD) is a mitochondrial enzyme found mainly in the:
  a. liver
  b. heart muscle
  c. kidneys
  but small amounts occur in other tissue, including
  d. brain
  e. skeletal muscle tissue
  f. leukocytes

Clinical significance

GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease, particularly when interpreted in conjunction with other enzyme test results. The key to this differential diagnostic potential is to be found in the intraorgan and intracellular distribution of the enzyme. As an exclusively mitochondrial enzyme, GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. GLD activity in serum is stable at 4°C for 48 hours and at -20°C for several weeks. The GLD upper reference limits are 6U/L (women) and 8U/L (men), when a method optimized at 37°C is used.
Muscle enzymes:

1. Creatine Kinase (EC 2.7.3.2; adenosine triphosphate: creatine N-phosphotransferase CK)

\[
\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}
\]

\[
\text{ATP} + \text{glucose} \xrightarrow{\text{HK}} \text{glucose-6-phosphate} + \text{ADP}
\]

\[
\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PD}} 6\text{-phosphogluconate} + \text{NADPH} + \text{H}^+
\]

CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.

The concentration gradients between some human tissues and serum for creatine kinase. The concentration gradient is logarithmic.
**Clinical significance**

Normal range for total CK: Male: 46-171 U/L = 0.78-2.90 µkat/L

Female: 34-145 U/L = 0.58-2.47 µkat/L

Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%-80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity. Quite high values of CK are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as:

a. Myasthenia gravis  
b. Multiple sclerosis  
c. Polimyeltis  
d. Parkinsonism

Serum enzyme activity is normal

**ISOENZYMES OF CK**

CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.
CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than five per cent in skeletal muscle: its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than six per cent of the total.

CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal.

Approximate concentrations of tissue CK activity (expressed as multiple activity concentrations in serum and cytoplasmic isoenzyme composition)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CK-BB</th>
<th>CK-MB</th>
<th>CK-MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle (red fibers)</td>
<td>&lt;1</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Skeletal muscle (white fibers)</td>
<td>&lt;1</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>Heart</td>
<td>5000</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Brain</td>
<td>5000</td>
<td>96</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>4000</td>
<td>92</td>
<td>6</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. **Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD⁺ oxidoreductase; LD)**

   catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely
distributed in the body, with high concentrations in cells of cardiac and skeletal
muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity
is therefore a non-specific marker of cell damage.

**LD** has a molecular weight of 134 kDa and is composed of four peptide chains of two types:
**M** (or **A**) and **H** (or **B**)

Each under separate genetic control

The subunit compositions of the five isoenzymes are listed below in order of their decreasing
anodal mobility in an alkaline medium.

LD-1  (HHHH; H₄) = migrates fastest towards the anode

LD-2  (HHHM; H₃M)

LD-3  (HHMM; H₂M₂)

LD-4  (HMMM; HM₃)

LD-5  (MMMM; M₄)

**Clinical significance**

**Normal range of total LDH:** 180-360 U/L= 3.1-6.1 µkat/L

**It is increased in plasma in M.I., acute leukemias, generalized carcinomatosis and in acute
hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic
disease and M.I.**

**CAUSES OF RAISED PLASMA TOTAL LD ACTIVITY**

- **Artefactual:**

  *Due to in vitro haemolysis or delayed separation of plasma from whole blood.*

- **Marked increase (more than 5 times the upper reference limit in adults):**

  - Circulatory failure with ‘shock’ and hypoxia:
  - Myocardial infarction
  - Some haematological disorders. In blood diseases such as megaloblastic anaemia,
  acute leukaemias and lymphomas. very high levels (up to 20 times the upper reference
  limit in adults) may be found. Smaller increases occur in other disorders of
erthropoiesis such as thalasaemia. myelofibrosis and haemolytic anaemias
  - Renal infarction, or occasionally during rejection of a renal transplant.
  - Moderate increase. viral hepatitis: malignancy of any tissue: skeletal muscle
disease: pulmonary embolism: infectious mononucleosis.
ISOENZYMES OF LD

LD₁ fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.

LD₅ is the most abundant form in the liver and in skeletal muscle. Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value.

• Predominant elevation of LD₁ and LD₅. (LD₁ greater than LD₅ occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.

• Predominant elevation of LD₂ and LD₃ occurs in acute leukaemia: LD₃ is the main isoenzyme elevated due to malignancy of many tissues.

• Elevation of LD₅ occurs after damage to the liver or skeletal muscle.

Other clinically important enzymes

1. Acid Phosphatase (EC 3.1.3.2; orthophosphoric acid-monoester phosphohydrolase [acid optimum]; ACP)

Acid phosphatase is present in lysosomes, which are organelles present in all cells with the possible exception of erythrocytes. Extralysosomal ACPs are also present in many cells:

   a. prostate,
   b. bone (osteoclasts),
   c. spleen
   d. platelets
   e. erythrocytes.

The lysosomal and prostatic enzymes are strongly inhibited by d-tartrate ions (tartrate-labile ACP), whereas the erythrocyte and bone isoenzymes are not (TR-ACP)

Normal range of TR-ACP: 1.5-4.5 U/L= 0.03-0.08 µkat/L

Elevated TR-ACP

   a. Paget disease
   b. Hyperparathyroidism with skeletal involvement
   c. Presence of malignant invasion of bones by cancers

The only nonbone condition in which elevated activities of TR-ACP are found in serum is Gaucher disease of the spleen, a lysosome storage disease.
The main indications for estimation are to help diagnose prostatic carcinoma and to monitor its treatment. The estimation is gradually being replaced by the measurement of plasma prostate specific antigen (PSA) a protein derived from the prostate. This test is more specific and sensitive for diagnosis and monitoring treatment. However, it may be raised in similar circumstances to those affecting prostatic ACP and is more expensive to estimate. ACP is more useful for monitoring the treatment of a known case of disseminated prostatic carcinoma than for making the diagnosis.

2. **Glucose -6-phosphate Dehydrogenase (EC 1.1.1.49); D-Glucose -6-phosphate: NADP+ oxidoreductase; G6PD)** is expressed in all cells and catalyzes the first step in the hexose monophosphate pathway, the conversion of glucose-6-phosphate to 6-phosphogluconate, generating NADPH. G6PD deficiency is the most common enzymeopathy, affecting 400 million people worldwide. More than 400 different types of G6PD variants have been described, leading to different enzyme activities associated with a wide range of biochemical and clinical phenotypes.

The majority of G6PD–deficient individuals develop hemolysis only when oxidative stress occurs, as with infections and after ingestion of certain drugs or fava beans. Outside these periods, they are usually asymptomatic; however, G6PD deficiency also leads to mild to severe chronic hemolysis, exacerbated by oxidative stress.

The reference interval for G6PD on erythrocytes is 8-14U/g Hb. Values >18 U/g Hb are often encountered in any condition associated with younger than normal RBCs but are of no clinical significance.
Summary

1. Enzyme concentrations are high in cells. Natural decay of these cells releases enzymes into the plasma. Plasma activities are usually low but measurable.

2. Plasma enzyme assays are most useful in the detection of raised levels due to cell damage.

3. Assays of selected enzymes may help to identify the damaged tissues and isoenzyme studies may increase the specificity. In general, knowledge of the patterns of enzyme changes, together with the clinical and other findings, are needed if a useful interpretation is to be made.

4. Non-specific causes of raised enzyme activities include peripheral circulatory insufficiency, trauma, malignancy and surgery.

5. Artefactual increases may occur in haemolysed samples.

6. Enzyme estimations may be of value in the diagnosis and monitoring of:
   a. • Myocardial infarction (CK, LD and its isoenzymes and sometimes AST);
   b. • Liver disease (transaminases, ALP and sometimes GGT):
   c. • Bone disease (ALP):
   d. • Prostatic carcinoma (tartrate-labile ACP):
   e. • Acute pancreatitis (α-amylase):
   f. • Muscle disorders (CK)
Plasma enzyme patterns in diseases

Muscle Disease

In the muscular dystrophies plasma levels of the muscle enzymes, CK and the transaminases, are increased, probably because of leakage from the diseased cells. Results of plasma CK estimation are the more specific.

Although plasma enzyme activities are usually normal in neurogenic muscular atrophy; the number of false positives makes such tests unreliable in differentiating these conditions from primary muscle disease.

Hematological Disorders

Very high activities of LD (HBD) may be found in megalohlastic anaemias and leukemias and in other conditions in which bone marrow activity is abnormal. Typically there is much less change in the plasma AST than in the LD (HBD) activities.

Severe in vivo haemolysis produces changes in both AST and LD (HBD) activities which mimic those of myocardial infarction.

Myocardial Infarction

All plasma enzyme activities (including that of CK-MB) may be normal until at least four hours after the onset of chest pain due to a myocardial infarction; blood should not be taken for enzyme assay until this time has elapsed. The simultaneous measurement of plasma CK-MB activity, which is shown to exceed six per cent of the total CK activity, may occasionally help in the early diagnosis: a raised plasma CK-MB activity or concentration alone is not diagnostic of an infarction.

Most of the CK released after a myocardial infarction is the MM isoenzyme, which is found in both skeletal and myocardial muscle and has a longer half-life than the MB fraction. After about 24 hours the finding of a high MM and
undetectable MB does not exclude myocardial damage as a cause of high total CK activities: by this time the plasma HBD activity is usually raised. In most cases of suspected myocardial infarction measurement of plasma total CK and LD_1 (HBD) activities, together with the clinical and ECG findings, are adequate to make a diagnosis. Plasma total CK activity alone can be very misleading.

A raised plasma total CK activity, due entirely to the MM isoenzyme, may follow recent intramuscular injection, exercise or surgery, this is more likely if associated with normal plasma LD_1 (HBD) or AST activity.

Newer markers for myocardial infarctions: troponin T and troponin I are regulatory proteins involved in myocardial contractility. both being evaluated as an early and specific marker of acute myocardial infarction. Elevated serum troponins are more predictive of adverse outcomes in unstable angina or myocardial infarction than the conventional assay of CK2.

**Enzymes in Malignancy**

*Plasma total enzyme activities may be raised or an abnormal isoenzyme detected, in several neoplastic disorders.*

- Serum prostatic (tartrate-labile) acid phosphatase activity rises in some cases of malignancy of the prostate gland.

- Any malignancy may be associated with a non-specific increase in plasma LD_1 (HBD) and occasionally, transaminase activity.

- Plasma transaminase and alkaline phosphatase estimations may be of value to monitor treatment of malignant disease. Raised levels may indicate secondary deposits in liver or of alkaline phosphatase, in bone. Liver deposits may also cause an increase in plasma LD or GGT.

- Tumors occasionally produce a number of enzymes, such as the 'Regan' ALP isoenzyme. LD (HBD) or CK-BB. assays of which may be used as an aid to diagnosis or for monitoring treatment.
Serum Patterns in different liver diseases


![Graph showing serum enzyme activities in obstructive jaundice](image)

Fig. Course of serum enzyme activities in obstructive jaundice
Fig 2. Course of serum enzyme activities in acute viral hepatitis.

Fig 3. Course of serum enzyme activities in acute alcoholic hepatitis.
Other Clinical correlations

1. **Niemann-Pick disease: Acid Sphingomyelinase Deficiency**
   - **Sphingomyelin**, a ubiquitous component of cell membranes, especially neuronal membranes, is normally degraded within lysosomes by the enzyme **sphingomyelinase**.
   - In patients with **Niemann-Pick disease**, inherited deficiency of this enzyme causes spingomyelin to **accumulate in lysosomes** of the brain, bone marrow, and other organs.
   - Enlargement of the lysosomes interferes with their normal function, leading to cell death and consequent **neuropathy**.
   - Symptoms include **failure to thrive** and **death** in early childhood as well as **learning disorders** in those who survive the postnatal period.

2. **Homocysteinuria: Cystathionine β-synthase Deficiency**
   1. **Cystathionine β-synthase** catalyzes conversion of homocysteine to cystathionine, a critical precursor of cysteine.
   2. Deficiency of this enzyme leads to the most common form of **homocystinuria**, a pediatric disorder characterized by accumulation of homocysteine and reduced activity of several sulfotransferase reactions that require this compound or its derivatives as substrate.
   3. **Accumulation of homocysteine and reduced transsulfation** of various compounds leads to abnormalities in connective tissue structures that cause altered blood vessel wall structure, loss of skeletal bone density (**osteoporosis**), **dislocated optic lens** (**ectopia lentis**), and increased risk of **blood clots**.

3. **Enzyme Replacement Therapy for Inborn Errors of Metabolism**
   - **Lysosomal enzyme deficiencies**, which frequently result in disease due to accumulation of the substrate for the missing enzyme, are suitable targets for **enzyme replacement therapy** (ERT).
   - In ERT, **intravenously administered enzymes** are taken up directly by the affected cells through a receptor-mediated mechanism.
   - ERT provides temporary relief of symptoms but must be given repeatedly and is not a permanent cure.
**Enzymes Assay**

**Enzyme assays** are laboratory methods for measuring enzymatic activity. They are vital for the study of enzyme kinetics and enzyme inhibition.

**Enzyme units**: Amounts of enzymes can either be expressed as molar amounts, as with any other chemical, or measured in terms of activity, in enzyme units.

**Measures of enzyme activity**

1. **Turnover number**, is the number of substrate molecules metabolized per enzyme molecule per unit time with units of min\(^{-1}\) or s\(^{-1}\).
   
   Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on conditions, which should be specified.
   
   The SI unit is the **katal**, 1 katal = 1 mol s\(^{-1}\), but this is an excessively large unit. One katal of trypsin, for example is that amount of trypsin which breaks a mole of peptide bonds per second under specified conditions.
   
   A more practical and commonly-used value is 1 **enzyme unit** (U) = 1 \(\mu\)mol min\(^{-1}\). 1 U corresponds to 16.67 nanokatals..

2. **Specific activity** is usually expressed as \(\mu\)mol of substrate transformed to product per minute per milligram of enzyme under optimal conditions of measurement. Specific activity gives a measurement of the purity of the enzyme, usually constant for a pure enzyme. It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of enzyme. Specific activity is equal to the rate of reaction multiplied by the volume of reaction divided by the mass of enzyme. The SI unit is katal kg\(^{-1}\), but a more practical unit is \(\mu\)mol mg\(^{-1}\) min\(^{-1}\),..

**Related terminology**

1. The **rate of a reaction** is the concentration of substrate disappearing (or product produced) per unit time (mol L\(^{-1}\) s\(^{-1}\)).

2. The **% purity** is 100% \(\times\) (specific activity of enzyme sample / specific activity of pure enzyme). The impure sample has lower specific activity because some of the mass is not actually enzyme. If the specific activity of 100% pure enzyme is known, then an impure sample will have a lower specific activity, allowing purity to be calculated.
Types of Enzyme assay

Enzyme assays can be split into two groups according to their sampling method: continuous assays, where the assay gives a continuous reading of activity, and discontinuous assays, where samples are taken, the reaction stopped and then the concentration of substrates/products determined.

1. Continuous assays

There are many different types of continuous assays.

a. Spectrophotometric: In spectrophotometric assays, you follow the course of the reaction by measuring a change in how much light the assay solution absorbs. If this light is in the visible region you can actually see a change in the color of the assay, these are called colorimetric assays. UV light is often used, since the common coenzymes NADH and NADPH absorb UV light in their reduced forms, but do not in their oxidized forms. An oxidoreductase using NADH as a substrate could therefore be assayed by following the decrease in UV absorbance at a wavelength of 340 nm as it consumes the coenzyme.

Direct versus coupled assays

Coupled assay for hexokinase using glucose-6-phosphate dehydrogenase.
Even when the enzyme reaction does not result in a change in the absorbance of light, it can still be possible to use a spectrophotometric assay for the enzyme by using a **coupled assay**. Here, the product of one reaction is used as the substrate of another, easily-detectable reaction. For example, figure 1 shows the coupled assay for the enzyme **hexokinase**, which can be assayed by coupling its production of glucose-6-phosphate to NADPH production, using **glucose-6-phosphate dehydrogenase**.

**b. Fluorometric**: **Fluorescence** is when a molecule emits light of one wavelength after absorbing light of a different wavelength. Fluorometric assays use a difference in the **fluorescence** of substrate from product to measure the enzyme reaction. These assays are in general much more sensitive than spectrophotometric assays, but can suffer from interference caused by impurities and the instability of many fluorescent compounds when exposed to light. An example of these assays is again the use of the nucleotide coenzymes **NADH** and **NADPH**. Here, the reduced forms are fluorescent and the oxidised forms non-fluorescent. Oxidation reactions can therefore be followed by a decrease in fluorescence and reduction reactions by an increase.

**c. Calorimetric**: is the measurement of the heat released or absorbed by chemical reactions. These assays are very general, since many reactions involve some change in heat and with use of a microcalorimeter, not much enzyme or substrate is required. These assays can be used to measure reactions that are impossible to assay in any other way.

**d. Chemiluminescent**: is the emission of light by a chemical reaction. Some enzyme reactions produce light and this can be measured to detect product formation. These types of assay can be extremely sensitive, since the light produced can be captured by photographic film over days or weeks, but can be hard to quantify, because not all the light released by a reaction will be detected.

**e. Light Scattering**
2. Discontinuous assays

Discontinuous assays are when samples are taken from an enzyme reaction at intervals and the amount of product production or substrate consumption is measured in these samples.

i. Radiometric:
Radiometric assays measure the incorporation of radioactivity into substrates or its release from substrates. The radioactive isotopes most frequently used in these assays are $^{14}$C, $^{32}$P, $^{35}$S and $^{125}$I. Since radioactive isotopes can allow the specific labelling of a single atom of a substrate, these assays are both extremely sensitive and specific. They are frequently used in biochemistry and are often the only way of measuring a specific reaction in crude extracts (the complex mixtures of enzymes produced when you lyse cells).

Radioactivity is usually measured in these procedures using a scintillation counter, which measures the ionizing radiation.

ii. Chromatographic
Chromatographic assays measure product formation by separating the reaction mixture into its components by chromatography. This is usually done by high-performance liquid chromatography (HPLC), but can also use the simpler technique of thin layer chromatography. Although this approach can need a lot of material, its sensitivity can be increased by labelling the substrates/products with a radioactive or fluorescent tag.
Factors to control in assays

- **Salt Concentration**: Most enzymes cannot tolerate extremely high salt concentrations. The ions interfere with the weak ionic bonds of proteins. Typical enzymes are active in salt concentrations of 1-500 mM. As usual there are exceptions such as the halophilic (salt loving) algae and bacteria.

- **Effects of Temperature**: All enzymes work within a range of temperature specific to the organism. Increases in temperature generally lead to increases in reaction rates. There is a limit to the increase because higher temperatures lead to a sharp decrease in reaction rates. This is due to the denaturating (alteration) of protein structure resulting from the breakdown of the weak ionic and hydrogen bonding that stabilize the three dimensional structure of the enzyme active site. The "optimum" temperature for human enzymes is usually between 35 and 40 °C. The average temperature for humans is 37 °C. Human enzymes start to denature quickly at temperatures above 40 °C. Enzymes from thermophilic archaea found in the hot springs are stable up to 100 °C.

- **Effects of pH**: Most enzymes are sensitive to pH and have specific ranges of activity. All have an optimum pH. The pH can stop enzyme activity by denaturating (altering) the three dimensional shape of the enzyme by breaking ionic, and hydrogen bonds. Most enzymes function between a pH of 6 and 8; however pepsin in the stomach works best at a pH of 2 and trypsin at a pH of 8.

- **Substrate Saturation**: Increasing the substrate concentration increases the rate of reaction (enzyme activity). However, enzyme saturation limits reaction rates. An enzyme is saturated when the active sites of all the molecules are occupied most of the time. At the saturation point, the reaction will not speed up, no matter how much additional substrate is added. The graph of the reaction rate will plateau.

- **Level of crowding**, large amounts of macromolecules in a solution will alter the rates and equilibrium constants of enzyme reactions, through an effect called macromolecular crowding.