These are essential compounds comprising 50-70% of the cell’s dry weight. These compounds are present in all of the body cells, fluids, secretions and excretions. Biologically active proteins are macromolecular and range in molecular weight from 6000 daltons for a simple protein i.e. insulin to several million for some structural proteins.

**Basic structure**

- All proteins have a covalently linked amino acid back bone.
- Amino acids are linked with the carboxyl group of one attached to the amino group of another. During which a peptide bond is created and a water molecule removed.
- Amino acids that have the amino group end free (unconjugated) is called N-terminal end, where as the amino acids whose Carboxyl group end is free is called the C-terminal end.
- Two amino acids joined together form a dipeptide bond where as three amino acids joined together form a tri-peptide bond and four joined together form a tetra-peptide bond. Several joined together will form a polypeptide chain.
There are four aspects of protein structures that relate to its shape:

**Primary structures** constitute of *covalent bonding only*. This structure is crucial for the function and molecular characteristics of the protein e.g. when amino acid Valine is substituted by Glutamic acid at position 6 of β-chain of hemoglobin A, hemoglobin S is formed which results in Sickle cell anemia.

**Secondary structures** is the winding of the polypeptide chain allowing *α-helix structures*. *Hydrogen-bonds occur* between the amino group (N-terminal) and carboxyl (COOH) groups within the same chain or between different chains within the same molecule.

**Tertiary structure** is the overlapping and folding of the already twisted chain to form a 3D structure. *Di-sulfide bonds, electro-static attractions, hydrogen bonds, hydrophobic interactions and Van der waals forces occur* between the R-groups. These structures are responsible for physical and chemical properties of the proteins.

**Quaternary structure** is the arrangement of *two or more polypeptide chains to form a functional protein*. Such as hemoglobin that has four globin chains, lactate dehydrogenase has five polypeptide chains and creatine kinase that has two polypeptide chains joined together. These chains arrange themselves in a complex manner to allow the formation of quaternary structured and functional protein. Where as *albumin, which only has a single polypeptide chain, will not form a quaternary structure and therefore is termed a simple but functional protein*. 
**General characteristics**

**Nitrogen content:**
Differentiates proteins from pure carbohydrates and lipids. Proteins are composed of C, O, H, **N** and S whereas others don’t contain nitrogen.

**Charge and iso-electric point:**
Because of the amino acid content proteins can carry a net –ve or +ve charge. This property of proteins is called *Amphoteric*.

**Immunogenicity:**
Most plasma proteins are effective antigens. When injected into another species, these antigens allow the formation of specific antibodies. New research allows the extraction of these antibodies to be used for specific *in-vitro* antigen-antibody binding assays (ELISA..etc).
**Classification**

**Simple proteins:**
Contain only polypeptide chain and yield amino acid upon hydrolysis. These may be **Globular** e.g. albumin or **Fibrous** in shape e.g. collagen and keratin.

**Conjugated proteins:**
These are composed of globular proteins plus a non-protein moiety that could be a lipid, a carbohydrate, porphyrins, or a metal such as metalloproteins; a group that consists of ferritin conjugated with iron or ceruloplasmin that's conjugated with copper. Also Lipoproteins have lipids conjugated to them e.g. cholesterol and triglycerides.
General functions of total proteins

- Inflammatory response and infection control
- Transport
- Colloid osmotic effect
- Building of body tissues and muscle mass
- Cellular structure building and repair
- Act as buffer to maintain pH
- Biological catalysts (enzymes)
- Hormone releasing factors
- Growth factors
- Cellular receptors and intracellular binding proteins (G-Proteins)
- Structural proteins
- Antigens
- Coagulation factors
These consist mainly of plasma / serum proteins and to a lesser extent urinary proteins. Concentrations of major constituents such as albumin and immunoglobulins must be monitored because changes in these protein fractions will most likely alter total protein concentrations significantly.

**Hyper-proteinemia:**
Marked increase in plasma proteins is observed in following pathological conditions:
Gain in protein concentration due to dehydration (water loss) e.g. vomiting, diarrhea, diabetic acidosis, hypoaldosteronism.
A significant increase in one or more of the immunoglobulins.

Slight to marked hyper-proteinemia may be observed in following non-pathological conditions:
- Increased protein due to excessive stasis during venepuncture.
- Use of wrong anti-coagulant
- Hemolysis
- Use of wrong gauge needle during sample collection
- Freezing & thawing of sample
**Hypo-proteinemia:**
low plasma protein concentrations may be due to following pathological reasons:
Hypoalbuminemia
Profound immunoglobulin deficiency.

Noticeable hypo-proteinemia may also be observed in non-pathological conditions such as:
Dilution, e.g if blood is obtained near the site of intravenous infusion.

**Metabolism of proteins**
*Catabolism or loss of protein:* most plasma proteins are catabolized by the process of pinocytosis into capillary endothelial cells or mononuclear phagocytes. Small proteins are lost passively through the renal glomeruli and intestinal wall. Some are reabsorbed either directly by renal tubular cells or after digestion in the intestinal lumen; some are catabolised by renal tubular cells.
**Anabolism or gain of protein:**
Many plasma proteins are synthesized in the liver and secreted in circulation by hepatocytes. However proteins of the complement system are synthesized by both these cells plus macrophages; whereas immunoglobulins are derived mainly from B cells of the immune system.

**Nitrogen balance:**
It can simple be defined as the balance between anabolism and catabolism of proteins (gain vs. loss of body nitrogen content).
Catabolism > Anabolism = overall nitrogen balance is negative because the rate of nitrogen excretion is greater than the rate of ingestion e.g. as seen in burns, vomiting, diarrhea, hyperalbuminemia.
Anabolism > Catabolism = overall nitrogen balance is positive because the rate of nitrogen ingestion is greater than the rate of excretion e.g. as seen in pregnancy, growth tissue repair, body building, high intake of proteins and amino acids.
Methods of analyzing total serum / plasma proteins

Blood sample collection criteria

• Fresh serum or plasma is the preferred sample for plasma protein determination. If analysis is delayed then freeze sample immediately until the day of assay.

Avoid the following:

• Gross hemolysis of blood sample, ictremia, lipidemia and high creatinine.
• Prolonged application of the tourniquet
• Repeated freezing and thawing of specimen
• Vigorous shaking of the samples allows denaturing of proteins.
• Patient posture while drawing the sample must be noted, the individual should be recumbent, ambulant individuals will cause the water to shift to the extra-vascular space hence producing falsely elevated protein levels in blood.

N.B. Sample used for plasma protein analysis in medical labs more often is serum rather than plasma. Patient need not be fasting; a random sample is good enough.
<table>
<thead>
<tr>
<th>METHOD</th>
<th>PRINCIPLE</th>
<th>COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjeldahl</td>
<td>Digestion of protein; measurement of nitrogen content.</td>
<td>Reference method</td>
</tr>
<tr>
<td>Refractometry</td>
<td>Measurement of refractive index due to solutes in serum.</td>
<td>Rapid and simple; assume non protein solids are present in same concentration as in the calibrating serum</td>
</tr>
<tr>
<td>Biuret</td>
<td>Formation of violet-colored chelate between Cu²⁺ ions and peptide bonds.</td>
<td>Routine method; requires at least two peptide bonds and an alkaline medium</td>
</tr>
<tr>
<td>Dye-binding</td>
<td>Protein binds to dye and causes a spectral shift in the absorbance maximum of the dye</td>
<td>Re-search use only</td>
</tr>
</tbody>
</table>
Proteins are also measured in Spinal fluid by using a turbidimetric procedure involving reaction with Trichloroacetic acid (TCA).

**Electrophoresis:**

- Electrophoresis alone provides *qualitative* analysis of proteins, which are separated according to their net electric charges.
- A strip of cellulose acetate or agarose can be used. Sample is applied and a controlled current is passed for a fixed time.
- A normal control serum sample is also fractionated and the five main groups of proteins could be distinguished after staining and may even be compared visually.
- This electrophoresis can also be *quantified* using densitometry. A technique used to measure the density of each band on the gel and provide a value for each of the five main protein fractions.
Agarose gel electrophoresis depends upon consistent current flow for a specific time period, this allows proteins to migrate towards anode

Stained protein gel shows various fractions. The main fractions being albumin, alpha 1, alpha 2, beta and gamma. Although several interzones exist but most important ones migrate in the main five regions.
A densitometry (quantitative) interpretation of total serum protein electrophoresis
**Serum / Plasma proteins**
Over 500 plasma proteins have been identified however the functionally important ones are divided into *five major fractions* according to their electrophoretic mobility (as shown in the densitometric graph above). Each fraction is further divided into sub-fractions according to their location within the electrophoretic band and their subsequent electrophoresis patterns.

<table>
<thead>
<tr>
<th>Protein type</th>
<th>% in blood</th>
<th>g/l blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td>60-84</td>
</tr>
<tr>
<td>1</td>
<td>Albumin</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Globulins</td>
<td>alpha 1</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>alpha 2</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>beta</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>gamma</td>
</tr>
<tr>
<td>Main Protein Fractions in serum/plasma</td>
<td>sub-fractions found in blood</td>
<td>COMMENTS</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>0 Pre-Albumin</td>
<td></td>
<td>Not stained in electrophoresis &amp; doesn’t appear upon densitometry</td>
</tr>
<tr>
<td>1 Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Alpha-1 globulins</td>
<td>$\alpha_1$-anti-trypsin</td>
<td>(detectable only during pregnancy)</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-feto protein</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\alpha_1$-acid glycoprotein</td>
<td></td>
</tr>
<tr>
<td>3 Alpha-2 globulins</td>
<td>Hepatoglobins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\alpha_2$-macroglobulin</td>
<td></td>
</tr>
<tr>
<td>4 Beta-globulins</td>
<td>Transferrin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Haemopexin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Complement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C- reactive proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myosin</td>
<td></td>
</tr>
<tr>
<td>5 Gamma globulins</td>
<td>IgA, IgD, IgE, IgG, IgM</td>
<td>Immunoglobulins</td>
</tr>
</tbody>
</table>
Pre-Albumin

• Migrates just ahead of albumin in serum electrophoresis but it's not stained and therefore is not a visible as a separate fraction.
• Separated distinctively by *immunoelectrophoresis*
• Rich in tryptophan and contains 0.5% carbohydrate groups

**Function:**
• Binds with T3 and T4 to assist their transport
• High binding affinity to retinol (vitamin-A). It form a complex that transports retinol (vitamin A)

**Pathology:**
• Decreased in hepatic damage, burns, salicylate ingestion, tissue necrosis, malnutrition
• Increased in some cases of nephrotic syndrome
Albumin

- Highest concentration in serum (~60%)
- Albumin levels change with age; 
  at birth ~39g/l decreasing at 9 months to ~28.4g/l and then returning in adults to levels 35-55g/l.
- Electrophoresis pattern is significant, as albumin is the major component of the total protein.

Function:
- Contributes in maintaining colloidal osmotic pressure of the intravascular fluid
- It contributes to the buffering activity in the blood maintaining at ~7.35
- Binds and transports generally insoluble substances in the blood e.g. bilirubin, salicylic acid, fatty acids, cortisol and other drugs.
- Binds and transports some metals such as Calcium and Magnesium
Pathology:

a)- Total absence can lead to very low concentrations of 0.4g/l, which occurs due to:
   • autosomal recessive genetic disorder
   • analbuminaemia (no albumin)
   • bisalbuminaemia (presence of unusual, non-functional albumin demonstrated by the presence of two bands instead of one in electrophoresis).

b)- Pathologically *hypoalbuminaemia* occurs in the following situation;
   • malnutrition
   • muscle wasting disease
   • liver cirrhosis
   • gastrointestinal loss (prolonged diarrhea & vomiting)
   • malabsorption
   • nephrotic syndrome
Alpha-1 Globulins

$\alpha_1$-anti-trypsin

- These are major component of proteins migrating immediately after albumin.
- It’s also an *acute phase reactant* protein

**Function:**
- Neutralizes enzymes such as elastase which causes hydrolytic damage to structural proteins

**Identification and quantification:**
Electrophoresis is important followed by one of the following methods:
- I)- radial immunodiffusion (widely used)
- II)- immunonephelometric assay (by automated instruments)
- III)- immunofixation (for phenotyping)
Pathology:

Deficiency or lack of functional $\alpha_1$-antitrypsin may be due to:

- Genetic disorder which results in severe degenerative pulmonary disease due to unchecked proteolytic activity of proteases from leukocytes in the lung during periods of inflammation. This type of genetic defect usually leads to reduction in functional alph-1 antitrypsin. Genotypes of $\alpha_1$-antitrypsin deficiency have been identified. The common genotype found in normal individual is $MM$, a homozygous genotype $ZZ$ have severe deficiency and suffer from serious liver and lung disease. Individuals with genotypes $MZ$ or $MS$ are defined as carriers and are usually not affected however they should be cautioned of having $ZZ$ offspring.

- Juvenile hepatic cirrhosis where the protein is synthesized but not released by the hepatocytes due to cirrhosis

Increased $\alpha_1$-antitrypsin levels are observed in:

- Inflammation
- Pregnancy
- Contraceptive use
**α₁-Fetoprotein (AFP)**

- Synthesized in the fetus by the yolk sac and then by the parenchymal cells of the fetus liver. Has a very crucial role in assessing normal pregnancy in high risk women.
- In normal pregnancy, it peaks at 13 weeks gestation and recedes at 34 weeks then at birth it goes to adult levels (negligible amount).
Identification and quantification
Haemagglutination, Radial immunodiffusion and ELISA

Function:
• The exact function is not very clear but it’s quite obvious, it has something to do with protecting the fetus from immunolytic attack from mother’s antibodies.

Pathology:
• AFP screening of maternal blood for proper assessment of any fetal condition or abnormality is carried out by determining AFP and it’s particularly important indicator for such fetal abnormality in high risk women with previous history of abortions or of producing abnormal children. However following points must be taken into consideration before determining AFP, otherwise AFP values will be of NO clinical significance:

  Prime time of screening AFP is between 15 and 20 weeks of pregnancy
  Accurate dating of pregnancy is important for accurate interpretation
• Other factors affecting AFP in pregnant women must be taken into consideration such as:

**Weight of the mother** (higher weight women > than 100kgs have higher AFPs during pregnancy compared to light weight women)

**Race of the mother** (dark skinned women have 10% higher AFP during pregnancy as compared to white Caucasian women)

**Diabetic women** (have lower AFP values during pregnancy)

**Presence of twins** (significantly increased AFP due to two fetuses will be observed) but this *increase is absolutely normal*.
Pathologically increased AFP in maternal blood would be observed in following fetal abnormalities:

- Fetal distress
- Spinal bifida
- Neural tube defect
- Hemolytic disease of the new born
- Ataxia-Telangiectasia

Pathologically decreased AFP in maternal blood would be observed in following fetal abnormality:

- 3-4 fold decrease in maternal AFP during 15 and 20 weeks will be observed in case of risk for Downs’ Syndrome

In adults AFP is non-existent, however increased AFP in non-pregnant adult females or males would be an indicator for underlying tumor i.e. it acts as a tumor marker for:

- Hepatocellular carcinomas (80% of the cases)
- Gonadal tumors
**Alpha-1 acid glycoprotein (orosomucoid)**

- It contains 5-carbohydrate units attached to a polypeptide chain
- It has low pI (2.7) and therefore it remains negatively charged even in acidic media, a property that eventually gave rise to its name.

**Function:**
- Formation of certain membranes and fibers in association with collagen
- Highest levels found in membranes of platelets

**Identifications and quantifications**
- Radial immunodiffusion
- Immunofixation

**Pathology**
- Increased levels are seen in cancer and other malignant conditions associated with cell proliferation. Also increased levels are seen in pneumonia and inflammatory disorders such as Rheumatoid arthritis.
Alpha-2 Globulins

Hepatoglobins

• Mainly synthesized in the liver however lesser quantities are synthesized by RES.
• Hepatoglobins are composed of two types of polypeptide chains: $2\alpha$ and $1\beta$ chain.
• At DNA level, due to polymorphic differences in $\alpha$-chains, three types of genotype patterns are seen by RFLP on agarose gel electrophoresis:
  HpT 1/1 (frequent homozygous $\alpha$-chain)......most common genotype
  HpT 1/2 (heterozygous $\alpha$-chain)...............less common genotype
  HpT 2/2 (rare homozygous $\alpha$-chain) ..........extremely rare genotype

• Identification and quantification;
  Radial immunodiffusion or
  Rocket electrophoresis.

٦٢
Agarose gel showing the three hepatoglobin genotypes for alpha chain. DNA is amplified by PCR using primers for the polymorphism. RFLP is used to cut the DNA in the following pattern. The gel is stained by ethedium bromide and seen under UV light using trans-illuminator.

<table>
<thead>
<tr>
<th>Lanes</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells for sample application</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A= DNA marker. Commercially available with known sizes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B= HpT 1/1 frequent homozygous genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C= HpT 1/2 Heterozygous genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D= HpT 2/2 rare homozygous genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E= Negative control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• Levels and concentrations show variations with age: **0.02 g/L at birth increasing to adult levels (0.8-2.7 g/L) within one year.** Highest levels are seen in males after the age of 60 yrs of age.

**Function:**
• Binding of free hemoglobin by the α-chain after the RBC destruction. The hepatoglobin-hemoglobin complex is removed from circulation by the reticuloendothelial system with in minutes of formation. *It prevents loss of hemoglobin and iron into urine.*
• Hepatoglobins are *increased* significantly in severe burns, inflammation and Nephrotic syndrome
• The protein is substantially *decreased* in hemolytic disease, transfusion reaction and any injury that causes mechanical break down of RBCs.
**Ceruloplasmin**

- It helps catalyze several enzymatic reactions in the form of Copper oxidase, Histaminase, Ferrous oxidase.
- *Major binding and transporting protein for Copper (90% or more of copper)*
- Levels and concentrations show variations with age and sex with low levels at birth that gradually increase with age.
  
  *Adult females have higher values than males.*

**Identification and quantification:**
- Radial immunodiffusion
- ELISA

**Pathology:**
- Increased levels are seen in inflammation, pregnancy, malignancy and while using oral contraceptives.
- Decreased in; **WILSON'S DISEASE** (copper deposition in the body), Menke's Kinky hair syndrome (genetic defect) that's due to decreased copper absorption or malnutrition.
α-2 macroglobulin

- It's a *dimeric large protein* and is mainly found in the intravascular spaces however much lower concentrations are found in CSF.

**Identification and quantification**

Radial immunodiffusion

- Levels and concentrations show variations with age and sex; where it's low at birth and peaks at age 2-4 yrs. Levels decrease to 1/3 concentrations by the age of 45 yrs with moderate increase in older age.
• Changes in concentrations are more evident in males.
• Adult females have higher levels of blood $\alpha$-2 macroglobulins as compared to males.

**Function:**
- Binds to hormones such as Insulin
- Inhibits proteases such as elastase, pepsin, plasmin

Due to their large size there is retention in the blood in case of Nephrotic Syndrome and the concentrations are 10x higher.
<table>
<thead>
<tr>
<th></th>
<th>Main Protein Fractions in serum/plasma</th>
<th>sub-fractions found in blood</th>
<th>COMMENTS</th>
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<tbody>
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<td>0</td>
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<td></td>
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<td>4</td>
<td>Beta-globulins</td>
<td>Transferrin Haemopexin Complement Fibrinogen C- reactive proteins Myosin</td>
<td></td>
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<tr>
<td>5</td>
<td>Gamma globulins</td>
<td>IgA, IgD, IgE, IgG, IgM</td>
<td>Immunoglobulins</td>
</tr>
</tbody>
</table>
Beta-globulins:

Transferrin

- *Major protein of the β-globulin fraction*
- Identification and quantification may be carried out Radial immuno-diffusion or ELISA

Function;
- Mainly binds to Iron and acts as a transporting vehicle for Iron.
- Prevents loss of Iron
- Prevents free Iron from depositing in the tissues
- Bacterio-static
- Transport Iron to its storage sites such as Liver and RES where it's incorporated into another protein i.e. Apoferritin and then Ferritin
- Transport Iron to bone marrow for the synthesis of hemoglobin and Iron containing compounds

Pathology:
- Normal to increased levels are seen in Iron deficiency anemia (hypochromic, microcytic).
- Decreased transferrin however could be due to; Reduced synthesis by reticulocytes or Hereditary transferring deficiency
Haemopexin

Synthesized by parenchymal cells of the liver and migrates in the $\beta$-globulin region

Identification and quantification:

- Radial immuno-diffusion
- Rocket electrophoresis

Function:

- Binding of heam (1:1 ratio) from the break down of hemoglobin, myoglobin and catalase
- Transporting Haem to the liver
- Binding of porphyrins
- Initially Haemopexin levels are low however they reach normal levels by the age of one.
- Hemopexin is however increased in inflammation, pregnancy, diabetes mellitus and muscle wasting disease such as Duchenne muscular dystrophy.
Complement

A collective name for a group of proteins that participate in sequential manner in case of a immune reaction. Normally they circulate in the blood as non-functional precursors. Increased levels are detected during inflammation and less than normal levels are detected during malnutrition, SLE, disseminated intravascular coagulopathies.

Function:
- Serve as a link to immune response
- Activation occurs when the first complement C1q binds to the antibody-antigen complex
- Each complement protein is then activated sequentially (C2 – C9)
- Bind to the foreign cell membrane
- Causes cell–lysis
- Allows the participation of humoral and cellular effector system in the process of inflammation
- Participate in the alternative pathway (properdine pathway) without the need for antigen-antibody binding

Identification and quantification:
- Titer measurement of complement using complement activity in a hemolytic system
**Fibrinogen**

- Present in plasma and *not serum*
- Migrates as a distinct band between beta & gamma bands of the plasma electrophoresis
- Immunoassays can be used to determine exact measurement of fibrinogen

**Function:**
- Formation of fibrin clot when activated by thrombin

Increased during pregnancy and inflammation
Decreased levels are found in extensive coagulation (post surgical trauma or heavy blood loss).

**C-Reactive protein**

- Normal individuals have very low levels because it’s synthesis increases during inflammation.
- Its function is recognition and binding to molecular toxin groups found on variety of bacteria and fungi.
- Also promotes the binding of complement proteins and promotes phagocytosis
**Myosin**

- Myosin is a large protein found in all types of muscle mass as it forms a thick filament
- It consists of six polypeptide chains: 2 heavy and 4 light chains

**Function:**
- ATPase activity for energy from muscle contraction
- Binds actin
- Pathologically light chains are detectable within 30 min of initial myocardial infarction
  
  Therefore its detection confirms ischemic heart disease or acute angina. The quantity is directly proportional to the amount of cardiac damage.
<table>
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</tr>
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<td>IgA, IgD, IgE, IgG, IgM</td>
<td>Immunoglobulins</td>
</tr>
</tbody>
</table>

[^1]
Gamma- Globulins (Immunoglobulins)

- Immunoglobulins are produced by B lymphocytes (B cells) and they are from the progeny of plasma cells.
- Migrate primarily in the gamma region and show antibody activity.
- Represent the ‘humoral’ component of the immune system.
- Bind to specific foreign substances (antigens) that elicit their synthesis.
- There are five major types: IgA, IgD, IgE, IgG, IgM

CHEMICAL STRUCTURE OF IMMUNOGLOBULINS
- The basic structural unit of immunoglobulins consists of four polypeptide chains held together by disulfide bonds.
- Two of these peptide chains are called “light chains,” kappa (κ) and lambda (λ) type, present normally in a ratio of 2:1, respectively.
- The other two peptide chains components of immunoglobulin are called “heaven chain;” these are unique for each immunoglobulin class.
• The basic immunoglobulin is a Y-shaped molecule and using papain as a proteolytic enzyme, the basic immunoglobulin structure is broken into three subunits:
  1. One Fc fragment of the heavy chain to the “hinge” and
  2. Two Fab subunits consisting of a light-chain portion and heavy-chain portion of the “hinge”.
  3. The Fd fragment consists of only the heavy-chain component of Fab moiety.
• Fab fragments are variable in amino acid make up and determine the ability of the immunoglobulin to bind specifically to antigens.
• The amino acid make up of the Fe portion is fixed for each immunoglobulin class and binds complement.
Some Important Immunoglobulino-pathies

MULTIPLE MYELOMA
Most myelomas involve the excessive production of intact immunoglobulin (Ig) molecules along with increased production of peptide fragments of Igs. In light-chain disease, however, more light-chain fragments of Igs are produced, filtered and released in the urine (owing to their low molecular weight). These light chains are detected in the urine as Bence Jones proteins.
Franklin’s disease (or heavy-chain disease) is a myeloma involving the overproduction and release of heavy-chain fragments.
Most myelomas are of IgG and IgA types, and Bence Jones protein is detected in the urine in 60% of all myelomas. Multiple myeloma incidence increases with age and is often associated with anemia, plasmacytosis with replacement of bone marrow with 10% plasma cells.
WALDENSTROM’S MACROGLOBULINEMIA

Proliferating cells resembling B-lymphocytes (rather than plasma cells) are seen in this disease, where complete IgM molecules as well as fragments thereof are found in the serum.

Increased viscosity reduces circulation and promotes thrombosis. Bence Jones protein is detected in only 10 to 20% of patients with Waldenstrom’s macroglobulinemia.

*Symptoms:* bleeding or cerebral ischemia, cold intolerance, hyperviscosity of serum, and heart failure are seen in these patients; treatment often includes plasmapheresis.

AMYLOID DISEASE

Amyloid is a proteaceous substance deposited throughout the blood vessels and organs. Organs particularly susceptible to amyloid deposits include the liver, kidney, spleen, and adrenal glands. Amyloid is identical to the variable portion of light chains and is possibly produced by plasma cells. Amyloidosis may exist as a primary disease or secondary to another disease involving prolonged stimulation of the immune system (i.e., rheumatoid arthritis, syphilis, etc.). Amyloid is identified on tissue slides by staining with Congo Red.
CRYOglobulinemia are formed due to polymerization of immunoglobulins. These abnormal globulins precipitate when a serum (or plasma) sample is cooled, then redissolved when the specimen is warmed. Cryoglobulins are rather common in adults beyond 60 years of age. Cryoglobulins are often due to polymers of IgM (most common), IgG, or IgA and hence are frequently seen in myeloma and macroglobulinemia. Cryoglobulins may also be pryclonal and are seen in rheumatoid arthritis, systemic lupus erythematosus (SLE), and other autoimmune diseases. Mixed cryoglobulin complexes deposit in vessel walls, fix complement with the resulting follows. Monoclonal cryoglobulin is more commonly associated with raynaud’s phenomenon or cascular purpura.

**ASSOCIATED RENAL DISEASE**

Increased production of abnormal proteins greatly affects the kidneys due to either (1) the deposition of amyloid in amyloidosis; (2) the vasculitis of cryoglobulinemia; or (3) the formation of urinary casts (due to Bence Jones protein), which block renal tubules.
In the assay of total protein determination, useful diagnostic information can be obtained determining the albumin fraction and the globulins, since these two fractions can cause total hyper or hypo proteinemia.

**Total protein – Albumin = Globulin fraction**

**Albumin / Globulin = A/G ratio**

Ratios are useful to determine the over all state of the disease rather than absolute values determined in individual assay.

Normal levels of A/G ratio range between 1.5 – 2.5. A significant change in the ratio of albumin and total globulin was first noticed in diseases of kidney and liver.

Decreased ratio is also seen in cases of malnutrition, burns, diarrhea, lymphomas, myeloma, and granulomatous disease.

*However total protein determination followed by electrophoresis has generally replaced A/G ratio as a diagnostic tool.*
In a Reference electrophoresis (densitometric) pattern:

- 62% albumin fraction
- 4% alpha 1-globulin
- 7.4% alpha 2-globulin
- 8.6% beta globulin
- 18% gamma globulin

Some Abnormal electrophoretic patterns

Monoclonal immunoglobulin disease
Protein electrophoresis is extremely useful
“M” spike is very distinct
Increase in immunoglobulin fraction is compensated by decrease in Albumin
Further investigation for immunoglobulins and clinical significance should be carried out

α1 – antitrypsin deficiency
A complete absence of alpha-1 globulin fraction is observed
This type of pattern is obvious in patients suffering from severe degenerative pulmonary disease with ZZ genotype.
**Nephrotic syndrome**
There is significant increase in $\alpha_2$ macroglobulin and $\beta$-lipoprotein fraction such as complement components and haptoglobin.
These events lead to a marked decrease in albumin fraction and an increase in $\alpha_2$ globulin and $\beta$-globulin bands.

**Inflammation**
There is increase in $\alpha_1$-globulin, $\alpha_2$ globulin and $\beta$-globulin fractions.
This type of pattern is also called acute-phase reactant pattern and is usually observed in burns, trauma, infarction, malignancy, and liver disease.

**Liver cirrhosis**
There is decrease in serum albumin levels and an obvious increase in $\gamma$ globulin fraction.
These patterns are very characteristic because of some fast migrating $\gamma$-fractions that prevent resolution of the $\beta - \gamma$ bands causing diffusion of the two bands called the $\beta - \gamma$ bridge of liver cirrhosis.