SERUM ENZYMES

* Factors affecting enzyme conc. in plasma/serum:

(i) leakage of enzyme from cells
(ii) Efflux of enzyme from damage cells
(iii) Altered enzyme production
(iv) Clearance of enzymes.

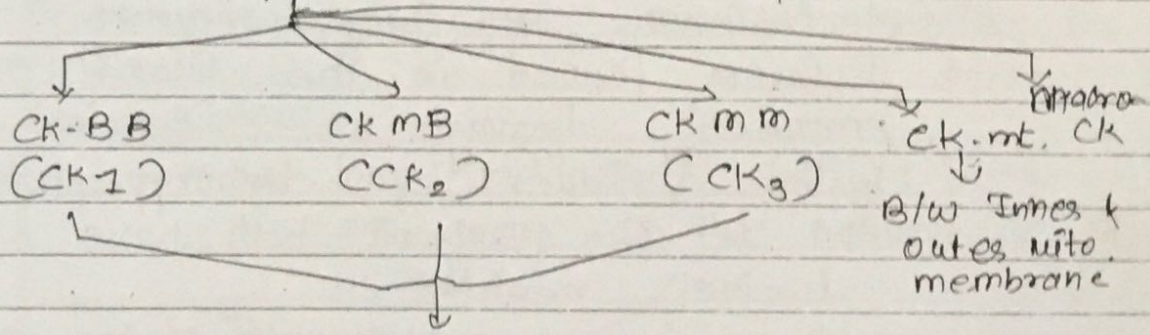
* CREATINE KINASE :-

→ Dimeric enzyme ← (active form)

→ 2 subunits

B m

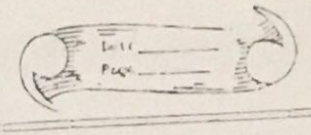
→ ~~dif. pair~~ or ~~CK~~ Isoenzymes of CK



→ 1, 2 & 3 → numbers on the basis of electrophoretic mobility due to presence of lysine residue with most anodal forms receiving lowest number.

→ most anodal → CKBB > CKMB > CKMM

CKmt Isoenzyme → sarcomeric, Ubiquitous



* Macro-CK :-

→ Type-1
- CKBB + IgG OR CKMM + IgA
- Can be cause of elevated CK
- causes diagnostic confusion

→ Type-2
- Oligomesic CK-mt
- seen in Malignancy, liver d'se

Isoenzyme → diff. in A.A seq, Product of diff. gene catalyze same reaction

* Isoforms of CK :→ highly related gene products perform same biological funct°, ≥2 functionally similar proteins. Similar but not identical AA seq,

→ CKMM & CKMB isoenzymes divided into subtypes — called Isoforms By electrophoresis or chromatofocusing
→ Isoforms formed in the Blood by irreversible enzymatic cleavage of C-terminal lysine residue by carboxypeptidase
→ M & B-Subunit both have C-terminal lysine residue.
→ Succesive removal of lysine residue makes 3 isoform of CKMM.

- $CKMM_3$ → 2 lysine +nt
- $CKMM_2$ → - 1 lysine +nt - 1 removed
- $CKMM_1$ → - NO Lysine +nt - Both removed.

CK MB

CK MB_1 → No lysine rnt
- 1 lysine removed from M subunit.

CK MB_2 → One lysine rnt.

→ Reaction catalyzed by CK :-

Creatine + ATP $\xrightarrow{pH = 9.0}$ Phosphocreatine + ADP

$\xleftarrow{pH = 6.7}$

Mg^{+2}

CK

→ At physiological pH - revesse reaction is favoured.

→ Inhibitors :-

→ metal ions (Mn^{+2}, Zn^{+2}, Ca^{+2}) : Binds to -SH gsoup on the active site oh enzyme

→ Idoacetate

→ Excess ADP → inhibit forward reaction

(?) → Urate & cystine (?)

→ Enzyme relatively unstable in sesum

↓

Because Oxidation oh -SH gsoup oh it.

↓

To prevent oxidation,

↓

Compounds use are : N-acetylcystein,
(cleland reagent) Dithiothretol,
Glutathion

↓

have reducing propestiy.

→ Serum concentration :—

CKMM > CKMB > CKBB

* Methods :—

Oliver & Rosalki method

① Creatine phosphate + ADP $\xrightarrow[pH=6.7]{CK}$ Creatine + ATP

② ATP + glucose $\xrightarrow{\text{Hexokinase}}$ glucose 6-P + ADP

③ G-6-P + NADP $\xrightarrow{G\text{-}6PD}$ 6-phosphogluconate + NADPH + H^+

NADPH → measured 340 nm

→ Based on reverse reaction

↓

it is 6 times fastes than forewasd reaction.

→ Reagent :— Contents :—

→ Creatine phosphate
- ADP
- Hexokinase
- G-6PD
- $NADP^+$
- glucose

} require for reaction

- N- acetylcysteine ⟶ to activate CK &
- EDTA ⟶ ~~maintain~~ to chelate Ca^{+2} as Ca^{+2} is inhibitor
- Adenosine pentaphosphate } to inhibito Adeny kinase
- AMP → ~~Product Inhibition~~

Prevent ~~false~~ false ← ~~Ad~~ to prevent use of ATP ~~during~~ which is formed during reaction
~~low~~ low result

~~2~~ ADP $\xrightarrow[AK]{}$ ATP + AMP ~~+ve interference at 2 step~~

⟶ IFCC → developed method at 37°C based on this reaction.

* Specimens :-

⟶ Hemolyzed sample :-

↓

Unsatisfactory

↓

enzymes & intesmediates (AK, ATP, G6P) libesated from esythrocytes.

As above ↑ 2nd step ↑ 2nd step ↑

⟶ Serum & plasma heparine — used

⟶ Other anticoagulant → can't be used

↓

may inhibit CK adiuity

~~* Metho~~

② Flourometric assay :-

Creatine p + ADP $\xrightarrow[CK]{PH-6.7}$ Creatine + ATP

Creatin + Ninhydrine $\xrightarrow{KOH}$ Flourescent compound.

③ Bioluminiscent assay :—

Creatine p + ADP $\xrightarrow{CK}$ Creatine + ATP

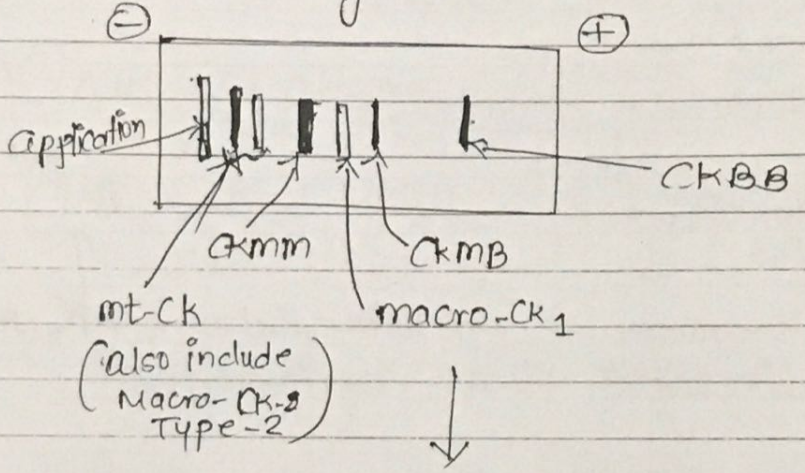
ATP + Luciferine + O_2 $\xrightarrow{\text{luciferase}}$ AMP + Oxyluciferin + phosphate + CO_2 + Light.

* Methods for Seperation of Isoenzymes :—

① electrophoresis :—

↓

At pH 8.3, isoenzymes have diff. mobility on 1% agarose



↓

after electrophoretic seperation (qualitative)

↓

add substrate for CK

↓

Incubate support medium

↓

Formation of NADPH

(amount of NADPH measured by flourescent)

(quantitative) ~~met~~ By densitometer

→ flouroscent ∝ CK amount

→ lengthy procedure
→ skill is required to interprete result.

② Ion exchange chromatography :—

↓

Seperation based on the charge

disadv : carryovers of CKMM into CKMB fraction.

↓

causing false high CKMB result

③ Immuno inhibition :—

↓

- ~~most frequently used~~
- Specific antibody toward CK-M fraction added

↓

CK-B subunit is unaffected, so retains its catalytic activity

↓

CK B activity then measured

↓

CKB - accounts for 50% activity of total CKMB enzyme

↓

doubling the CK-B activity gives total CKMB activity

→ if we assume that CK-BB & CK-MCARD not present in the sample

↓

Otherwise - it raises in CKMB result

↓

Falsely high result given.

④ Immunological Mass Assay :-

→ Ab - directed against CKMB covalently linked to the solid surface

↓

CKMB in the sample reacts & bound ab.

↓

Ag + Ab complex

↓

2nd Ab conjugated & enzyme is added

↓

2nd Ab is directed against another antigenic site on the CKMB molecule different from 1st Ab

↓

Ab + Ag + Ab-E complex (sandwich)

↓

washing - remove unbound labelled ab.

↓

add substrate

↓

↓

detectable product.

↓

rate of product formation ∝ CkMB activity.

→ Minimal interference

→ No effect of hemolysis, Drugs, inhibitors

* Reference Interval :-

Male :

Female :

* Clinical Significance :-

↑ Ck :- Rhabdomyolysis
Crush injury
Intramuscular injection
muscular dystrophies
Drugs like statins, Fibrates, ARV

Ck level → Not affected in neurogenic muscle d/se like polio, mysthenia gravis, multiple sclerosis, parkinsonism.

→ During childbirth → 6 fold elevation in mothers.

→ during due to surgical intervention

→ Ck-BB :- ↑↑ → in neonates
↓
mainly in brain damaged, VLBW-Babies.