

Plasma Proteins

* **Normal level:-**

- Adult plasma proteins: 6-8 g/dl (recumbent).
Fluids 6.8-8.5 (ambulatory) due to more water extravasation in the extremities.

* **Functions of Proteins:-**

1. Oncotic P (Mainly albumin)
2. Nutretive (Mainly albumin) [degradation of albumin] AAJ.
3. Buffering effect.
4. Coagulation and fibrinanalysis
5. Defense (functions that depend on Ig, synthesized in the :-
 - Lymphoreticular system
 - The complement system
6. Transport (drugs and hormones)
7. Enzymes and hormones.

* **Origins:-**

- Albumin, globulin and globulin –formed in the liver.
- Globulin –are formed by plasma cells all over the body in:
 - Bone Marrow
 - Lymph node

* **Plasma Proteins:-**

a) **Biophilic and Suicidal:**

- Fibrinogen
- Component of complement
- Ig
- Haptoglobin.

b) **Perform transport (carrier)**

eg. Albumin, pre-albumin, hormone-binding, Metal binding protein and apo-lipoprotein

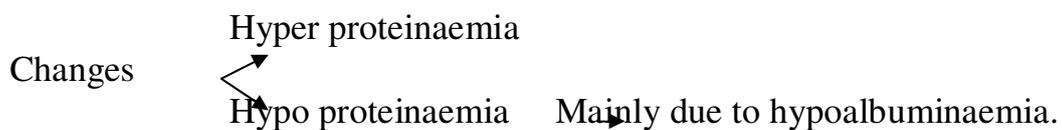
○ **Transport Function of Plasma Proteins:**

Carrier Protein	Carrier function
Pre-Albumin	- Retinol (Vit A) - T4&T3
Albumin	- Inorganic constituent of plasma(Ca) - Free fatty acid - Hormones (T4&T3) - Excretory product(Unconjugated bilirubin) - Drugs & other toxic substances
Hormone-binding protein	- Corticoids - Sex hormone -Thyroid homones each have their own specific binding proteins
Metal-binding protein	Copper; by ceruloplasmin Iron; by transferrin
Apo-Lipoproteins	- Lipids (transport of essential metabolites)

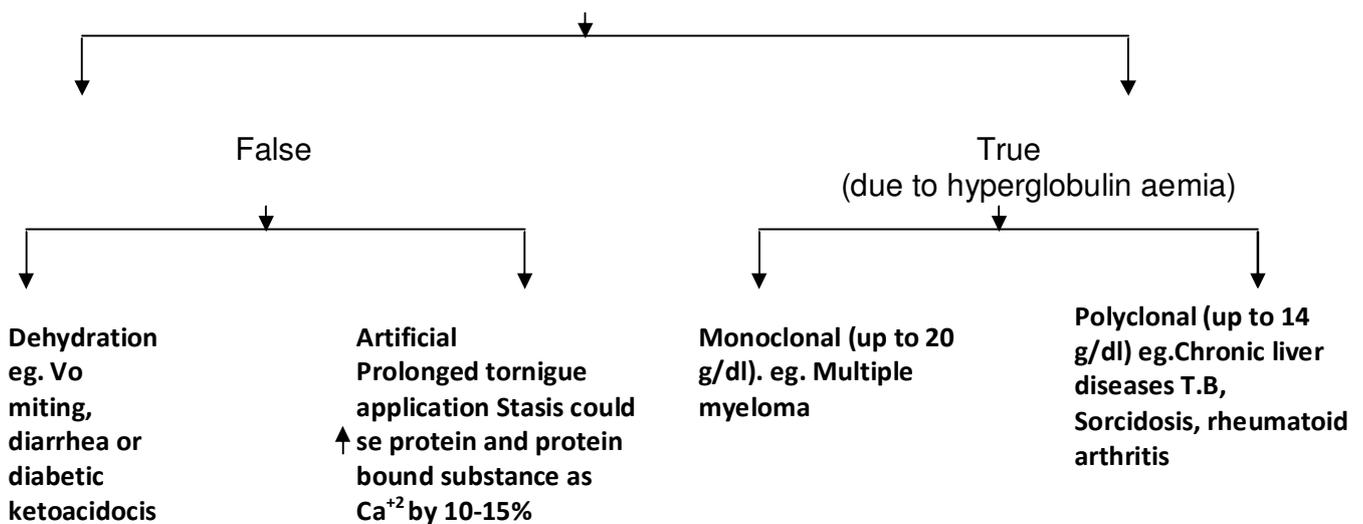
* CHANGE IN THE TOTAL SERUM PROTEINS:-

- Measuring of total serum proteins is misleading:

- Hypo-albuminaemia may be balanced by hypergammaglobunaemia.
- Proteins other than albumin may have large % change in conc still not detectable as a change in total serum protein.

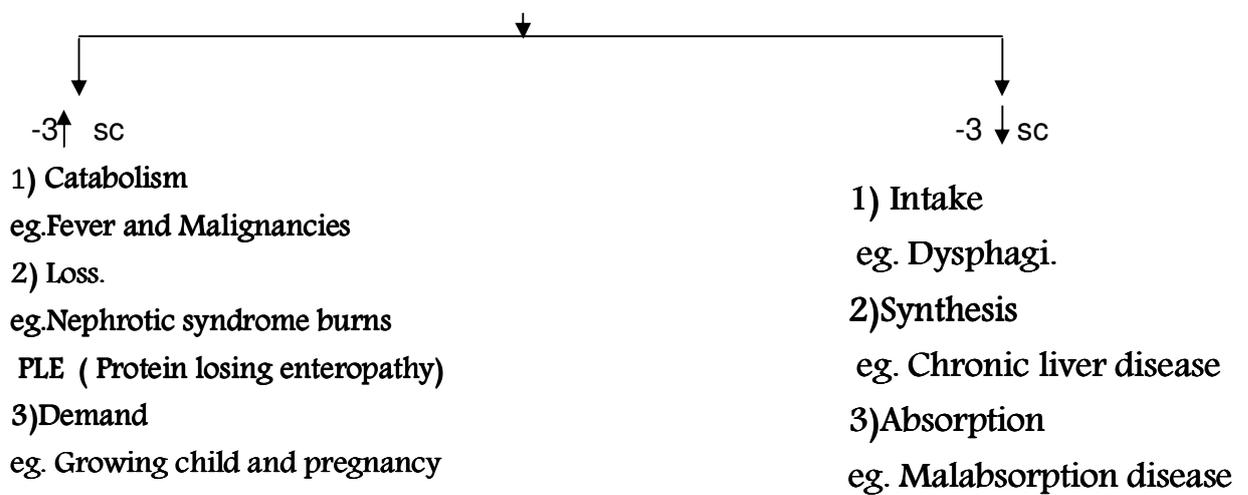


Hyper proteinaemia :- (more than 9 g/dl).



Hyper proteinaemia: -3 ↑ sc

-3 ↓ sc



- **Serum albumin :**

- one of the most protein concentration .
- lost early in nephritic syndrome .
- anything happen in the body will be reflected on albumin .

Fraction of Serum Proteins :-

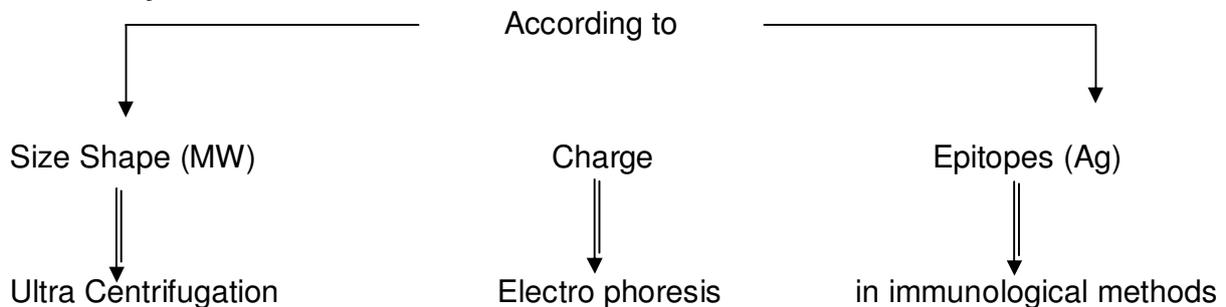
1. Chemical Precipitation methods :

- By Biuret's methods followed by protein, globulin & in estimation of albumin

2. Non- specific precipitation method :

- A number of colloid solutions are stabilized by albumin & are ppt. by globulins especially abnormal globulin this is the principal of the flocculation tests used as liver function test.

3. Physical method :



N.B.

Measurment of proteins

Albumin → Solubelization

Globulin → Precipitation

(1) RID

(2)Immuno electrophoresis

Ultra Centrifugation :-

- Proteins will separate in to fractions depend on their sedimentation constants which are the property of MW, shape & density of proteins

- At 60,000 rev/min the refractive index of the boundary b/w solvent & the protein is visualized by an optic system

- The results given is Srrrbeg units,

- **Advantage** : Most useful for the determination of MW of protein

- **Disadvantage** : high cost of each analysis & poor resolving capacity

Electrophoresis :-

- It depends on **the charge** carried by protein carries no charge

- **2 types** :

- **Boundary technique** : Free fluid

- **Zone electrophoresis** : Stabilizing media, cellulose acetate (Hb), Starch gell, Polyacrylamid gel 'have small pores, best for separation of genetic proteins & isozymes. Fraction total proteins liver

Q- what u prefer to measure (total protein or fraction) ?

We prefer fraction cuz total protein is misleading .

e.g. liver disease P.t if we measure the total we find it normal but, (A/G ratio changed) (decrease albumin & increase globulin) So, its apparently normal but actually not .

- also when we measure the percentage change its normal 4 total protein but, actually speaking its changed .

Individual Protein Fractions

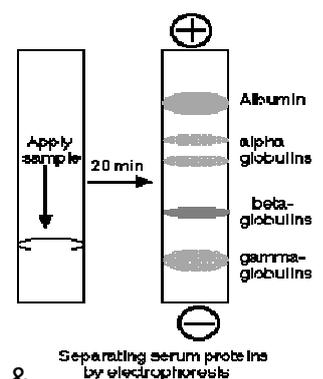
- Most plasma proteins are glycoproteins, the amount of CHO varying form 1% (albumin) to 40% (acid glycoprotein also called or somucoid)
- Most plasma protein are synthesized in liver except.
 - Ig by the lymphocytes – Apolipoprotein by Enterocytes.
- Catobolism of plasma proteins is degraded throughout the body after being taken up by cells

❖ Individual Protein fractions :-

- 1) Albumin
- 2) Globulins : Heterogeneous Gp. + α_1 , α_2 , β & γ Globulins

✓ Alpha1 Globulins :-

1. alpha1 Anti trypsin constitutes about 90% of this fraction "Main"
2. Also include : alpha1 acid glycoprotein, trans cortin, corticosteroid Binding protein, prothrombin, alpha1 fetoprotein & alpha1 lipoprotein : HDL .



1. alpha1 Anti trypsin (alpha1 protease inhibitor AP₁):

- Produced by : Hepatocyte & Macrophages.
- Proteases as Trypsin, chymotrypsin, Elastase & Thrombin III continually being released in circulation, AP : inhibits the activity of these proteases.
- In congenital deficiency (homozygous state) patients are prone to pulmonary emphysema, Neonatal hepatitis may proceed to cirrhosis.
- It is increased in acute inflammation & infection etc. It increases in acute phase destruction.

2. Alpha 1 Fetoprotein (AFP)

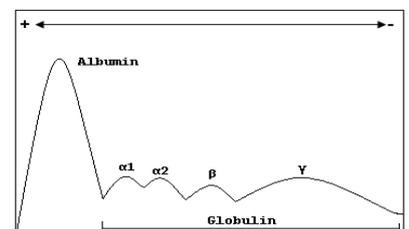
- It is a normal fetal protein, which starts to appear at 6th week. Maximum at 12-15 weeks. It decreases after birth to reach up to 15 mg/l at adult hood.
- It's function is unclear, But it may play an important immunoregulatory role during pregnancy.
- Screening programs during pregnancy involve measurement of AFP in maternal serum where it ↑↑↑ in open neural defects while it ↓↓ Down's syndrome.
- AFP detection is very useful in Primary liver cancer
- Other causes of increase include : gonadal teratoma, germ-cell tumor, hepatoma "used as tumor marker"
- Non malignant causes: hepatitis, cirrhosis, & pregnancy. ⇒ ↑ AFP.

N.B. Embryonic & Fetal protein associated with human neoplasia :
- Several fetal proteins are synthesized in human tumors : they are released in biological fluid & are useful in diagnosis of malignancy (But not specific)
eg.alpha1 fetoprotein & alpha2 ferroprotein.

* Alpha2 – ferroprotein :-

- It's a 17 S iron –containing protein synthesized in liver.
- Found in fetal organs & serum
- Increase in Child hood : nephroblastoma, leukemia, hepatoma.
Adult : Hepatoma, cholangio, carcinoma, lymphoma.

* Carcino-embryonic antigen :

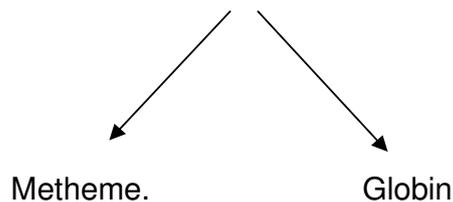


- It is normally up to 2.5 mg/l
- Elevated levels produced by tumors of ectoderm origins.
- Elevated levels also occur in smoker & with inflammatory disease of the bowel, lung & chronic liver diseases.

- * **Alpha2 Globulin** : this includes ;
 Hepatoglobulin (H_p), alpha₂ Macroglobulin, Ceruloplasmin, alpha₂ lipoprotein (Pre B : VLDL) which in Pre-β-lipoproteinemia.
- In alpha₂ Globulins : Hepatoglobulin : Carries Hb “free Hb”
 Ceruloplasmin : Carries metal which is copper.

1. Hepatoglobulin (Hp) :

- It's major synthesis occurs in hepatocytes.
- It binds to free Hb) any to only 2% destruction of RBC /day will completely deplete plasma Hp in the absence of a stimulus for production.
- In case of hepatoglobulin depletion, with further lysis, Hb may be oxidized methemoglobin



- The hemoexin – heme complexes are removed by RES (as in case of Hp- Hb complexes) while albumin (lower affinity).
 Metheme → methemalbumin : releases heme to hemoexin directly or go to the liver

N.B : 1) RES . Reticulo-endothelial system (macrophagis, spleen, Bone marrow)

2) hemoexin has high affinity to Hb.

- ↑↑levels of Hp : tissue destruction & malignancy, Recovery stage of burns.” b/c of loss of proteins.”
- ↑↑Levels of Hp: intravascular hemolysis, ineffective erythropocisis also hepato cellular damage

N.B: 1) Hp. Is an acute phase protein (↑inflammation & infection)
 2) In chronic liver disease : (pattern); ↓albumin. Begin between B & gamma ← Polyclonal gammopathy

3) Other polyclonal : TB, arthritis, sarcoidosis.

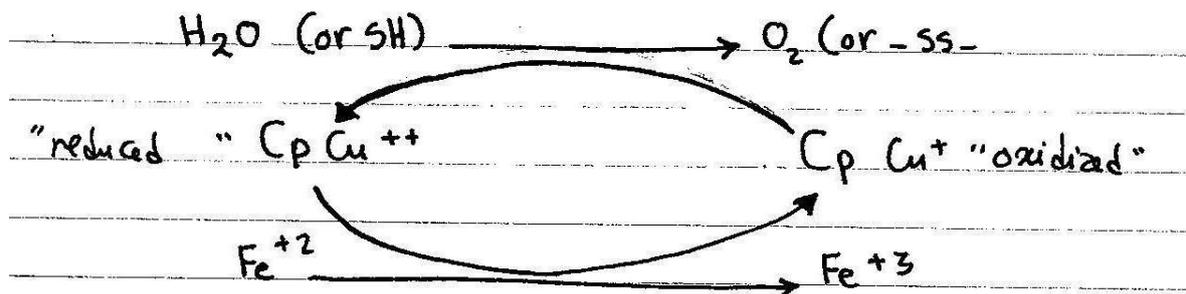
2. α_2 Macroglobulin :

- It is a very large molecule, with a Molecular mass ~ 750 dalton thus doesn't diffuse from plasma to extra cellular fluid.
- It's produced by liver
- Other proteinase inhibitors: alpha1 anti-chymotrypsin, anti-thrombin III e 1 esterase inhibitor, Protein C & Plasmonogen activator inhibitor.
- $\uparrow\uparrow$ Levels : nephritic syndrome & estrogen intake (\uparrow synthesis)
- It also binds many cytokines & help their uptake by cells.

N.B. : 1- It's retained with the loss of other low MW proteins.
2- Not lost easily.
3- Pattern of nephrotic syndrome;
albumin : \downarrow alpha2 globulin : (mainly increased alpha2 macroglobulin)
B- globulin: \uparrow / gamma - region: \downarrow

3. Ceruloplasmin (Cp) : "Blue in color , b/c of copper"

- It's a copper binding protein that has the ability to "scavenges" O_2 derived free radicals.
- It has the capacity to catalyze oxidation of Fe^{+2} (ferrous) to Fe^{+3} (ferric)



- $\uparrow\uparrow$ Levels: acute phase response (modest \uparrow), estrogen intake, & pregnancy
 - $\downarrow\downarrow$ Levels: Wilson's disease (hepatolenticular) malabsorption.
- N.B. 3 Proteins \uparrow ed in pregnancy: alpha1 –fetoprotein – Cp – Alkaline phosphatase.

* **B- Globulin** : (\uparrow ed in nephritic syndrome pattern)
 (In the serum is fresh \uparrow 2 B bands are seen B₁ & B₂)

❖ If the serum is Fresh \uparrow 2 B bands are seen : B₁ & B₂ (C3 complement component)

- It includes : Transferrin, hemopexin, C₄ B lipoprotein : LDL fibrinogen, C-reactive protein, some immunoglobulin are B-globulin.
- In non-fresh (old) sample only 1 Band for B glob lin.
- In B-globulin : hemopexin carries Hb.
 Transferrin carries metal which is iron.



1. **Fibrinogen** \rightarrow 400 mg/dl (1.4 g/l) \rightarrow "important in coagulation + in Plasma Not serum"
 (900 \rightarrow 400 mg/dl) (1.4 g/l) absent in serum.

- In many inflammatory diseases, rheumatic fever, pneumonia.
- In Congenital as afibrinogenemia, acquired in terminal liver disease, premature separation of placenta (DIC) Placenta will detach from the wall
- $\uparrow\uparrow$ runs between B & gamma globulins.
- \downarrow transformed in to fibrin in process of blood clotting.
- Fibrinogen gives viscosity to Bl. Plasma helping the maintenance of blood viscosity & pressure.



2. C- reactive protein:-



- First was found in reaction with bacterial pneumococci, then with other antigens.

- It occupies any where from the slow gamma to mid B region
- CRP is some how involved in body in response to foreign material.
- It is considered in immune regulatory function participation.
- ~~Level in MI: stress, trauma, infection, inflammation~~ ^{1st} protein to detect early inflammatory reactions, that's why it's 1st one that returns to normal levels!!



β_2 microglobulin:

- In surface of **nucleated cell**
- HLA human lymphocyte antigen
- ↑↑ level in renal failure , inflammation & neoplasm
- It's clinical value in malignant to test renal tubular function , particularly in **Kidney transplant** recipients

Pre-Albumin	Albumin
<ul style="list-style-type: none"> -Low concentration -T 1/2 : 2 days -Reflex acute deficiency (+ve) -T3&T4 carrier -Liver disease -Bind to retinol -Not seen normally BUT it seen by high resolution of electrophoresis 	<ul style="list-style-type: none"> -large 66000 MW -T 1/2 : 20 days - Reflex acute deficiency (- ve) - bilirubin , FFA , Ca++ transport -special for Liver - colloid oncotic pressure -A.A > for tissue - chronic deficiency stasis - ↑ : Albuminuria – nephritic – nephritic – dehydration - ↓ : Edema – blood volume - fraction of electrophoresis supporting cellulose acetate

- Band of Albumin : Homogeneous - α1 Globulin the band is : Heterogeneous

Immunoglobulin

- They are group of structurally related proteins that function as Anti-bodies.
- They are synthesized by lymphoreticular system.
- Immunoglobulin are made of 4 polypeptide chains : 2 heavy chains are composed of 5 classes, while the light chains are 2 types

- The heavy chains (5);



Ig M : are pentamers found of 5 units, connected through J-protein (acute phase)
Ig G : Can cross the placenta (chronic inflammation)

- Ig A : present secreted in the alimentary tract is a dimer
- Ig E : low fraction, delay hypersensitivity. It's binding site is on basophiles.
- Ig D : as Ig E.

Note: T-cell (lymphocytes) → cell mediated immunity

B-cell (lymphocytes) → Humeral immunity

Gamma Globulin:- →

- ↓ Agammaglobulinemia : less than 0.1g/dl
- ↓ Hypo gammaglobulinemia : 0.1-0.7g/dl

Causes:-

In infants : it's normal to have transient hypo gammaglobulinemia till 6 month (ed infection) after 6 months abnormal acquired : nephritic syndrome.



Acute Phase Reaction

(not indicative only to acute reactions but to other diseases)

- These are groups of plasma proteins which show marked $\uparrow > 50\%$ in concentration. During the early stages of disorders with tissue lesions accompanied by inflammation (trauma, septic necrosis & infection) whether it is acute or chronic.
- There are $+^{ve}$ & $-^{ve}$ acute phase Reactants.

1. eds synthesis & release of other proteins ($+^{ve}$ phase proteins) as:

- 1) C-reactive protein
- 2) Protease inhibitor (α_1 Antitrypsin – α_2 macroglobulin – Antithrombin111 – α_1 elastase inhibitor)
- 3) α_1 Glycoprotein
- 4) Fibrinogen
- 5) Ceruloplasmin
- 6) Complement Component ($C_3 + C_n$)
- 7) Hepatoglobulin

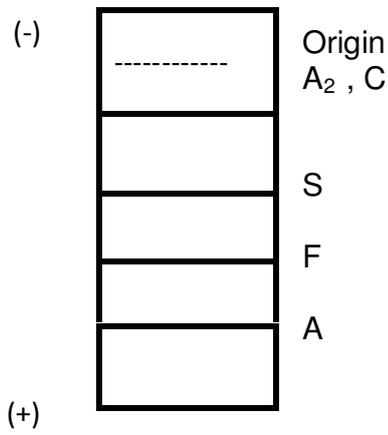
2. ($-^{ve}$ acute Reactant) : \downarrow ed in inflammation;

- 1) Prealbumin “also \downarrow ed in \downarrow ed nutrition”
- 2) Albumin “ free albumin”
- 3) Transferrin

Methods of Estimation of Hb

1. Electrophoresis.

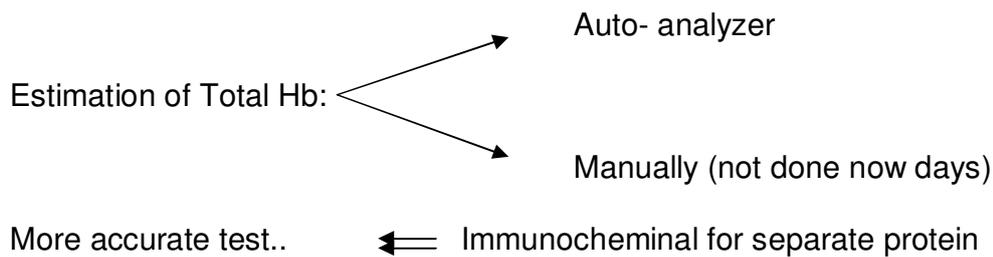
- at alkaline pH : 8.6
- at acidic pH: 6.0



2. Iso electric focusing

- Separate on the basis of iso electric pH of Hb variation
- Simple method
- Doesn't separate all variation

- Simple procedure
- Accurate
- Useful for identification of count & variation.
- Can be quantified by Densitometer.



1. Immuno diffusion (RID) : Radial Immuno Diffusion.

- For specific protein identification.
- Simple.
- Proteins are identified on terms of precipitant react respective Anti bodies.

2. Immuno electrophoresis :

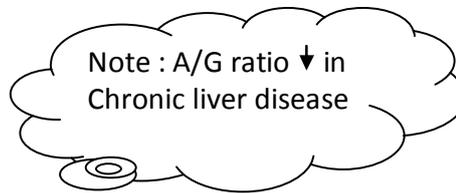
- More sophisticated test.
- Immune diffusion plus electrical field
- Rocket immuno electrophoresis.
- Quantitative method
- Accurate but complex
- Take a long time.

Examples of Organ-Specific Profiles:-

Liver function test :

Bilirubin, Alkaline phosphates transaminase, Albumin.

Enzymes of liver:



On biliary canicili
" used in case of Sterisis
eg. Stones" .
1) Alkaline phosphates
2) gamma g-t

Inside the cell AST. ALT
"used when liver cells
are affected.
ALT : more liver
specific.

- * Electrolyte profile : Na^+ , K^+ , Cl^- , HCO_3^-
- * Acid –base balance ; pH, PCO_2 , HCO_3^-
- * Cardia profile : CPK, CPK MB, LDH, AST.

⇒ in Myocardial infarction :-

- CPK “total “ & AST ↑ together & decrease together.+
- LDH last one to ↑ & last one to ↓
- CPK MB ; 1st to ↑ & 1st to ↓
- Troponin are used in MI

Note that CPK MB/ CPK total ration in ♥ more than 30%.

Note:

AST : Aspartate transaminase ; ALT : Allanine transaminase ; A/G : Albumin/Globulin

LDH : lactate dehydrogense ; CPK : Creative phosphokinase ; MB : sub units

- * Endo crier profile ; T_3 , T_4 , TSH for thyroxin function
 - * Muscle function : Mg^{+2} , K^+ , aldolase
- Note: in skeletal MS. LDH,AST.
the CPK MB/CPK “total” ration doesn’t exceed 5%

- * Diabetes Mellitus : Acid-base balance used in diabetic ketoacidosis for diagnosis: fasting g/c.level or GTT(g/c . Tolerance test) for follow up : Hb A_{1c}
- * Kidney function : Uric acid, urea, creatinine. Electrolytes (Na^+ , K^+)
- * In Rickets :
 - Ca^{++} “ ↓ ed”
 - Vit. D
 - PTH “parathyroid hormone = Parathormone”
 - ↑ Phosphate.

* Note : Chronic Renal Failure :-

- ↓ Ca^{+2}
- ↑ Phosphate
- Metabolic Acidosis ⇒ ionized form

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