PLASMA PROTEINS AND IMMUNOGLOBULINES – An Overview

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How is blood plasma different from serum?

- Plasma is fluid portion of whole blood,
  - Plasma is obtained when whole blood containing anti-coagulant is centrifuged,
  - Plasma contains clotting factors,
- Serum is fluid portion of clotted blood,
- Serum is obtained after centrifuging clotted blood
  - Serum does not contain clotting factors that are normally present in plasma,

What are some of the proteins in blood plasma or serum?

- Plasma contains a variety of proteins with different functions and some proteins of unknown functions
- "Total Protein" in plasma is made up of **Albumin** and **Globulins,**
- Clinical Biochemistry labs routinely measures **Total Protein** and **Albumin** usually in serum,
- **Globulin** fraction = **Total protein – Albumin**

- Other plasma proteins (e.g., Immunoglobulins) are measured as Classes,
- Immunochemical methods are use to measuring specific plasma proteins, hormones or enzymes;
- Enzymes in serum or plasma are also measured by their activity;
- Electrophoresis can be used to separate protein components in Serum or Plasma,
  - About 5 or more major bands can be used to demonstrate the presence of Paraproteins;

What are the functions of proteins in blood plasma?

- **Some functions include**:
- **Blood clotting factors:** proteins in coagulation cascade
- **Immune defense**: Immunoglobulins, Complement proteins involved in Inflammatory responses:
  - Acute phase response proteins: C-reactive protein, alpha-acid glycoprotein (Orosomucoid);
- **Transport /binding proteins**: Albumin, Ceruloplasmin, Haptoglobin, Retinol Binding Protein, Sex Hormone Binding Globulin, Thyroid Hormone Binding Protein, Transferrin, etc.
- **Anti-proteases**: Anti-Chymotrypsin, Antithrombin, α_2-Macroglobulin,

What are some diagnostic significance of Total Protein?

- Total Protein in plasma is about **7 – 7.5 g/dL**
  - Plasma proteins includes: Simple Proteins, Mixed or Conjugated Proteins, Glycoproteins and various types of Lipoproteins,
- Changes in amount of Total Protein in plasma are common in some disease conditions;
- Elevated amount of Total Protein in plasma may indicate presence of Paraproteins,
- Decrease amount of Total Protein in plasma may indicate low level of Albumin,

What are some of the functions of Albumin?

- Albumin is one of the major plasma proteins; it is synthesized and secreted by the Liver,
  - Biological half-life of Albumin in plasma: 20 days
  - Significant decrease in amount of Albumin in plasma is usually slow to occur if it is due to reduction in biosynthesis of Albumin,
- Albumin makes the biggest contribution to plasma Oncotic Pressure ,
  - Edema may occur when plasma Albumin level falls very low,
- Albumin is one of the major binding /transport proteins in blood plasma,

What are some of the possible causes of Hypoalbuminemia?

- Three main reasons for **Hypoalbuminemia**:
- **Decreased synthesis**:
  - May be due to malnutrition or mal-absorption,
  - May be a feature of advanced liver disease,
- **Abnormal distribution or dilution**:
  - May be induced by overhydration,
  - May be caused by increased capillary permeability as occurs in septicemia,
- **Abnormal excretion or degradation**:
  - May be causes by: Nephritic Syndrome, Protein-losing Enteropathies, Burns, Hemorrhage, Catabolic states,

How important are some of the Specific Serum/Plasma Proteins?

- Measurement of some specific plasma proteins gives useful information for diagnosis and management of some diseases: Examples:
  - Transferrin receptors, Ferritin,
  - Thyroid Binding Globulin (TBG)
  - Sex Hormone Binding Globulin (SHBG),
  - Haptoglobin,
  - Albumin,
  - Globulins,
  - C-reactive protein (CRP),
  - Immunoglobulins (Ig); etc.

- Characteristic changes in amount of certain plasma proteins are seen after Surgery or Trauma, or during Infection or Tumor growth:
  - Proteins involved are called **Acute Phase Proteins;**
  - Acute Phase Protein response leads to greatly increased De Novo biosynthesis (mainly in Liver) of some plasma proteins along with decease in levels of other proteins in plasma,
  - Response is stimulated by release of Cytokines: **Interleukin-1**, **Interleukin-6** and **Tumor necrosis factor (TNF)** and increased plasma [**Cortisol]** and [**Glucagon]**

- Acute Phase Protein response is an adaptive response to diseases;
- Example:
  - Increases in plasma levels of CRP and Complement will contain and eliminate infection,
  - Increased Coagulation Factors will aid and prevent excess blood loss,
  - Protease Inhibitors will prevent the spread of tissues necrosis when damaged cells at the site of injury release Lysosomal enzymes,
- Clinically some Acute Phase proteins are used to monitor progress of some disease condition or its response to treatment;

How useful is Electrophoresis of serum/plasma proteins (pH 8.6)?

- Electrophoresis may be used to study protein abnormalities;
  - **Serum is a better choice for Electrophoresis, because the Fibrinogen of Plasma gives a discrete band, which can easily be mistaken for Paraproteins**
- General pattern of electrophoresis result (**Fig. 1**):
  - Shows order of migration along Horizontal Axis with proteins of highest mobility **closest to Anode**,
  - Height of the band along the Vertical Axis shows the protein concentration,
  - Location of some major proteins are indicated underneath their Electrophoretic mobility peaks;

- Electrophoresis can also show gross deficiency or excess of Immunoglobulins and whether Paraproteins are present (**Fig. 1**);
- Quantitative measure of each protein class may be obtained by scanning Electrophoretic strip (**Fig. 2**)

Fig. 1: Electrophoresis of Serum proteins: (a) Normal pattern, (b) Presence of Paraproteins, (c) Presence of Paraproteins

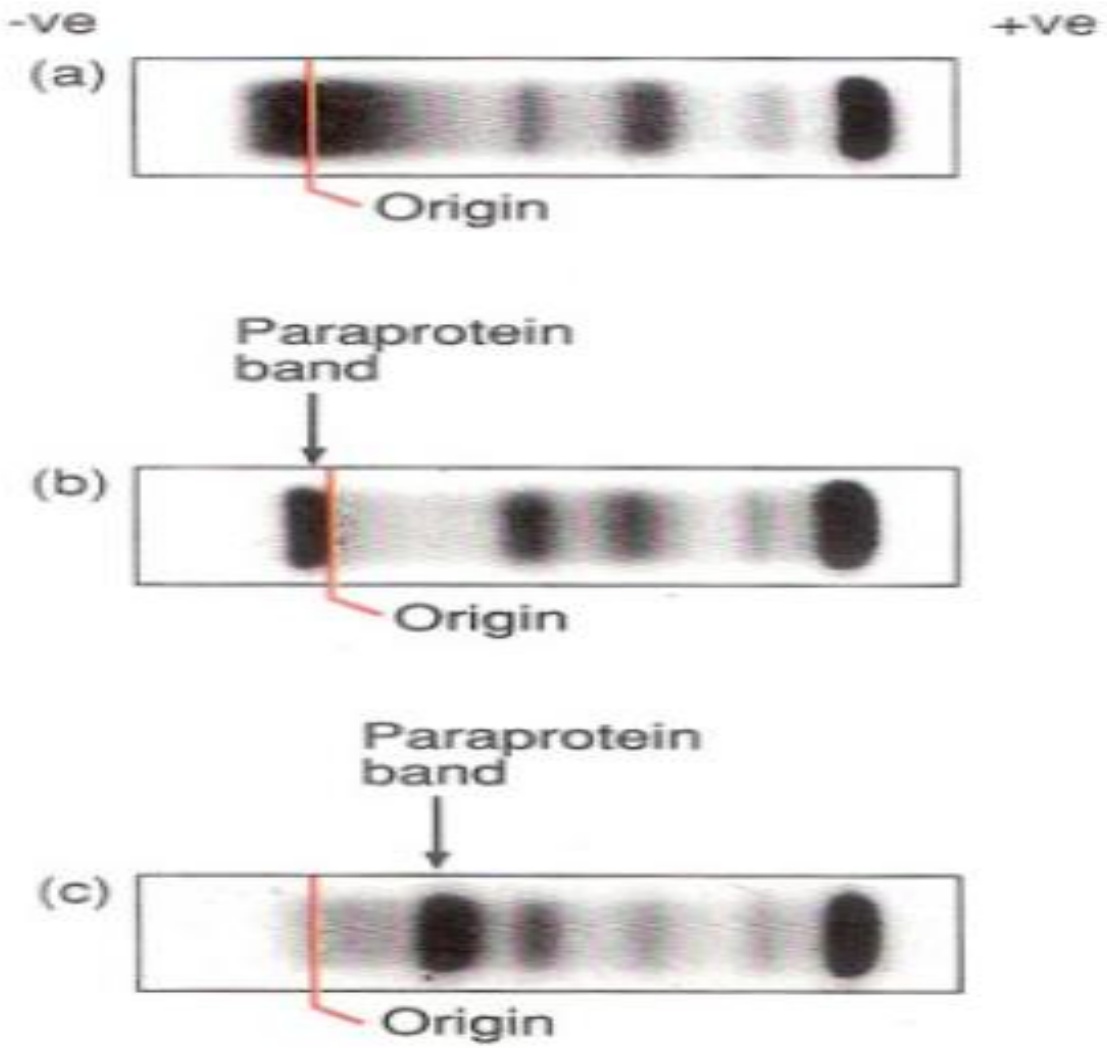


Fig. 2: Scan of an Electrophoretic strip (Gaw et al 1999)

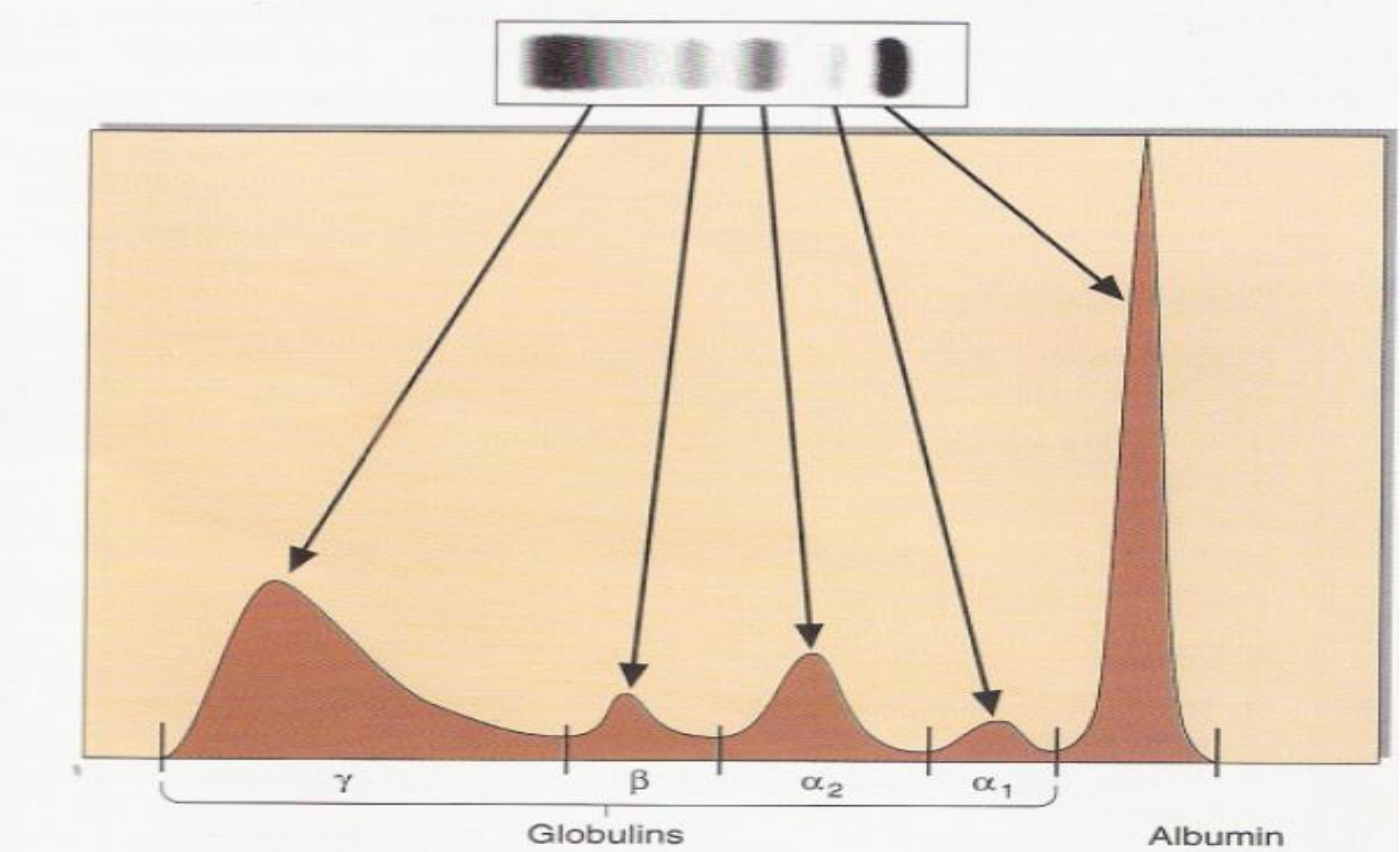


Fig. 3 **Scan of an electrophoresis strip.**

What are Immunoglobulins (Ig)?

- Immunoglobulins are a group of structurally related proteins that function as Antibodies;
- Immunoglobulins are produced by cells of the Lympho-reticular System,
- Immunoglobulins are also produced by Plasma Cells, which are B-lymphocytes transformed after exposure to foreign (occasionally an endogenous) Antigen;

- **What is an Immunogen**?
  - **IMMUNOGEN** is a molecule that can generate an Immune response (cellular or Humoral);
- **What is Antigen**?
  - **ANTIGEN** is a molecule that reacts with Antigen Receptors, irrespective of its ability to generate an Immune Response,
  - Antigen may, or may not be an Immunogen,

What is Hapten?

- **HAPTEN** is a small molecule that is able to react with preformed Antibodies, i.e.,
  - Hapten has Antigenicity, but is not capable, by itself, to stimulate specific Immune Response, i.e., is not Immunogenic,
  - Haptens are only Immunogenic when coupled to a large protein called a carrier;
- **All Immunogens are therefore Antigenic but not all Antigens are Immunogenic,**

What are Epitopes?

- **Epitopes or Antigenic determinants:**
  - Antigen Receptors on Lymphocytes recognize discrete sites on an Antigen called Epitopes or Antigenic Determinants,
  - Antigen recognition by B-cells and T-cells is fundamentally different and does not involve the same Epitopes,

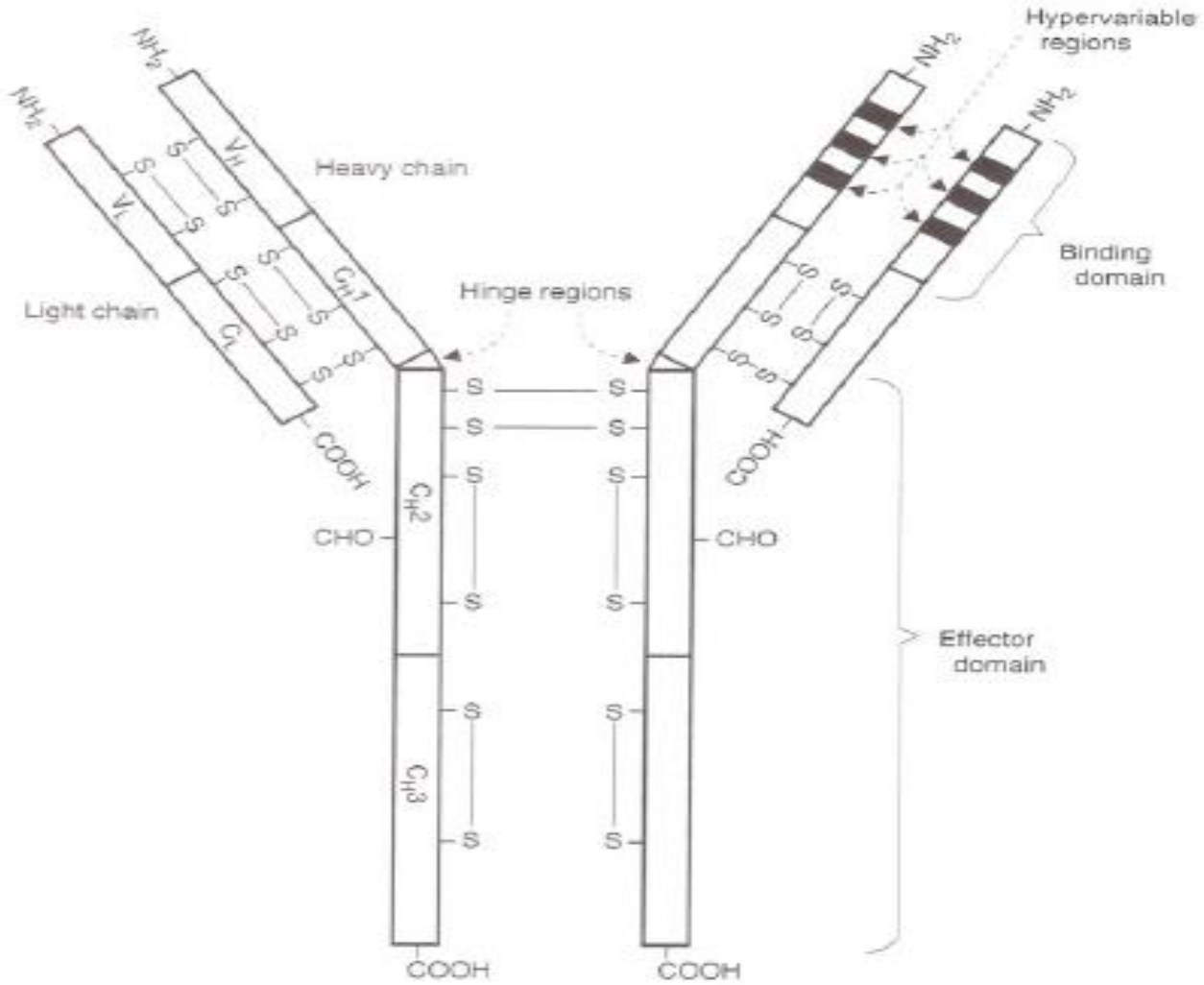
What is the basic structure of Immunoglobulins (Ig)?

- Basic structure of Immunoglobulin (Ig):
  - **2 Identical "Heavy" Polypeptide Chains, and**
  - **2 Identical "Light" Polypeptide Chains;**
  - Both Chains have Inter-chain and Intra-chain Disulfide (S-S) Bonds and Non-covalent Interactions

- Two types of "**Light**" Polypeptide Chains:
  - **Kappa "Light" Chains**
  - **Lambda "Light" Chains**

- Five principal types of **Heavy** Polypeptide Chains:
  - **Alpha,**
  - **Gamma,**
  - **Delta,**
  - **Epsilon,**
  - **Mu**

Fig 3: Schematic diagram of an Immunoglobulin



What are the classes of Immunoglobulins?

- Immunoglobulins are named and classified by their heavy chain type;
- Five types of Heavy chains gives Five Classes of Immunoglobulins:
  - **IgA,**
  - **IgG,**
  - **IgD,**
  - **IgE,**
  - **IgM**

What products are obtained when IgG is hydrolyzed by (a) Papain and (b) Pepsin?

- **Action of Papain on IgG**:
  - Papain a protease enzyme acts on the **Hinge region** (**in front of inter chain S-S bonds**) in IgG,
  - Hydrolysis of IgG by Papain gives 3 components:
    - **Two Identical Fab** (Fragment antigen-binding) fragments,
    - **One Fc** (Fragment-crystalizable) fragment,

- **Action of Pepsin on IgG**:
  - Pepsin a protease enzyme acts on **Hinge region (behind the inter chain S-S bonds**) in IgG,
  - Hydrolysis of IgG by Pepsin produces:
  - **A single divalent F(ab')2** and
  - **A pFc' fragment**

What are the regions in the structure of an Immunoglobulin?

- **V regions: Amino-terminal portions** of Heavy and Light chains show considerable **variability** in Amino Acid composition;
- **Hyper-Variable Regions** or **Complementarity-Determining Regions**: Three areas in V regions of Light and Heavy chains that have remarkably diverse amino acid sequences;
- **C region:** Parts of Heavy and Light chains that are relatively **Constant** in terms of Amino Acid composition;
- **Light chains** contain One Variable Domain (VL) and One Constant Domain (CL);
- **Heavy chains** contain One Variable Domain (VH) and 3 or 4 Constant Domains designated CH 1 – 3 or CH 1 – 4) accordingly

TAKE NOTE

- Ig molecules contain **two functional areas**:
  - **Fab, or Variable end** – is the area that recognizes and binds to Antigens;
  - **Fc end** – is responsible for interaction with other components of Immune system, e.g., Complement and T helper cells;
  - **Hyper-variable Loops** form Antigen-Binding Site of an Immunoglobulin molecule, i.e.,
    - Each Hyper-variable Loop contributes to the Antigenic Specific or Complementarity of the binding site for Antigen;

- Various Classes/Types of Immunoglobulins have different Tertiary structure and Functions;
- **Major Immunoglobulins in plasma are**:
  - **IgG:** neutralizes toxins, activates complement, capable of crossing Feto-placental barrier;
  - **IgA:** contains J chain and secretary component, part of defense against viral and bacterial infections;
  - **IgM:** usually first to be made in immune response, contains J chain, in presence of complement are very effective in producing Lysis of cells;

What is the significance of increase in Ig level in plasma?

- **Ig** may be increased non-specifically in a wide variety of Infections and in Autoimmune diseases
- Increase biosynthesis of Ig may be cause by **several Cell Lines**, each producing specific type of Immunoglobulins (Hyper-Gamma-Globulinemia)
  - Such response is said to be "**Polyclonal**" and results in diffuse increase in protein mass throughout the Gamma Globulin region;
  - Appears as broad band during Electrophoresis of Serum protein;

- Increase biosynthesis of Ig may be cause by a Single Clone of cells making Identical **Ig;**
  - Such is said to be "**Monoclonal**"
  - **Ig** production may increase, becomes large enough to be observed as a single discrete band on electrophoresis of the serum,
  - Such single discrete band may be due to increase in Intact Immunoglobulin or fragments called **Paraproteins**

What are Paraproteins?

- **Paraproteins** (**Monoclonal components**): discrete Ig bands, seen on electrophoresis of Serum,
- Paraproteins are due to production of Single type of **Ig** or **Ig** fragments (Light-chain or Heavy-chain fragments) by a Single clone of B cells,
- Paraproteins may arise from any of the Ig classes
- Detection of Paraprotein in blood or urine requires further investigation to determine if the **Paraproteinemia** is caused by Benign or Malignant condition

- Benign Parpaproteinemia may occur transiently during acute infection and in autoimmune disease due to Antigen stimulation,
- Paraproteins are found in malignant conditions such as:
  - Multiple Myeloma,
  - Macroglobulinemia,
  - Heavy chain diseases, etc.

- Monoclonal Light Chains are produced in excess of Heavy chains in about 50% of cases of Myeloma, and in about 15% of cases only Light chains are found;
  - These **light chains** are small enough to spill into urine, they are known as **Bence-Jones Protein**,
  - Serum electrophoresis may not show the presence of light chains,
  - Urine electrophoresis after concentration may be required to demonstrate the Paraproteins,

IMPORTANT TO NOTE

- Myeloma is characterized by Bony Metastases,
- Bone pain is often the presenting symptom,
- In the face of increasing synthesis of abnormal Immunoglobulins, other bone marrow function is reduced, and there is a decline in Red and White cell and Platelet formation and decreased production of normal Immunoglobulins,
- Anemia and susceptibility to infection are the usual consequences;

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