Enzymes in Clinical Biochemistry

You are supposed to know …

- Enzymes – main features, properties
- Coenzymes – structures, functions
- Enzyme kinetics
- Enzyme activity

Isoenzymes – General Features

- Genetically determined differences in primary structure
- Catalyze the same reaction
- May have **different subcellular distribution**
  (cytoplasm mitochondria)
- May have **different tissue distribution**
- May be combined from more subunits (quarternary structure)
- May differ in kinetic properties (K_M)
- Usually are determined by electrophoresis

Q. 2 (p. 27)

Explain the terms: proenzyme, isoenzyme, isoform

A. 2

- **Proenzyme** (zymogen) – inactive form of enzyme that becomes active after partial proteolysis

  example: pepsinogen → pepsin

- **Isoenzyme** – see previous page

- **Isoform** – more general term, includes true isoenzymes and pseudoisoenzymes (posttranslational variations)

Lactate dehydrogenase (LD)

- **Tetramer**
- Two different chains (H - heart, M - muscle)
- Five isoenzymes:

  LD_1 (H_4), **LD_2** (H_3M), **LD_3** (H_2M_2), **LD_4** (HM_3), **LD_5** (M_4)
- Widely distributed in body
- Total activity determination – nonspecific finding
- $LD_1 + LD_2$ ….. marker of myocardial infarction (MI)
- Today is LD assay considered out-of-date

Creatine kinase (CK)

- **Dimer**
- Two different chains (M – muscle, B – brain)
- Three isoenzymes: **MM** (muscle), **MB** (heart), **BB** (brain)
- Major isoenzyme in blood is MM (95 %)
- MB form in blood: 0 – 6 %
- BB in blood: traces (BB cannot pass across blood-brain barrier)
- MB isoenzyme …. excellent marker of myocardial infarction

Enzymes in Blood

Feature	Plasmatic enzymes	Secretory enzymes	Intracellular enzymes
Example	coag. factors	amylase, lipase	AST, …
Source organ	liver	pancreas	various
Function in	blood	GIT	cells
Enzyme activity in blood after source organ damage	?	?	?

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Q. 6

Why are low activities of cellular enzymes detected even in serum of healthy people?

A. 6

Low activities of **intracelular** enzymes

in **extracelular** fluid (blood plasma, serum)

are the consequence

of physiological cell disintegration.

Main Tissue Distribution of Enzymes

AST	liver, myocard
ALT	**liver**
LD	not specific
CK	**myocard, muscles**
GMT	**liver**
ALP	biliary tract, bones
ACP	**prostate**
AMS	**pancreas**
LPS	**pancreas**
CHS	**liver**

Intracellular Location of Enzymes

Intracellular Location	Enzymes
Cytoplasm	LD, **ALT**, **30 % AST**
Mitochondria	**70 % AST**
Golgi complex, ER	CHS, AMS
Lysosome	ACP
Membrane	**GMT, ALP**

Consider the AST/ALT ratio

- AST/ALT > 1 ……. severe liver damage

- AST/ALT < 1 ……. mild liver damage

Enzymes of Clinical Significance

Enzyme	Source of blood elevation
ALT	hepatopathy
AST	MI, hepatopathy
GMT	hepatopathy (alcohol, drugs)
ALP	biliary tract diseases, bone diseases
ACP	prostatic cancer
CK	MI (CK-MB), muscle diseases
AMS	pancreatitis
LPS	pancreatitis
CHS	hepatopathy (alcohol, drugs) – decreased

Catalytic concentration of some enzymes

Enzyme	Reference values (serum)
ALT	0.1 - 0.9 μkat/l
AST	0.1 - 0.7 μkat/l
LD	up to 7.5 μkat/l
CK	up to 4 μkat/l

see also the lab manual

Q. 7

What enzymes might appear in blood

a) In mild hepatocellular damage

b) In serious hepatocellular damage

A. 7

a) Mild hepatocellular damage:

enzymes from cytoplasm and/or membrane are released into ECF – ALT, GMT, ALP

b) Severe hepatocellular damage:

enzymes from mitochondria are released into ECF – AST

Q. 8

Write equations of reactions catalyzed by:

ALT

AST

LD

ALT Reaction

alanine + 2-oxoglutarate ⇆

ALT Reaction

alanine + 2-oxoglutarate ⇆ pyruvate + glutamate

AST reaction

aspartate + 2-oxoglutarate ⇆

AST reaction

aspartate + 2-oxoglutarate ⇆ oxaloacetate + glutamate

LD reaction

lactate + NAD^+ ⇆

LD reaction

$$\text{lactate} + NAD^+ \leftrightarrows \text{pyruvate} + NADH + H^+$$

Q. 9

The levels of most blood enzymes are increased in newborns and infants. What enzyme persists elevated till puberty?

A. 9

ALP – the bone isoenzyme activity persists till puberty

Biochemical Diagnostic of MI

Enzyme / Protein	Half-time (hrs)
Myoglobin	0,25
Troponine T cardial form	2
CK-MB	13
AST	17
LD_{12}	110