قسم الكيمياء الحيوية
Biochemistry Department
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BCH 471

Experiment (5)

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.

PLASMA ENZYMES

- **Plasma enzymes are classified into:**

  1. Functional plasma enzymes

  2. Non functional plasma enzyme

Differences between functional and non-functional plasma enzymes

	Functional plasma enzymes	**Non-functional plasma enzymes**
Concentration in plasma	Present in plasma in higher concentrations in comparison to tissues	Normally, present in plasma in very low concentrations in comparison to tissues
Function	Have known functions	No known functions
The substrates	Their substrates are always present in the blood	Their substrates are absent from the blood
Site of synthesis	Liver	Different organs e.g. liver, heart, brain and skeletal muscles
Effect of diseases	Decrease in liver diseases	Different enzymes increase in different organ diseases
Examples	Clotting factors e.g. prothrombin, Lipoprotein lipase and pseudo-choline esterase	ALT, AST, CK, LDH, alkaline phosphatase, acid phosphatase and amylase,

SOURCES OF NON FUNCTIONAL 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 ENZYMES

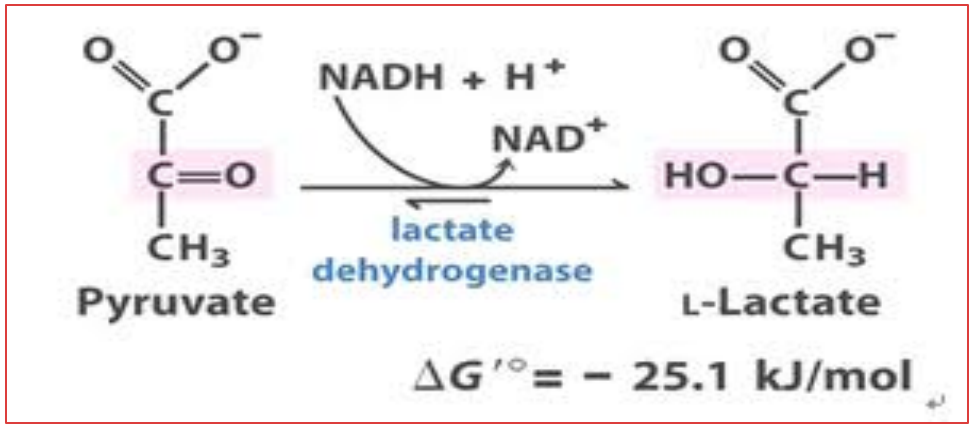
- Measurement of non functional enzymes is important for:
  - Diagnosis of diseases
  - Prognosis of the disease: following up of the treatment by measuring plasma enzymes before and after treatment.

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.
- Lactate is released into the blood and is eventually taken up by the liver.
- In the liver, it catalyzes the oxidation of L-lactate to pyruvate (L→P) with the mediation of NAD as hydrogen acceptor, eventually converting pyruvate to glucose.
- Glucose is released into the blood and then taken up by resting muscles, red blood cells, and other tissue.



- Exercising muscles convert (and red blood cells metabolize) glucose to lactate.
- 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 such as P-chloromercuribenzoate 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

-Lactate Dehydrogenase 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	3ml
Pre- warm at 37 c for 3 minutes	
Sample (serum)	0.1 ml(100 µl)
Mix and incubate at 37 c for 1 min . Read the absorbance at 340 nm against distilled water every minute for 3 minute Determine ΔA/min	

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) = $\Delta A \times 4984$

- Normal Values 109 to 245 U/L