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BCH 471

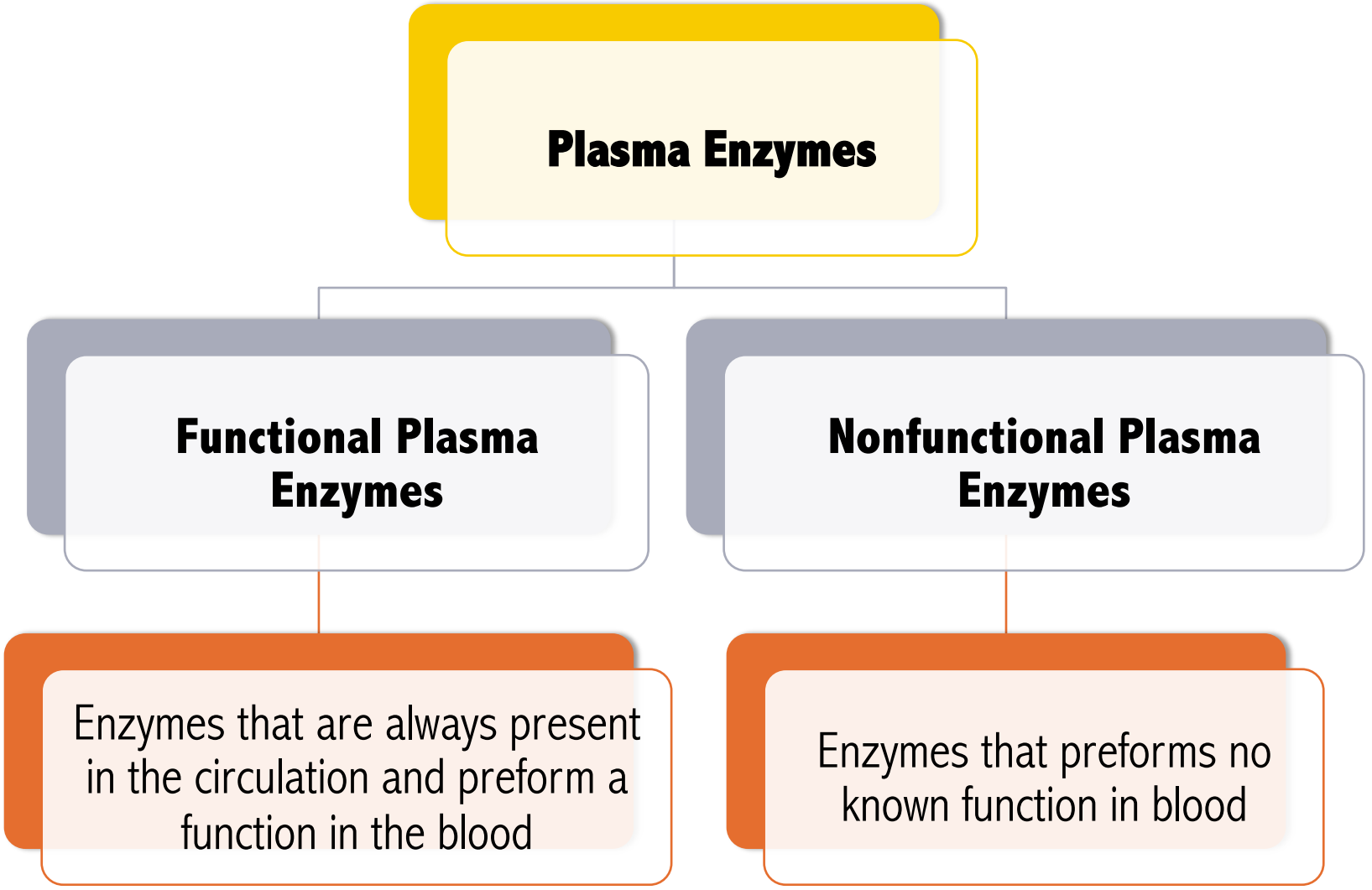
Determination of plasma enzymes

Determination of LDH in serum

Objectives

- To determine the level of LDH in serum.
- To evaluate the presence of tissue damage.

Most clinical enzyme measurements using serum or plasma, occasionally other fluids, such as urine and gut secretions, are investigated.

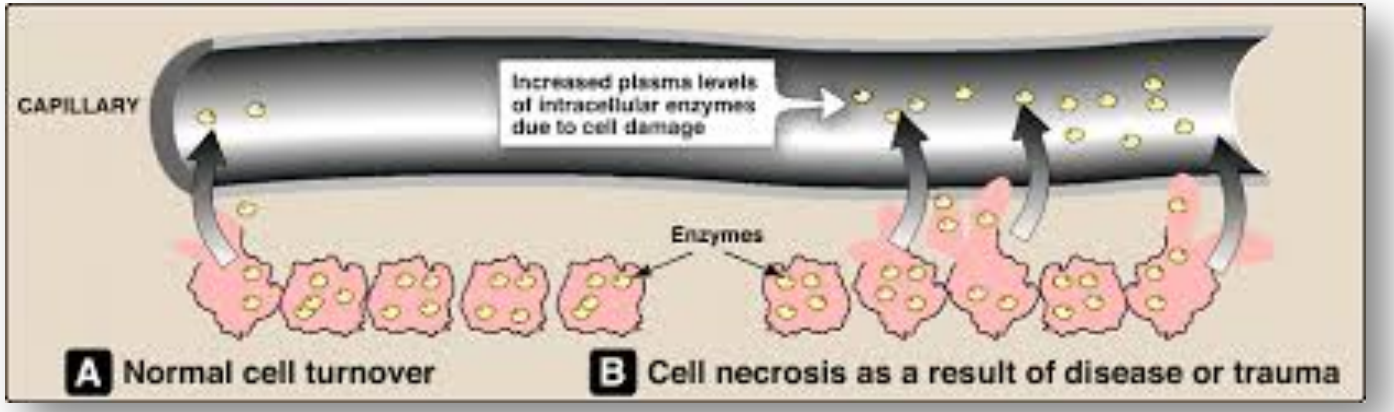


Differences of Functional and Nonfunctional plasma enzymes

	Functional plasma enzymes	Nonfunctional 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	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Clotting factors Lipoprotein Lipase	ALT LDH Acid Phosphatase Amylase

Sources of Nonfunctional Plasma Enzyme

- Cell damage with the release of its content of enzymes into blood e.g. Myocardial infarction and viral hepatitis
- Obstruction of normal pathways e.g. Obstruction of bile duct increases alkaline phosphatase
- Increase of the enzyme synthesis e.g. bilirubin increases the rate of synthesis of alkaline phosphatase in obstructive liver disease
- Increased permeability of cell membrane as in hypoxia



Medical Importance of Non Functional Plasma Enzymes

Measurement of non functional enzymes is important for:

- Diagnosis of diseases

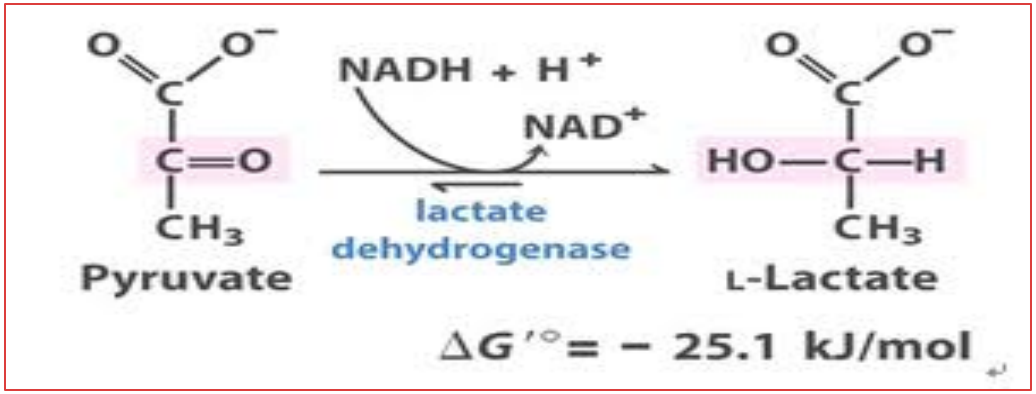
- Prognosis of the disease: following up of the treatment by measuring plasma enzymes before and after treatment.

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.



LDH Isoenzyms

- 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.

LDH isoenzyme	Tissues
LDH-1	is found primarily in heart muscle and red blood cells.
LDH-2	is concentrated in while blood cells.
LDH-3	is highest in the lung
LDH-4	is highest in the kidney, placenta, and pancreas
LDH-5	is highest in the liver and in skeletal muscle

↑LDH in plasma

Diseases	Examples
Myocardial infarction	
Liver Disease	Toxic jaundice
	Viral hepatitis
	Obstructive jaundice
Anemia	Pernicious anemia
	Megaloblastic anemia
Renal Diseases	Tubular necrosis
	Pyelonephritis
Malignant Disease	Lung Cancer
	Hodgkin's disease

LDH Assay

Principle:

LDH catalysis the following reaction:

$$\text{L-Lactate} + \text{NAD+} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H+}$$

The rate of NADH formation is indicated by **increase** the **absorbance** at 340nm and it is **directly proportional to serum LDH activity.**

If:

NADH is **product : increase** the absorbance /min

NADH is **reactant: decrease** the absorbance /min

Method

	Tube
LDH reagent	1 ml
Pre-warm at 37 °C for 3 minutes and add	
Sample (serum)	25 µl
Mix and incubate at 37 °C for 1 minutes, then read the absorbance at 340 nm against distilled water (blank) every minute for 3 minutes and determine ΔA/min.	

2) Applications → 2) Simple Kinetics → wave length (340 nm) → 1) Seconds → Duration (180 sec = 3 min) → Intervals (60 sec= 1 min) → Print Data Table (off) → Press start (2 times)

Results

	Time (min)	Absorbance at 340 nm
A1	1	
A2	2	
A3	3	

Calculations

$\Delta A_1, = A_2 - A_1$

$\rightarrow \quad \Delta A/\text{min} = (\Delta A_1 + \Delta A_2) / 2$

$\Delta A_2 = A_3 - A_2$

LDH (U/L) = $\Delta A \times 6592$

- Normal Values 109 to 245 U/L