Clinical Enzymology
Enzymes

- Biological catalysis
- Very efficient – can increase reaction rates at the order of $\times 10$
- Most of the enzymes are proteins - so liable to denaturation
- Specific to substrates
- Partly specific to tissues
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
- **Mostly Organ specific** - Not All
- Most enzymes are present in most cells----differing amounts
Information from enzymes measurements in serum

- Presence of disease
- Organs involved
- Aetiology /nature of disease
- Extent of disease - more damaged cells - more leaked enzymes in blood
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
## 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>
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</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>γ glutamyl Transferase (γ 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>α Amylase</td>
<td>Pancreas</td>
</tr>
</tbody>
</table>
LACTATE DEHYDROGENASE (LDH)

Pyruvate $\leftrightarrow$ Lactate (anaerobic glycolysis)

- LDH is elevated in myocardial infarction, blood disorders
- It is a tetrameric 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>
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</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>myocardial infarction</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 \(\rightleftharpoons\) phosphocreatine + ADP

(Phosphocreatine – serves as energy reserve during muscle contraction)

- Creatine kinase is a dimer made of 2 monomers
- **Skeletal muscle** contains **M subunit**, **Brain** contains **B subunits**
- CK has three different isoenzymes.
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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>
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**ALANINE TRANSAMINASE (ALT) AND ASPARTATE TRANSAMINASE (AST)**

\[ \alpha\text{- Oxoglutarate} + \text{L-aspartate} \quad \text{Aspartate aminotransferase (AST)} \quad \text{L-glutamate} + \text{oxaloacetate} \]

\[ \alpha\text{- Oxoglutarate} + \text{L-alanine} \quad \text{Alanine aminotransferase (ALT)} \quad \text{L-glutamate} + \text{pyruvate} \]

- **the most abundantly present in the liver**
- 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
ALKALINE PHOSPHATASE (ALP)

- 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
ACID PHOSPHATASE (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.
γ glutamyltransferase (γ GT)

- 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.
- γ-GT increased in liver diseases especially in obstructive jaundice.
- γ-GT levels are used as a marker of alcohol induced liver disease and in liver cirrhosis.
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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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<td>Myocardial infarction, Liver disease especially with liver cell damage</td>
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<tr>
<td>Alkaline Phosphatase (ALP)</td>
<td>Liver disease- biliary obstruction, Osteoblastic bone disease-rickets</td>
</tr>
<tr>
<td>Acid Phosphatase (ACP)</td>
<td>Prostatic carcinoma</td>
</tr>
<tr>
<td>(\gamma) glutamyl Transferase ((\gamma) GT)</td>
<td>Liver disorder like liver cirrhosis</td>
</tr>
<tr>
<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>(\alpha) Amylase</td>
<td>Acute pancreatitis</td>
</tr>
</tbody>
</table>
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.
ENZYMES IN THERAPY
• Substitution of missing production of digestive enzymes – digestive enzymes – pepsin trypsin…

• Removal of deposits of death tissue or fibrin (e.g. in lungs, eyes),

• treatment of skin defects – proteinases, nucleases, collagenase

• Acceleration of fibrinolysis in lungs embolization (activation of plasmin and plasminogen) – streptokinase, urokinase