Lactate Dehydrogenase

→ LDH is a hydroge transfes enzyme that catalyzes the oxidation of L- lactate to pyruvate & the mediation of NAD^+ as a hydrogen acceptor.

$$\text{L-Lactate} + NAD^+ \underset{\text{pH = 7.4 to 7.8}}{\overset{\text{LDH, pH = 8.8 to 9.8}}{\rightleftharpoons}} \text{Pyruvate} + NADH$$

→ more favourable reaction is P → L

→ Optimal pH varies & predominant isoenzyme in the sample & depands on the temp. & on substrate & Buffer concentration.

→ LDH can act on 2-Hydroxy acid & 2-Oxo-acid.

* Isoenzymes :-

4 peptides chains or 2 types under seperate genetic control

M → chromosome 11

H → chromosome 12

In alkaline pH

(−) ——————→ (+) (most anodal)

LD-5 LD-4 LD-3 LD-2 LD-1 (Highest -ve)

LDH-1 ⟶ HHHH ⟶ H_4

LD-2 ⟶ HHHM ⟶ H_3M

LD-3 ⟶ HHMM ⟶ H_2M_2

LD-4 ⟶ HMMM ⟶ HM_3

LD-5 ⟶ MMMM ⟶ M_4

⟶ LD-X : X_4 : XXXX : 6th isoenzyme

↓

+nt in postpubertal human testes

⟶ 7th Isoenzyme : LD-6 : ⟶ tnt in sera of severely ill pt.

* Inhibitors of LDH :—

⟶ mercuric ions @ p- :— reagent ~ reactivity against thiol groups of LDH

↓

inhibition is reversed by addition of cysteine or glutathione

⟶ Borate / oxalate :—

↓

- competitive inhibitors
- competes ~ lactate to binds ~ LDH

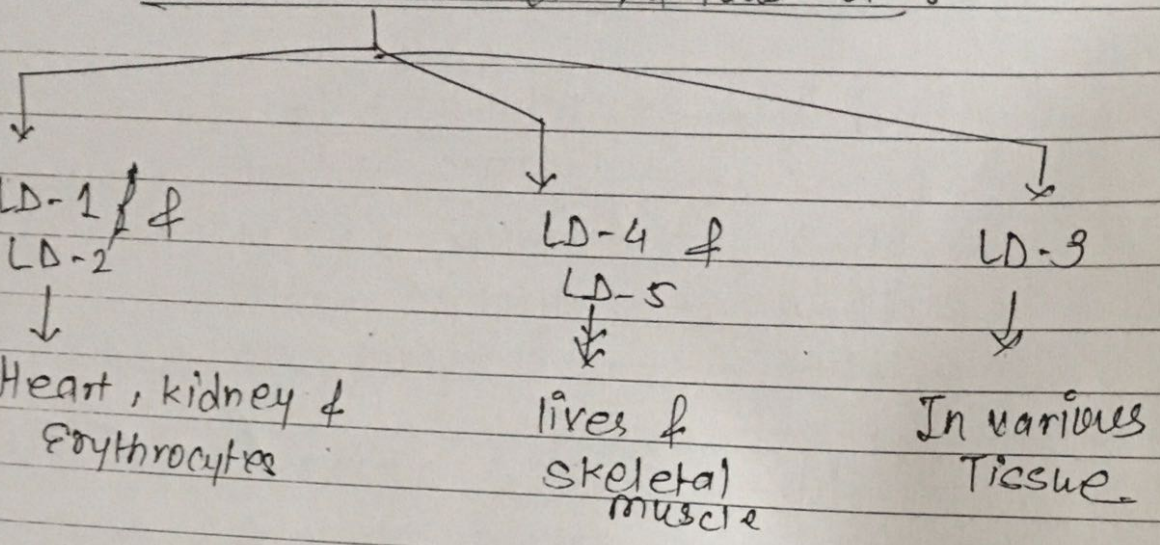
⟶ Oxamate :—

- competes ~ pyruvate for its binding to LDH in reverse reaction

⟶ Excess of pyruvate & lactate : also inhibits LDH

⟶ EDTA :— Bind ~ Zn^{+2}

* Tissue distribution of various LD :—



→ mainly ent in the cytoplasm.
→ 1500 to 5000 times greater conc. in cell than serum
↓
therefore, leakage of enzyme from even a small mass of damaged tissue ↑ the observed serum activity of LDH

* Clinical Significance :—

① Hemolytic anaemia :—
↓
↑ upto 50 times the URL

② → Megaloblastic anaemia :—
↓
large quantities of LD-1 & LD-2 is due to break down of ↑ precursor erythrocyte in Bone marrow

elevation rapidly return to (N) after appropriate Rx

→ (3) Malignant useful to predict d'se activity & survival rate & duration in Hodgkin's & non-Hodgkin's d's e

→ (3) Malignant d'se like lives metastases & nonhepatic metastasis

* → Macro-LD :-
↓
- LDH + autoantibody complex
- gives ab(n) migrating band at electrophoresis

* Methods :-

① kinetic spectrophometric measurement :-
↓
measures ↑ing absorbance due to formation of NADH from of NAD^+ at 340 nm

② Reference method :-
- IFCC L → P at 37°C

* Sample type :-
→ serum is preferred.

→ plasma sample should not be used
↓
as it may be contaminated ⊂ platelete ⊂ have high conc. of LD.

→ Sesum should be seperated from the clot as soon as possible after specimen has been obtained.

→ Hemolyzed sesum must not be used
↓
as esythrocyte coutain 4000 times more LD activity than sesum.

→ LD4 & LD5 are more sensitive too cold
↓
Thus sesum should kept at room temp.
(if stored at -20°C → activity of LD4 & LD5 lost)

* Reference intesval :—
→ Acc. to IFCC Ref. method : 125 to 220 U/L
→ highes in children ⊂ gradual ↓ noted oves the whole childhood period.

* Methods for seperation & Quantification of LDH :—

① Electrophoresis on agarose gel or cellulose acetate membrane

↓

Isoenzymes are seperated By electrophoresis

↓

Reaction mixture (L-Lactate, NAD+ dissolved in a suitable pH 8.0 Buffer)

↓

NADH generated over LD zone

↓

detected By its flouroscence when excited by long wave UV light (365 nm)

OR

By its reduction of tetrazolium salt to form colored formazan

② Agarose gel technique with fluorometric quantitation of generated NADH