Clinical Enzymology
Objectives

• List the clinically important enzymes and isoenzymes.

• State which of the enzymes and isoenzymes are found in which tissues

• Describe plasma enzyme changes in myocardial infarction and liver disease

• Outline different ways of measuring plasma enzymes
Enzymes

- Biological catalysis
- Very efficient – can increase reaction rates at the order of x 10
- All are proteins - so liable to denaturation
- Specific to substrates
- Partly specific to tissues
- Assay by measure of rate of specific reaction catalyzed by that enzyme
Measurement of serum enzymes

- Diagnostic enzymology
- Enzymes are normally intracellular and LOW concentration in blood
- Enzyme release (leakage) in the blood indicates cell damage (cell death, hypoxia, intracellular toxicity)
- Quantitative measure of cell/tissue damage
- Fairly non invasive possible to do repeated tests
- **Organ specificity** - but not absolute specificity in spite of same gene content.
- Most enzymes are present in most cells-differing amounts
Information from enzymes measurements in serum

- Presence of disease
- Organs involved
- Etiology / nature of disease: differential diagnosis
- Extent of disease - more damaged cells - more leaked enzymes in blood
- Time course of disease
# Enzymes routinely measured

<table>
<thead>
<tr>
<th>NAME OF THE ENZYME</th>
<th>PRESENT IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate Amino transferase (AST)</td>
<td>Heart and Liver</td>
</tr>
<tr>
<td>Serum glutamate-oxaloacetate transaminase (SGOT)</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Bone, intestine and other tissues</td>
</tr>
<tr>
<td>Acid Phosphatase (ACP)</td>
<td>Prostate</td>
</tr>
<tr>
<td>glutamyl Transferase (GT)</td>
<td>Liver</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td>Muscle Including cardiac muscle</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>Heart, liver, muscle, RBC</td>
</tr>
<tr>
<td>Amylase</td>
<td>Pancreas</td>
</tr>
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</table>
Isoenzymes

- Catalyse same reactions but are formed from structurally different polypeptides.
- They perform the same catalytic function.
- Different isoenzymes may arise from different tissues and their specific detection may give clues to the site of pathology.
- Various isoenzymes of an enzyme can differ in three major ways:
  - Enzymatic properties
  - Physical properties (e.g. heat stability)
  - Biochemical properties such as amino acid composition and immunological reactivities.
Measurement of enzyme activity

- Enzyme activity is expressed in International unit (IU)
  - It corresponds to the amount of enzymes that catalyzes the conversion of one micromole (µmol) of substrate to product per minute
LACTATE DEHYDROGENASE (LDH)

- Pyruvate $\rightarrow$ Lactate $\rightarrow$ (anaerobic glycolysis)

- LDH is elevated in myocardial infarction, blood disorders

- It is a tetrametric protein and made of two types of subunits namely H = Heart, M = skeletal muscle

- It exists as 5 different isoenzymes with various combinations of H and M subunits
<table>
<thead>
<tr>
<th>Isoenzyme name</th>
<th>Composition</th>
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<th>Present in</th>
<th>Elevated in</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH1</td>
<td>(H₄)</td>
<td>HHHH</td>
<td>Myocardium, RBC</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>LDH2</td>
<td>(H₃M₁)</td>
<td>HHHM</td>
<td>Myocardium, RBC</td>
<td></td>
</tr>
<tr>
<td>LDH3</td>
<td>(H₂M₂)</td>
<td>HHMM</td>
<td>Kidney, Skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>LDH4</td>
<td>(H₁M₃)</td>
<td>HMMM</td>
<td>Kidney, Skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>LDH5</td>
<td>(M₄)</td>
<td>MMMM</td>
<td>Skeletal muscle, Liver</td>
<td>Skeletal muscle and liver diseases</td>
</tr>
</tbody>
</table>
CREATINE KINASE (CK)

- Creatine + ATP $\rightarrow$ phosphocreatine + ADP
- (Phosphocreatine – serves as energy reserve during muscle contraction)
- Creatine kinase is a dimer made of 2 monomers
- occurs in the tissues
- **Skeletal muscle** contains M subunit, **Brain** contains B subunits
- Three different isoenzymes are formed
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<th>Composition</th>
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<th>Elevated in</th>
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<tr>
<td>CK-1</td>
<td>BB</td>
<td>Brain</td>
<td>CNS diseases</td>
</tr>
<tr>
<td>CK-2</td>
<td>MB</td>
<td>Myocardium/Heart</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>CK-3</td>
<td>MM</td>
<td>Skeletal muscle, Myocardium</td>
<td></td>
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ALANINE TRANSMINASE (ALT) AND ASPARTATE TRANSMINASE (AST)

- Oxoglutarate + L-aspartate
- Oxoglutarate + L-alanine

L-glutamate + oxaloacetate

Aspartate aminotransferase (AST)

L-glutamate + pyruvate

Alanine aminotransferase (ALT)
Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes are the most abundantly present in the liver and is elevated in blood as a result of leakage from damaged cells. Measurement of these transaminases is useful for the diagnosis of liver diseases. In viral hepatitis the enzyme levels are increased 20-50 times above the upper limit of the normal range. Alanine transaminase (ALT) increase is specific for liver damage involving hepatocellular damage. Aspartate transaminase (AST) is moderately increased in Muscular dystrophy and acute myocardial infarction.
In viral hepatitis, rapid rise in transaminases (AST & ALT) in serum occurs even before bilirubin rise is seen.
LEVELS OF ENZYMES IN MYOCARDIAL INFARCTION

AST and CK rise in 6 hours following acute myocardial infarction.

HBDH and LDH are elevated much later and remains high for a longer period of days.
ALKALINE PHOSPHATASE (ALP)

- Is a group of enzymes that have maximal activity at a high pH 9.0-10.5
- Widely distributed throughout the body
- High levels are seen in liver, bone, placenta and intestine and useful to assess hepatobiliary and bone diseases
- In hepatobiliary obstruction, hepatocytes lining the biliary ducts induces the ALP synthesis.
- High levels of ALP is indicative of extrahepatic obstruction rather than intrahepatic obstruction.
- In bones, the enzyme is derived from osteoblasts. Hence increased in bone diseases like rickets, osteomalacia, neoplastic diseases with bone metastases and healing fractures.
ALKALINE PHOSPHATASE (ALP) conti

\[ \text{p-NPP} + \text{H}_2\text{O} \stackrel{\text{ALP, Mg}^{2+}}{\rightleftharpoons} \text{p-NP (benzenoid form)} + \text{PO}_4^{3-} \]

\[ \text{p-NP (quinonoid form)} + \text{PO}_4^{3-} \]

\[ \text{Colorless} \quad \xrightarrow{\text{Rearrangement}} \quad \text{Yellow} \]

Color read at 405nm
The activity of the bone isoenzyme can be estimated by heat treating a serum sample at 56°C. The bone ALP is heat labile and is destroyed or heat inactivated at this temperature.

Measurement of ALP before and after heat treatment gives a measure of bone ALP.
ACID PHOSPHATASEASE (ACP)

- Is a group of enzymes that have maximal activity at pH 5.0-6.0.
- It is present in prostate gland, liver, spleen and RBC.
- The main source of ACP is prostate gland and so can be used as a marker for prostate disease.
AMYLASE

- Is the digestive enzymes from the pancreas and salivary glands to digest complex carbohydrates.
- Elevated in acute pancreatitis.
- It is used as a marker to detect acute pancreatitis AND appendicitis.
glutamyltransferase (• GT)

- Amino acid + Glutathione → -glutamyl Cysteinylglycine
- It is involved in aminoacid transport across the membranes.
- Found mainly in biliary ducts of the liver, kidney and pancreas.
- Enzyme activity is induced by a number of drugs and in particular alcohol.
- GT increased in liver diseases especially in obstructive jaundice.
  - GT levels are used as a marker of alcohol induced liver disease and in liver cirrhosis.
MEASUREMENT OF ENZYMES

- Enzymes are measured
- End point assay
- Kinetic assay

Measurement of enzymes are affected by the presence of inhibitors or activators. Hence most of the enzymes are measured by coupled assay.
A **coupled assay** is one in which a second enzyme is used to act on the product of the enzyme of primary interest. Second enzyme used NADH as coenzyme. The rate can be followed by measuring oxidation of NADH which can be done conveniently at 340nm.
Principle involved in AST estimation

- Oxoglutarate + L-aspartate

\[
\begin{align*}
&\text{Aspartate aminotransferase (AST)} \\
&\text{L-glutamate + oxaloacetate} \\
&+ \\
&\text{NADH + H}^+ \\
&\text{Malate dehydrogenase (MDH)} \\
&\text{L-malate + NAD}^+
\end{align*}
\]
Figure 6.5 Ultraviolet absorption spectra of NADH and NAD+
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<th>NAME OF THE ENZYME</th>
<th>Conditions in which level of activity in serum is elevated</th>
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<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Liver disease- biliary obstruction Osteoblastic bone disease-rickets</td>
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<tr>
<td>Acid Phosphatase (ACP)</td>
<td>Prostatic carcinoma</td>
</tr>
<tr>
<td>glutamyl Transferase (\ GT)</td>
<td>Liver disorder like liver cirrhosis</td>
</tr>
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<td>Creatine kinase (CK)</td>
<td>Myocardial infarction and skeletal muscle disease(muscular dystrophy)</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>Myocardial infarction, other diseases like liver disease.some blood diseases</td>
</tr>
<tr>
<td>Amylase</td>
<td>Acute pancreatitis</td>
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SUMMARY

- Enzymes are biological catalysts present in every cell of the body.
- An enzyme will act on a specific substrate yielding a product.
- An isoenzyme is a genetic variant produced largely within a specific tissue.
- Isoenzyme patterns can give information about organ-specific disease.
- Important enzymes in the investigation of heart disease are CK, LDH and AST.
- Important enzymes in the investigation of liver disease are AST, ALT, alkaline phosphatase and GGT.
- Creatine kinase has three isoenzymes: CK-MM, CK-MB and CK-BB.
- LDH has five isoenzymes.
- Alkaline phosphatase can be used in the investigation of liver and bone disease.
- Increased levels of acid phosphatase are found in prostate cancer.
- GGT is induced by alcohol and is useful in monitoring alcohol abuse.
- Enzyme measurements should be performed using zero order kinetics, i.e. using excess substrate.
- Determinations of enzyme activity can be performed using an end-point or kinetic method.
Thank you