Blood Biochemistry BCH 471[Practical]

Lab (2) Determination of Non-functional Plasma Enzymes in Serum

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Objectives

- To determine the level of **alanine transaminase (ALT)** in serum.
- To evaluate the presence of tissue damage.

Blood Enzymes

- Plasma, serum or **blood proteins**, are proteins present in blood plasma which have several functions.
- Some blood proteins also act as **enzymes.**
- **Enzymes** are biocatalysts that increase the rate of the chemical reaction.
- **Clinical enzymology** refers to measurement of enzyme activity in body fluids for the diagnosis and treatment of diseases.
- Most clinical enzyme measurements using **serum** or **plasma**, occasionally other fluids, such as urine and gut secretions are also investigated.
- The most commonly used body fluid for this purpose is **SERUM**. (Why?)

Differences Between Plasma Enzymes

Plasma Enzymes

1. **Plasma-specific Enzymes (Functional)**

Enzymes that are normally present in the plasma and perform their primary function in the blood.

2. **Non-plasma specific Enzymes (Non functional)**

Intracellular enzymes that are normally present in very small amount in blood and perform no known function in blood.

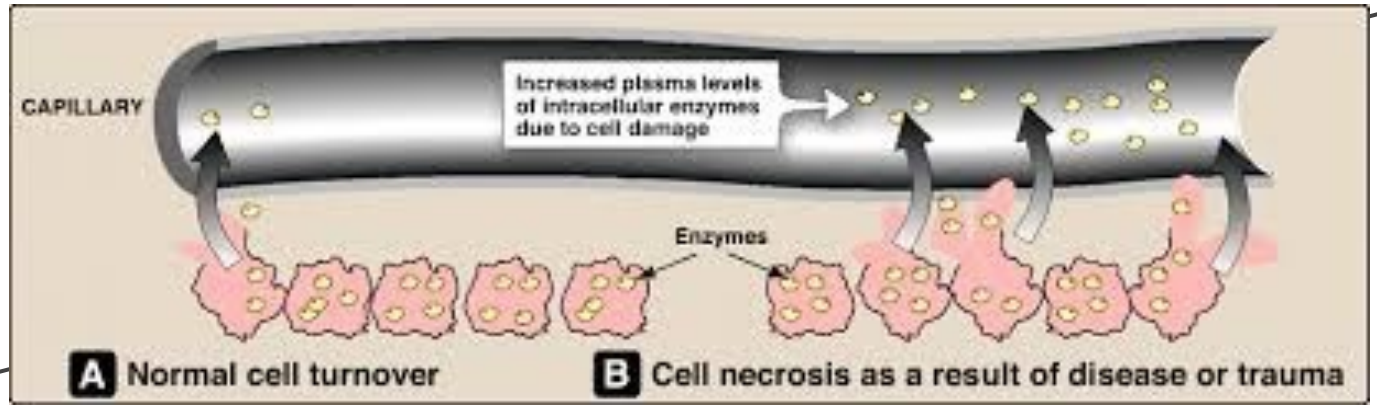
	Functional plasma enzymes	Non functional plasma enzymes
Their substrate	Always present in the blood	Absent from the blood
Site of synthesis	Liver	Different organs e.g. liver, heart, muscles, and brain
Effect of diseases in its plasma levels	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Thrombin Plasmin Ceruplasmin	ALT LDH Acid Phosphatase Amylase

Pause and Think **Which of these enzymes is a better diagnostic indicator ? Why?**

Sources of Non functional Plasma Enzyme

1. **Cell damage** with the release of its content of enzymes into blood e.g. **Myocardial infarction** and **viral hepatitis**.
2. **Block in the secretory pathway** e.g. elevation of blood pancreatic amylase and lipase in **pancreatitis**.
3. **Increase enzyme synthesis** e.g. elevation of serum alkaline phosphatase in **bone cancer**.

So estimation of the plasma concentration of these enzymes in blood is useful for the diagnosis of disease depending on their tissue origin.



Clinical Significance of Non-Functional Plasma Enzymes

Measurement of non-functional enzymes is important for:

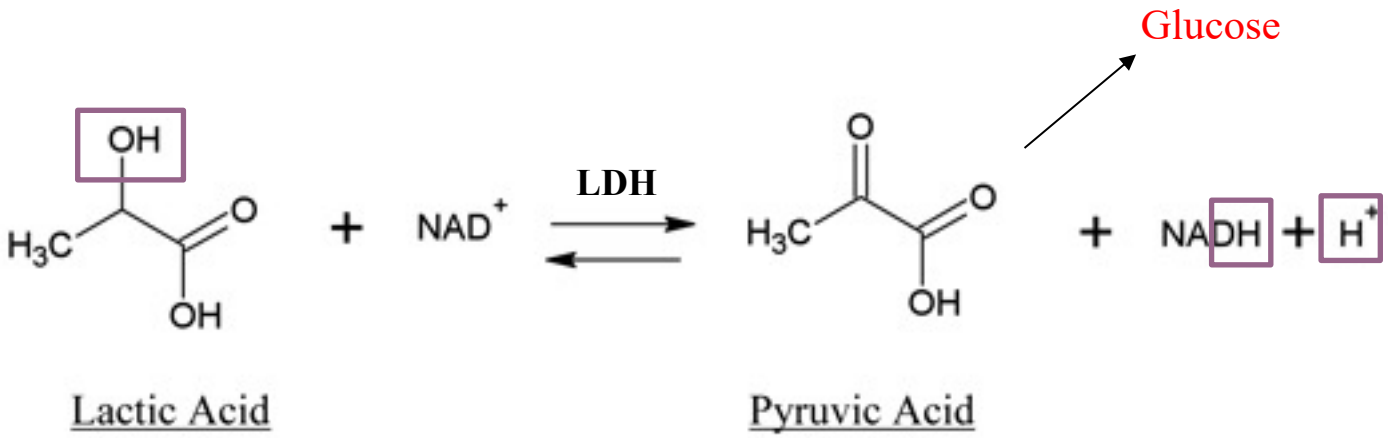
1. **Diagnosis of diseases.**
2. **Prognosis of the disease:** following up of the treatment by measuring plasma enzymes before and after treatment.

Lactate Dehydrogenase (LDH)

- **Lactic acid dehydrogenase (LDH)** is an enzyme that helps produce energy.
- LDH is most often measured to evaluate the presence of tissue damage (diagnostic).
- The enzyme LDH is in many body tissues, especially the **heart, liver, kidney, skeletal muscle, brain, blood cells and lungs.**

LDH Reaction

- LDH is a hydrogen transfer enzyme which catalyzes the **interconversion of pyruvate and lactate** with the mediation of **NAD^+** as hydrogen acceptor, eventually converting pyruvate to glucose.
- The optimum pH for lactate pyruvate (L→ P) reaction is **8.8 – 9.8,** While for pyruvate to lactate (P→ L) is **7.7 – 7.8.**
- The enzyme is inhibited by **sulfhydryl reagents** and **mercuric ions.**

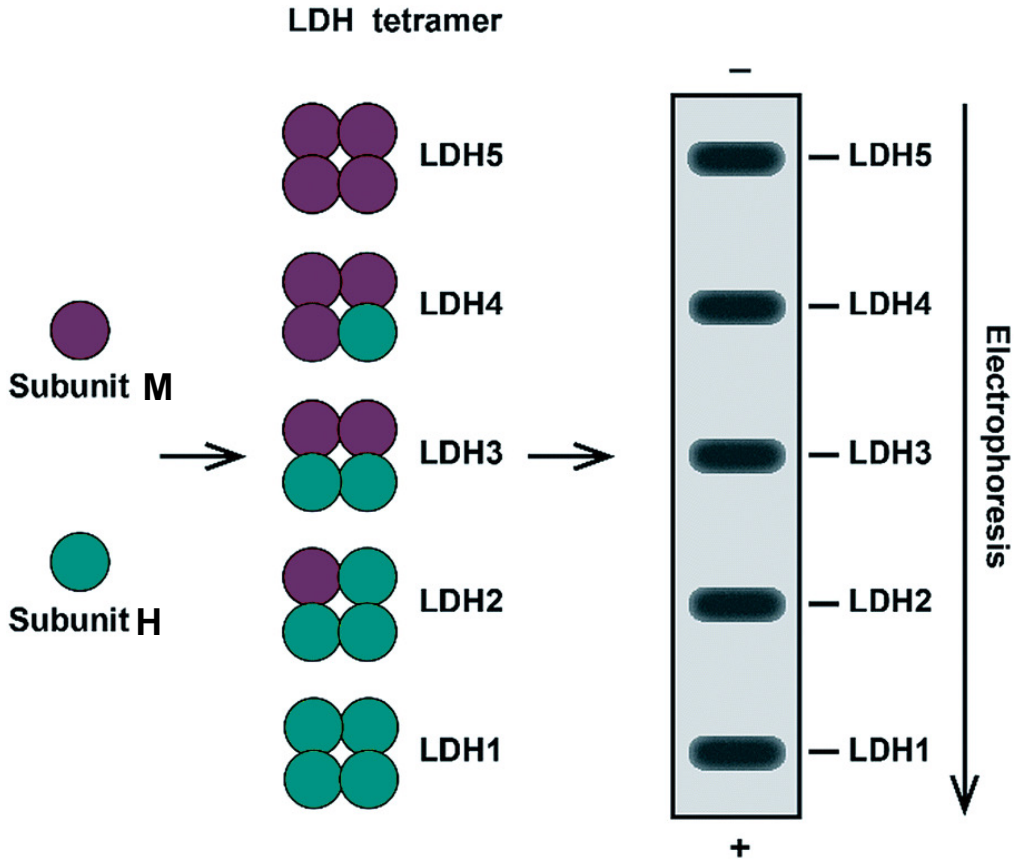


Lactic Acid Pyruvic Acid

LDH Isoenzymes

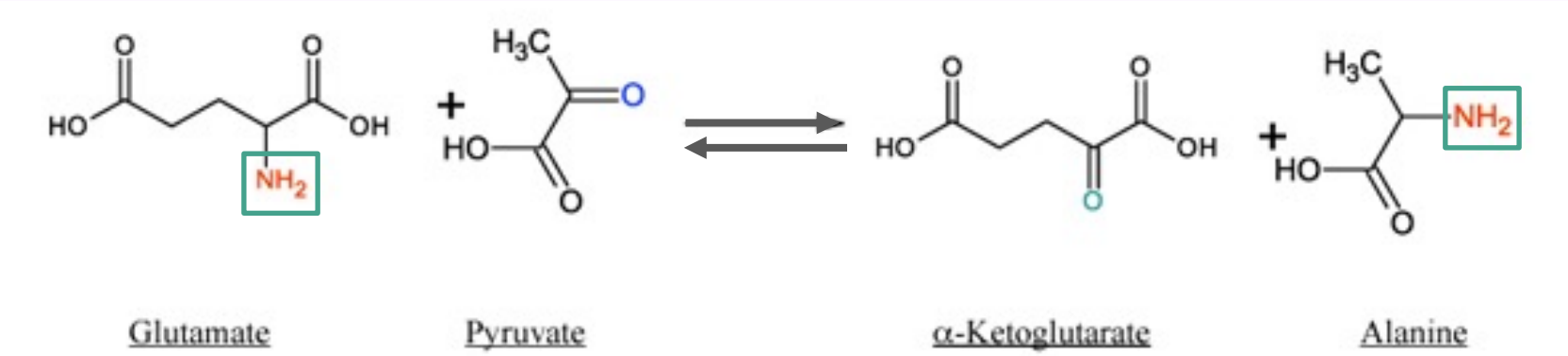
- LDH exists in **5 forms** (isoenzymes), which differ slightly in structure.
- All of these isoenzymes can be measured in the blood, and can be separated by electrophoresis.

Isoenzyme	Tissues	Diseases associated
LDH-1	Found primarily in **heart muscle** and RBC	Myocardial infarction
LDH-2	Highest in **WBC**, heart and **RBC**	➢ Megaloblastic anemia ➢ leukemia
LDH-3	Found in **lung** tissue	Pulmonary embolism
LDH-4	Highest in the kidney, placenta and **pancreas**	Pancreatitis
LDH-5	Highest in the **liver** and **skeletal muscle**	➢ Toxic hepatitis with jaundice ➢ Muscular dystrophy



Alanine Transaminase

- ALT is an enzyme that catalyzes a type of reaction (**transamination**) between an amino acid and α-keto acid.
- It is important in the production of various amino acids.
- Also called alanine transferase (**ALT**), serum glutamate-pyruvate transaminase (**SGPT**).



Glutamate Pyruvate α-Ketoglutarate Alanine

- **Transamination reaction** is the process by which amino groups are removed from amino acids and transferred to acceptor keto-acids to generate the amino acid version of the keto-acid and the keto-acid version of the original amino acid.

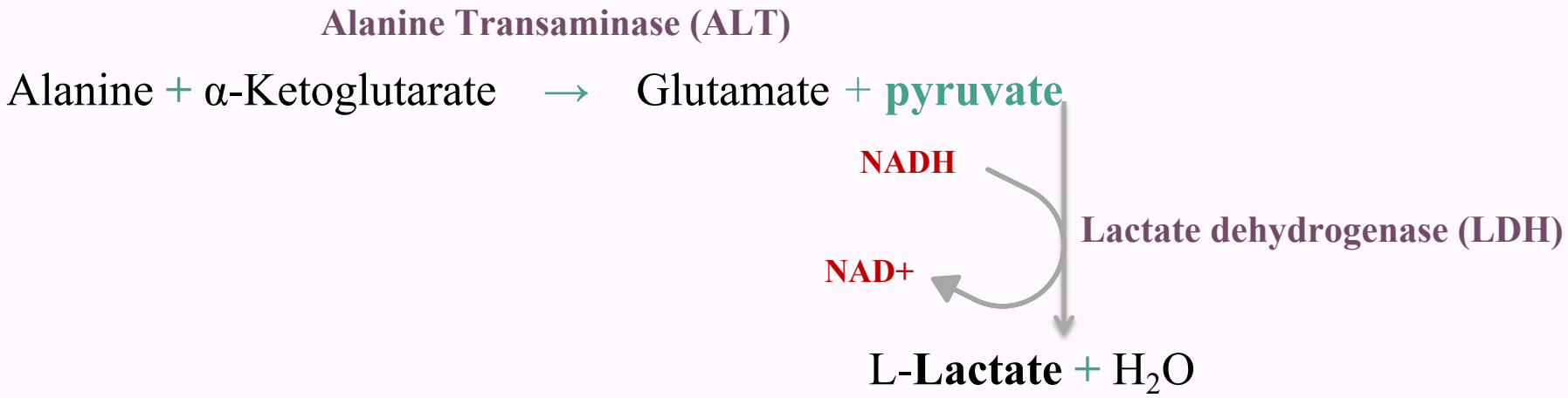
ALT Diagnostic Importance

- ALT is found in serum (**at low level**) but is most commonly associated with the liver.
- Thus, an elevated level ALT is a sensitive index of acute hepatocellular injury.
- Elevated serum ALT (SGPT) level are found in **hepatitis, cirrhosis** and **obstructive jaundice.**
- Levels of ALT are only slightly elevated in patient following a **myocardial infarction.**

Practical Part 💉

Alanine Transaminase Assay

Principle



- The rate of NAD^+ formation is indicated by **decreased the absorbance at 340 nm** and it is indirectly proportional to serum LDH activity.

If:

- NADH is **product** → **increase** the absorbance/min
- NADH is **reactant** → **decrease** the absorbance/min

Method

	Tube
ALT reagent	1 ml
Pre-warm at 37 °C for 3 minutes and add	
Sample (serum)	100 µl
Mix and incubate at 37 °C for 1 minutes, then read the absorbance at 340 nm against distilled water (blank) every minute for 2 minutes and determine ΔA/min.	

Measure enzyme kinetics using UV-visible spectroscopy:

2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (120 sec = 2 min) → Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times)

Results and Calculations

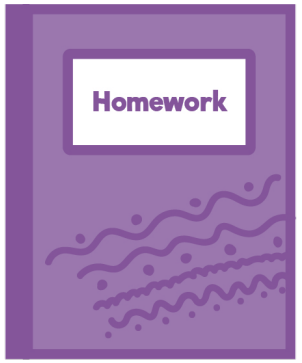
Results

	Time (min)	Absorbance at 340 nm
A_1	1	
A_2	2	
A_3	3	

Calculations

- $\Delta A_1 = A_1 - A_2$ $\Delta A_2 = A_2 - A_3$
- $\Delta A/min = (\Delta A_1 + \Delta A_2) / 2$
- ALT(U/L) = **ΔA x 1746**

Normal Values Males: **up to 42(U/L)** Female: **up to 32(U/L)**



Homework:

a. Name five plasma enzymes that can be used for diagnosis and name the disease associated with.