

## **Plasma Proteins**

### **\* Normal level:-**

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

### **\* Functions of Proteins:-**

1. Oncotic P (Mainly albumin)
2. Nutretive (Mainly albumin) [degredation of albumin] AAJ.
3. Buffering effect.
4. Coagulation and fibrinalysis
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

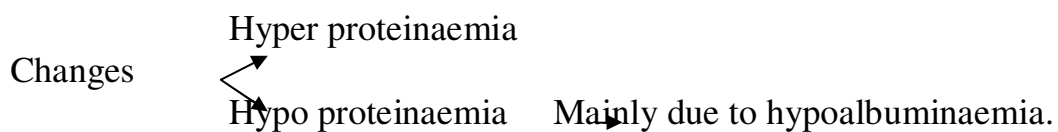
○ **Transport Function of Plasma Proteins:**

<b>Carrier Protein</b>	<b>Carrier function</b>
<b>Pre-Albumin</b>	<ul style="list-style-type: none"> <li>- Retinol (Vit A)</li> <li>- T4&amp;T3</li> </ul>
<b>Albumin</b>	<ul style="list-style-type: none"> <li>- Inorganic constituent of plasma(Ca)</li> <li>- Free fatty acid</li> <li>- Hormones (T4&amp;T3)</li> <li>- Excretory product( Unconjugated bilirubin)</li> <li>- Drugs &amp; other toxic substances</li> </ul>
<b>Hormone-binding protein</b>	<ul style="list-style-type: none"> <li>- Corticoids</li> <li>- Sex hormone</li> <li>-Thyroid homones</li> </ul> <p>each have their own specific binding proteins</p>
<b>Metal-binding protein</b>	<p>Copper; by ceruloplasmin</p> <p>Iron; by transferring</p>
<b>Apo-Lipoproteins</b>	<ul style="list-style-type: none"> <li>- Lipids (transport of essential metabolites)</li> </ul>

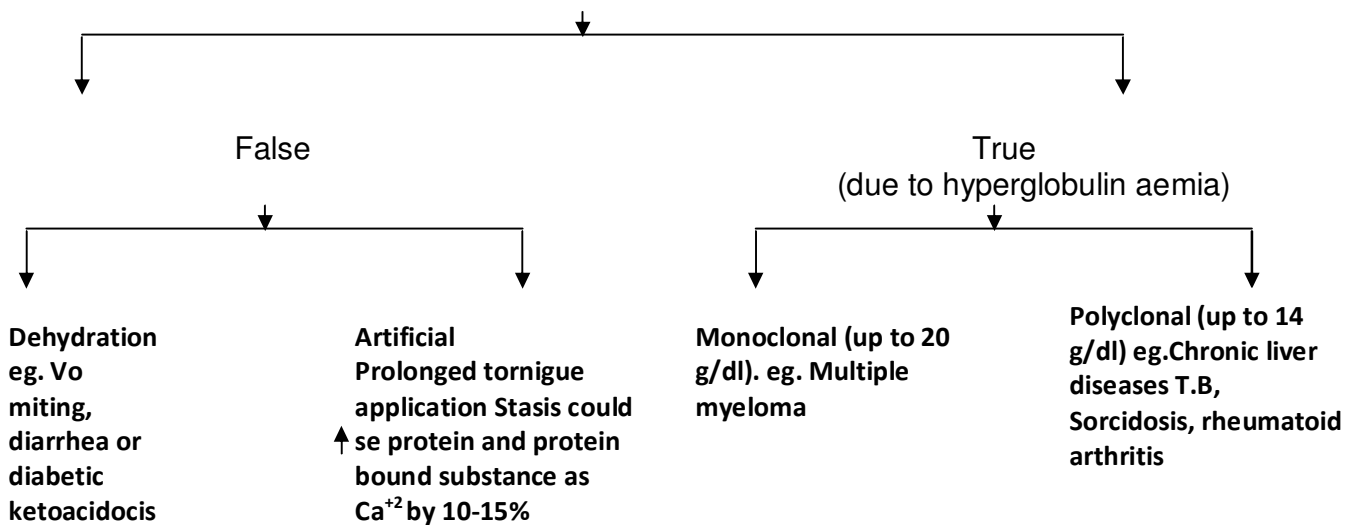
## \* CHANGE IN THE TOTAL SERUM PROTEINS:-

- Measuring of total serum proteins is misleading:

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

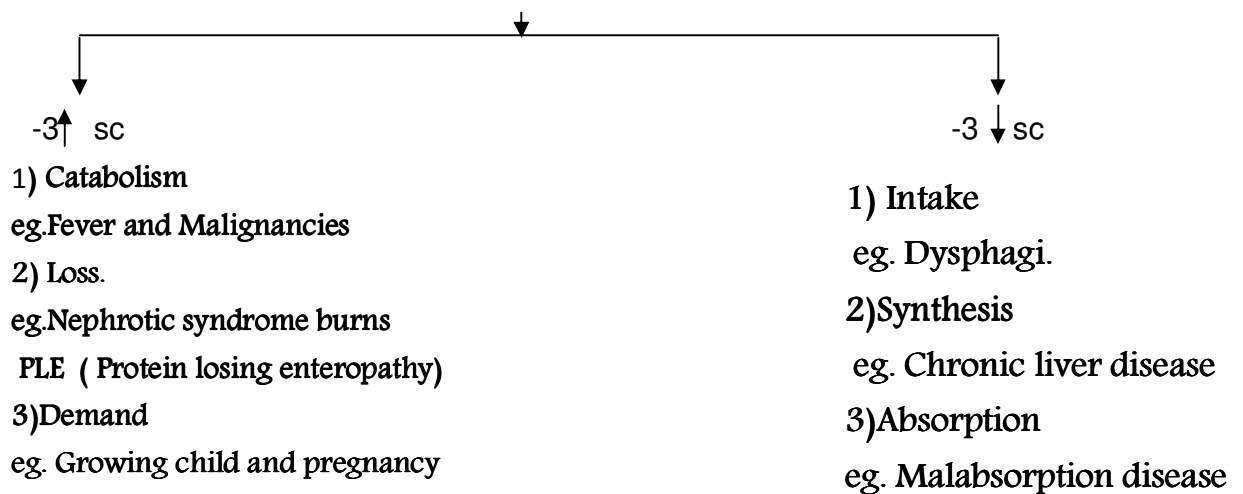


**Hyper proteinaemia :- ( more than 9 g/dl).**



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 :-

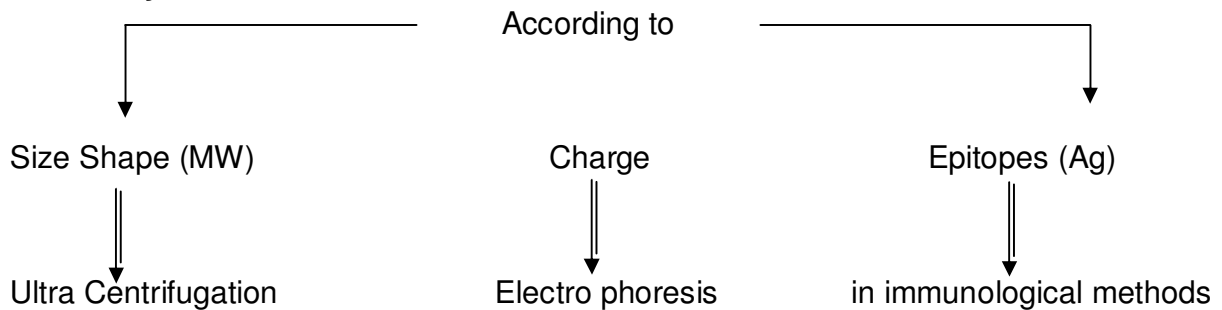
### 1. Chemical Precipitation methods :

- 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 gel, 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

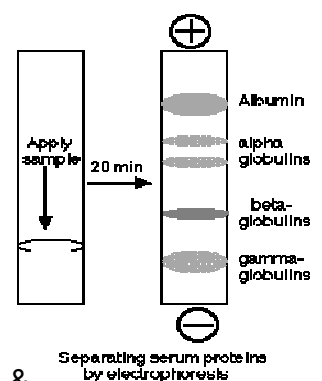
- Most plasma proteins are glycoproteins, the amount of CHO varying form 1% (albumin) to 40% (acid glycoprotein also called or somucoid)
- Most plasma protein are synthesized in liver except.
  - Ig by the lymphocytes – Apolipoprotein by Enterocytes.
- Catobolism of plasma proteins is degraded throughout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

### ❖ Individual Protein fractions :-

- 1) Albumin
- 2) Globulins : Heterogeneous Gp. +  $\alpha_1$  ,  $\alpha_2$  ,  $\beta$  &  $\gamma$  Globulins

### ✓ Alpha1 Globulins :-

1.  $\alpha_1$  Anti trypsin constitutes about 90% of this fraction "Main"
2. Also include :  $\alpha_1$  acid glycoprotein, trans cortin, corticosteroid Binding protein, prothrombin,  $\alpha_1$  fetoprotein &  $\alpha_1$  lipoprotein : HDL .



## 1. alpha1 Anti trypsin ( alpha1 protease inhibitor AP<sub>1</sub> ):

- 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 6<sup>th</sup> 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.

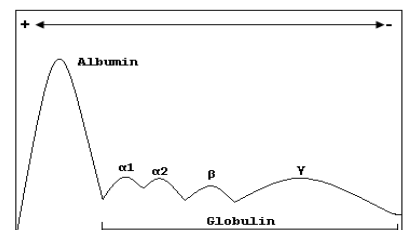
N.B. Embryonic & Fetal protein associated with human neoplasia :

- 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 :

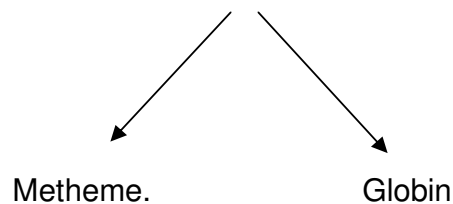


- It is normally up to 2.5 mg/l
- Elevated levels produced by tumors of ectoderm origins.
- Elevated levels also occur in smoker & with inflammatory disease of the bowel, lung & chronic liver diseases.

- \* **Alpha2 Globulin** : this includes ;  
 Hepatoglobulin (H<sub>p</sub>), alpha<sub>2</sub> Macroglobulin, Ceruloplasmin, alpha<sub>2</sub>  
 lipoprotein (Pre B : VLDL) which in Pre-B-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 homopexin – heme complexes are removed by RES ( as in case of Hp- Hb complexes ) while albumin (lower affinity ).  
 Metheme → methemalbumin : releases heme to homopexin directly or go to the liver

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

### 2) homopexin 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 erythropoiesis 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. $\alpha_2$ Macroglobulin :

- It is a very large molecule, with a Molecular mass  $\sim 750$  dalton thus doesn't diffuse from plasma to extra cellular fluid.
- It's produced by liver
- Other proteinase inhibitors:  $\alpha_1$  anti-chymotrypsin, anti-thrombin III &  $\alpha_2$  esterase inhibitor, Protein C & Plasminogen 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.

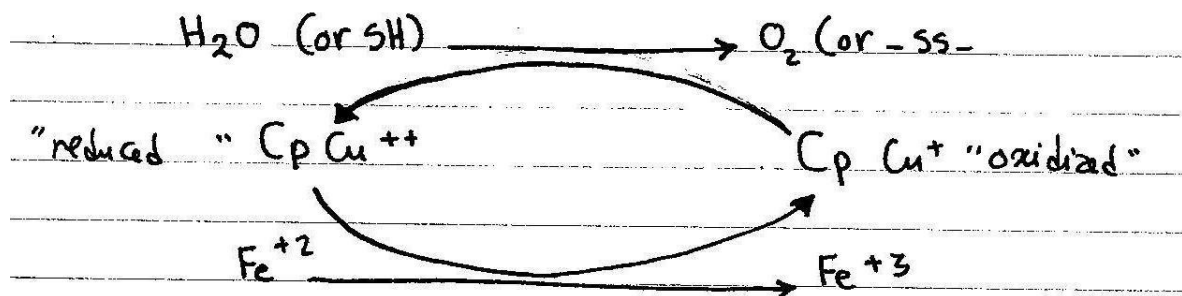
3- Pattern of nephrotic syndrome;

albumin :  $\downarrow$   $\alpha_2$  globulin : (mainly increased  $\alpha_2$  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** : (  $\uparrow$  in nephritic syndrome pattern ) In the serum is fresh. 2 B bands are seen B<sub>1</sub> & B<sub>2</sub>

❖ If the serum is Fresh  $\uparrow$  no B bands are seen : B<sub>1</sub> & B<sub>2</sub> ( C3 complement component )

- It includes : Transferrin, hemopexin, C<sub>4</sub> 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**  $\div$  200 – 400 mg/dl or ( 1 – 4 g )  $\Rightarrow$  "important in coagulation + in Plasma Not serum"

- In many inflammatory diseases, rheumatic fever, pneumonia.
- In Congenitals 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  $\Rightarrow$  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 anywhere from the slow gamma to mid B region
- CRP is somehow involved in body in response to foreign material.
- It is considered in immune regulatory function participation.
- ~~Level in MI: stress, trauma, infection, inflammation~~ Detect early inflammatory reactions, that's why it's 1<sup>st</sup> protein to increase in inflammation & 1<sup>st</sup> one that returns to normal levels!!



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

- 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

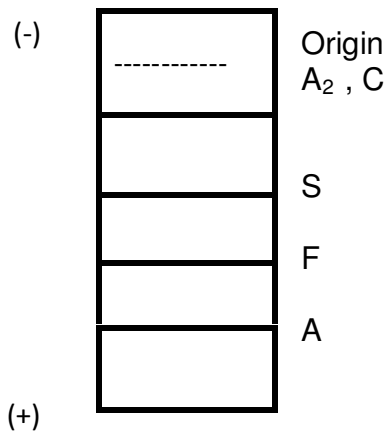
(not indicative only to acute reactions but to other diseases)

- 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  $+^{\text{ve}}$  &  $-^{\text{ve}}$  acute phase Reactants.
1. eds synthesis & release of other proteins ( $+^{\text{ve}}$  phase proteins) as:
    - 1) C-reactive protein
    - 2) Protease inhibitor ( $\alpha_1$  Antitrypsin –  $\alpha_2$  macroglobulin – Antithrombin111 –  $\alpha_1$  elastase inhibitor )
    - 3)  $\alpha_1$  Glycoprotein
    - 4) Fibrinogen
    - 5) Ceruloplasmin
    - 6) Complement Component ( $C_3 + C_n$ )
    - 7) Hepatoglobulin
  2. (  $-^{\text{ve}}$  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.

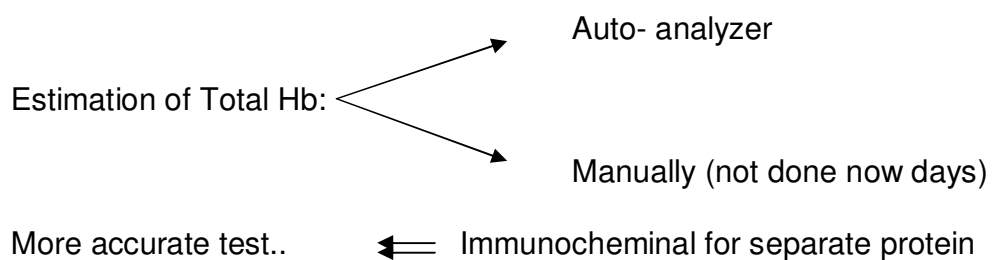
- at alkaline pH : 8.6
- at acidic pH: 6.0



### 2. Iso electric focusing

- Separate on the basis of isoelectric 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:

Note : A/G ratio ↓ in Chronic liver disease

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 :  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$
- \* Acid –base balance ; pH,  $\text{PCO}_2$ ,  $\text{HCO}_3^-$
- \* 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 ; 1<sup>st</sup> to ↑ & 1<sup>st</sup> 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 dehydrogenase ; CPK : Creative phosphokinase ; MB : sub units

- \* Endo crier profile ;  $\text{T}_3$ ,  $\text{T}_4$ , TSH for thyroxin function
- \* Muscle function :  $\text{Mg}^{+2}$ ,  $\text{K}^+$ , 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<sub>1c</sub>
- \* Kidney function : Uric acid, urea, creatinine. Electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ )
- \* In Rickets :
  - $\text{Ca}^{++}$  “ ↓ ed”
  - Vit. D
  - PTH “parathyroid hormone = Parathormone”
  - ↑ Phosphate.

- \* Note : Chronic Renal Failure :-

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

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