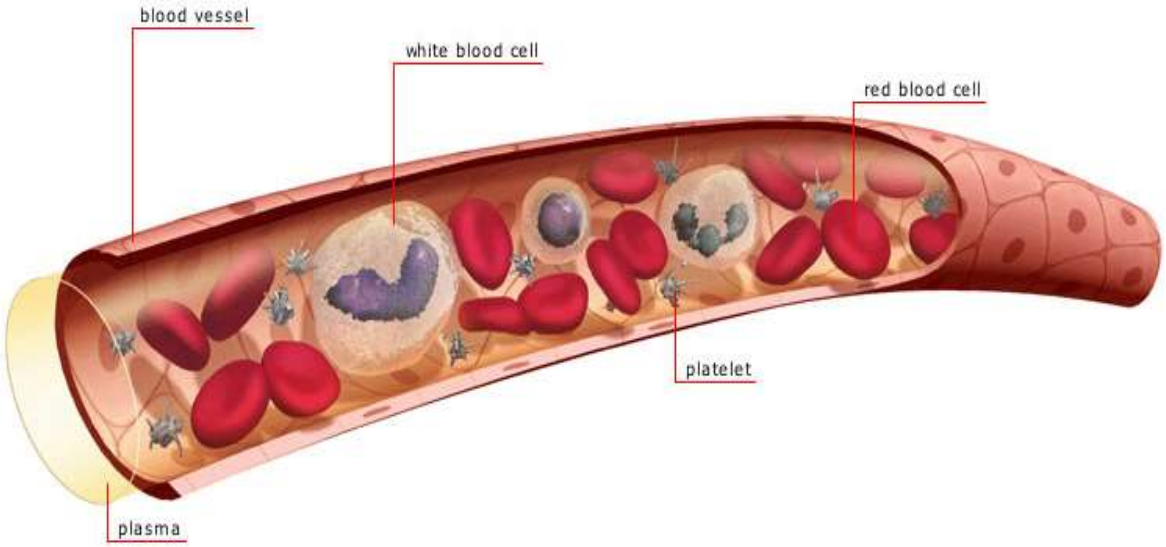
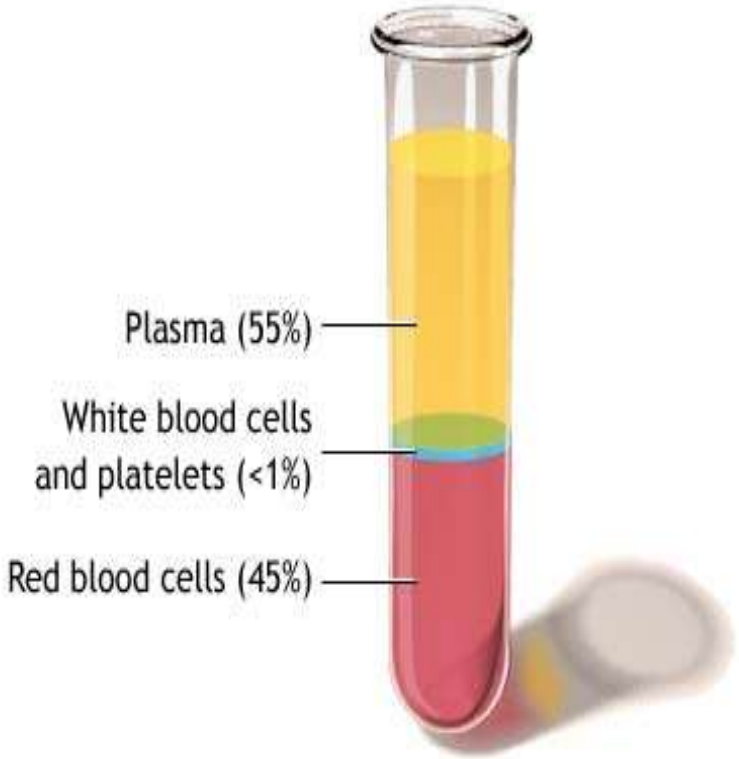
Plasma Proteins

Dr Aparna Chaudhari



Plasma Proteins



Blood - Functions

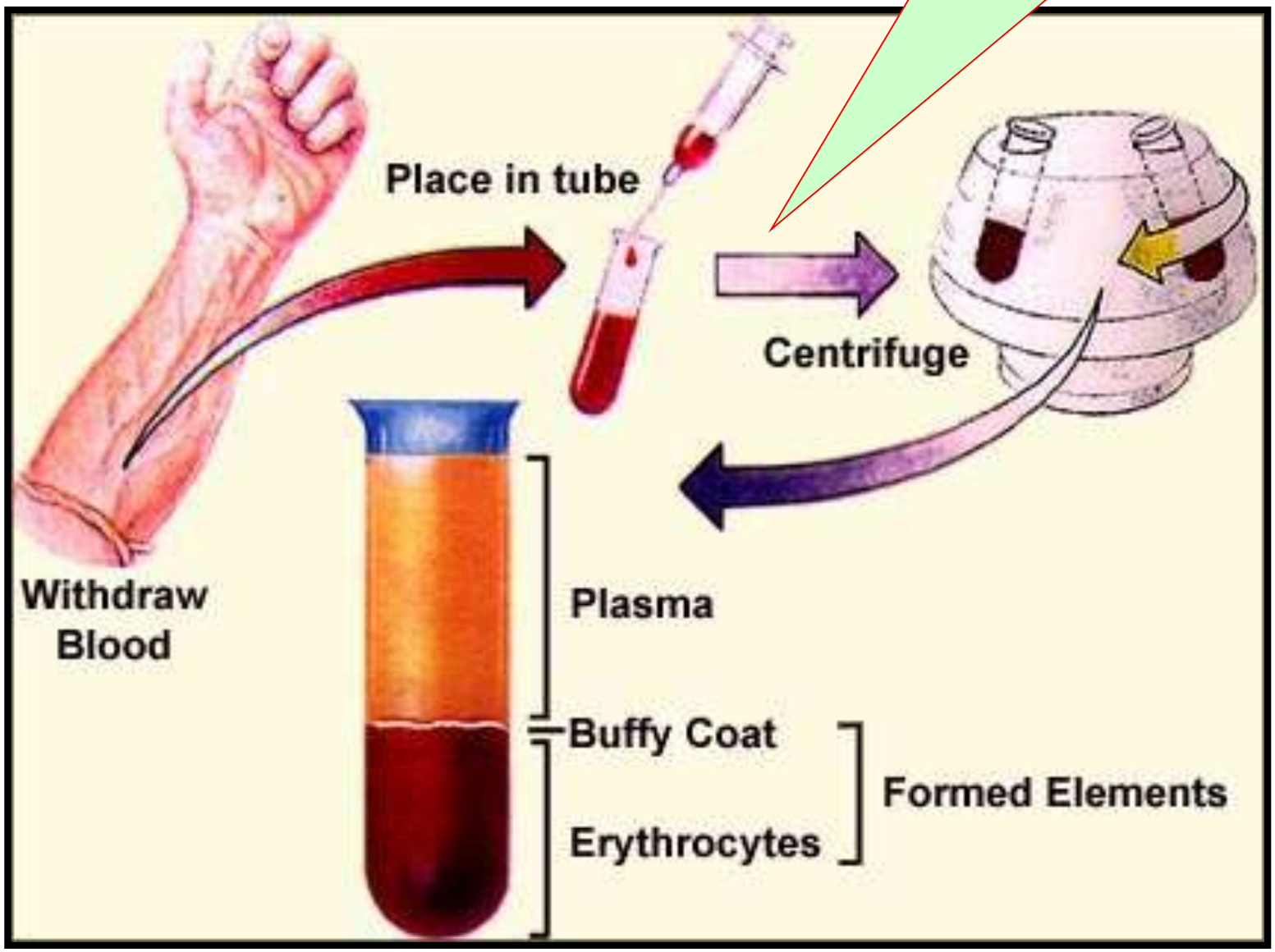
- **Respiratory**
  - Transport O_2 from lungs to tissues
  - Transport CO_2 from tissues to lungs
- **Nutrition**
  - Transports "food" from gut to tissues (cells)
- **Excretory**
  - Transport waste from tissues to kidney (urea, uric acid, water)

- **Regulatory**
  - **Water Content of Tissues**
  - **Water exchanged through vessel walls to tissue**
- **Body Temperature**
- **Protective**
  - **Antibodies, antitoxins, white blood cells (WBC)**
- **Acid-base balance**
  - **pH 7.35~7.45, $NaHCO_3/H_2CO_3$**
- **Coagulation**

- **Blood composition**
  - **70 mL/kg of body weight**
  - **5 L (average) in an adult**
  - **Suspension of *cells* in a carrier fluid (*plasma*)**
    - **Cells - 45% by volume**
    - **Plasma - 55% by volume**
- **Cells**
  - **Red cells (erythrocytes):**
    - **$5x10^6/\mu L$**
  - **White cells (leukocytes)**
    - **$7x10^3/\mu L$**
  - **Platelets (thrombocytes)**
    - **$3x10^5/\mu L$**

Centrifuged Blood Sample

Add anticoagulants (heparin, potassium oxalate)

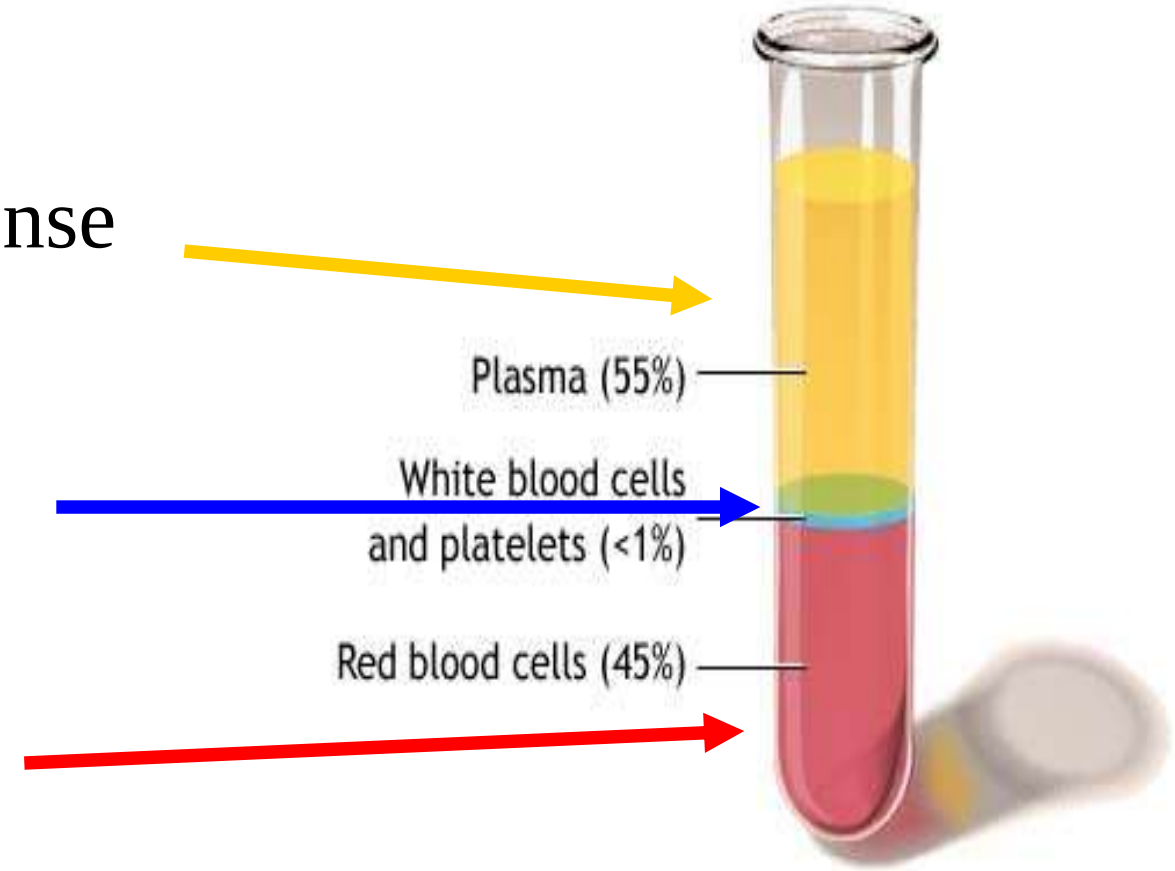


Separation of Components

Plasma = Less Dense

Platelets / WBCs

RBCs
More Dense



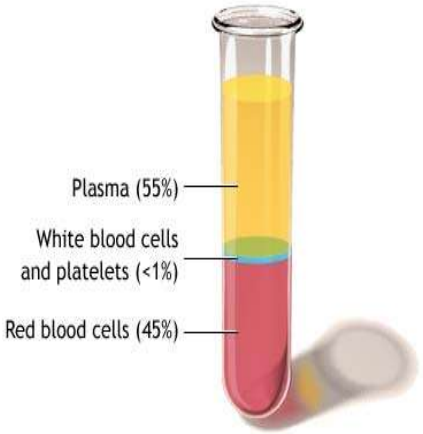
Plasma vs serum

Plasma is the liquid, cell-free part of blood, that has been treated with anti-coagulants.

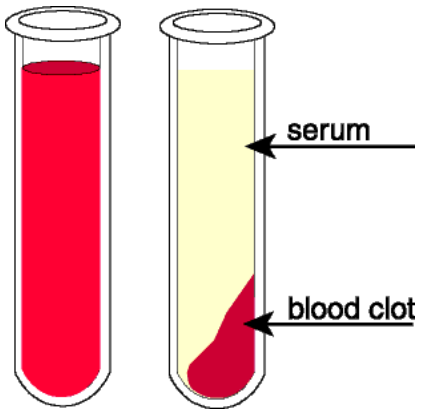
Serum is the liquid part of blood AFTER coagulation, therfore devoid of clotting factors as fibrinogen.

Anticoagulated

Clotted



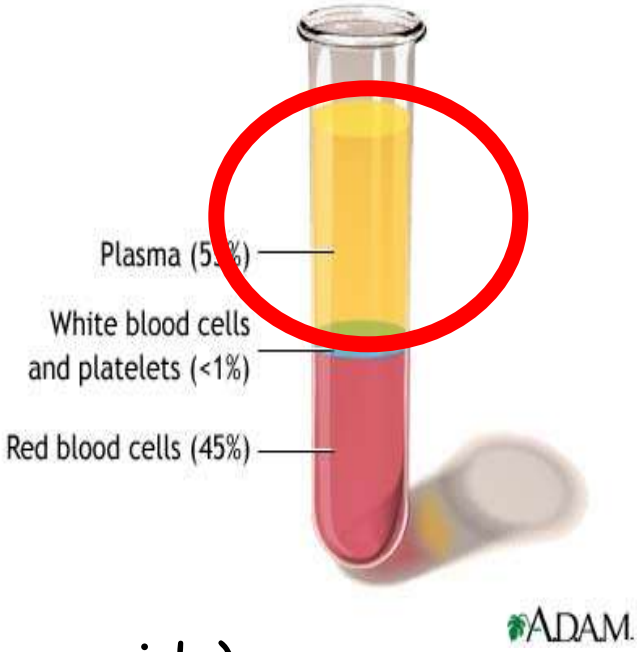
Serum = plasma - fibrinogen



Components of Plasma

Blood plasma Consists of:

- Water 90%
- Plasma Proteins 6-8 %
- Electrolytes (Na^+ & Cl^-) 1%



Other components:

- Nutrients (e.g. Glucose and amino acids)
- Hormones (e.g. Cortisol, thyroxine)
- Wastes (e.g. Urea)
- Blood gases (e.g. CO_2, O_2)

Significance

- The concentrations of various plasma proteins and enzymes give definite clues and **aid in the diagnosis** of diseases.

Plasma proteins

- ***A large number*** **of dissolved proteins of the plasma**
- **includes**
  - **simple proteins, conjugated proteins**
- **act by holding fluid in the blood vessels by osmosis.**
- **carry out a number of different functions.**

Plasma proteins

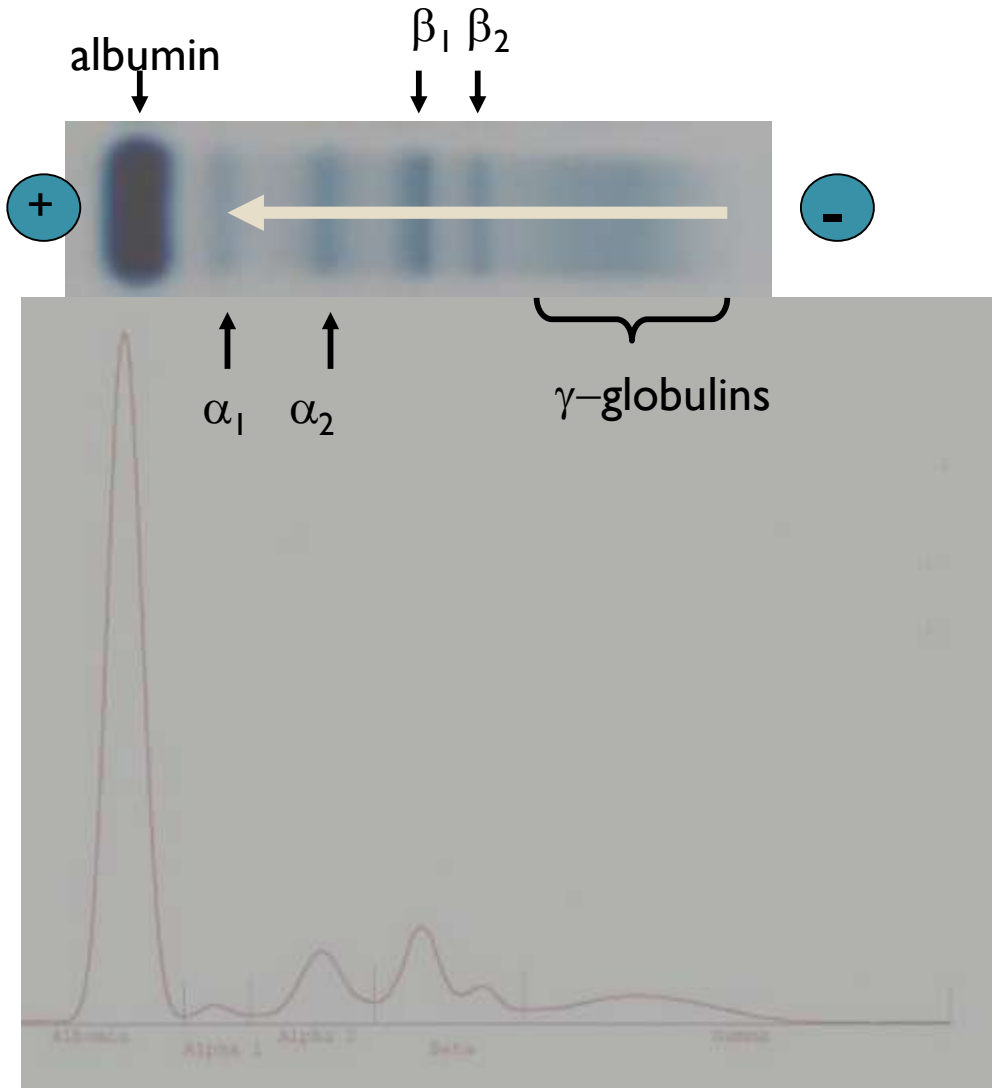
Plasma Proteins			
Protein	**Percentage of Total**	**Origin**	**Function**
Albumin	60%	Liver	Helps maintain blood osmotic pressure
Globulin	36%		
Alpha globulins		Liver	Transport lipids and fat-soluble vitamins
Beta globulins		Liver	Transport lipids and fat-soluble vitamins
Gamma globulins		Lymphatic tissues	Constitute a type of antibody
Fibrinogen	4%	Liver	Blood coagulation

Separation of plasma proteins

- Salting out technique.
- Precipitation by dehydration.
- Ppted by ammo. sulfate
  - Albumin – full saturation
  - Globulins – half saturation
  - Fibrinogen – 1/5 saturation

Electrophoresis of plasma proteins

Proteins move in an electric field according to their charge and size.



- Ultracentrifugation
  - Based on difference in the densities of proteins.
- Cohn's fractionation
- Gel filtration

General characteristics of plasma proteins

1. They are synthesized in liver except immunoglobulin.
2. Almost all plasma proteins are glycoproteins.
3. Many plasma proteins exhibit polymorphism such as α_1-antitrypsin, transferrin, haptoglobin.
4. Each plasma protein has a characteristic half-life in the circulation.
5. Acute Phase Proteins, APP

Albumin

Albumin (69 kDa), single polypeptide chain having 585 aa with 17 disulfide bonds, is the most abundant protein (60%) in the blood plasma. (3.5-5.0 g/dl)

Synthesis of albumin:

- *Liver* produces about 12g albumin per day which represent 25% of total hepatic protein synthesis and 50% of secreted protein. half-life: 20 days
- For this reason, measurement of serum albumin concentration is used to assay liver function test.

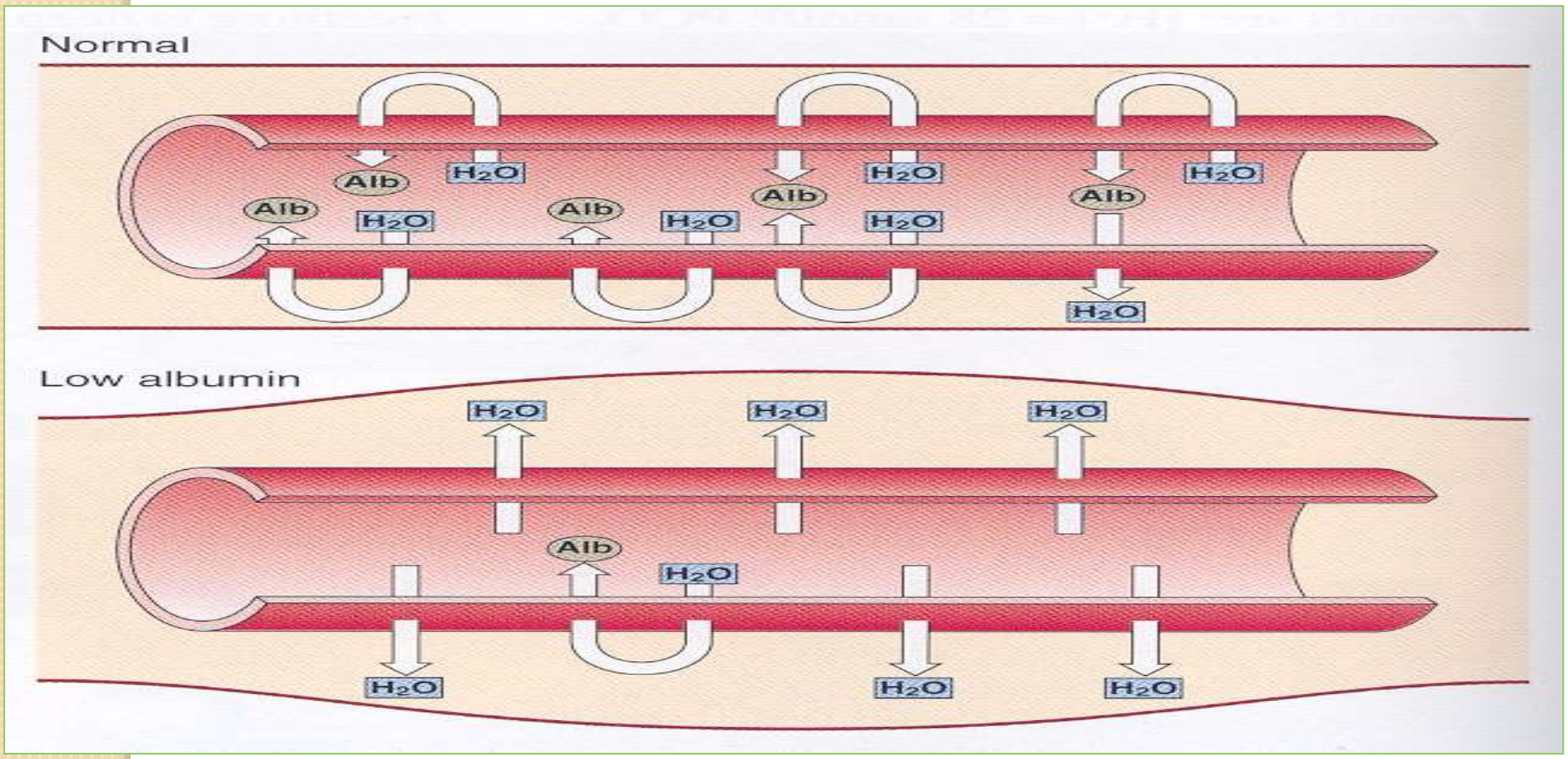
Functions

1. Maintains colloid osmotic pressure

- **Colloid osmotic pressure, is a form of osmotic pressure exerted by proteins in blood plasma that usually tends to pull water into the circulatory system.**

- **In conditions where plasma proteins are reduced,**
  - **e.g. from being lost in the urine (proteinuria) or from malnutrition,**
  - **there will be a reduction in osmotic pressure, leading to enhanced fluid retention in tissue spaces (edema).**

Colloid osmotic pressure



Low albumin, causing edema.

2. ***Transport:*** **It can bind and transport many diverse molecules and serve as low-specificity transport protein, which include:**

- **a. *Metal ions:* such as calcium and copper.**
- **b. *Free fatty acid:* albumin binds to free fatty acid released by adipose tissue and facilitates their transfer to other tissue.**
- **c. *Bilirubin:* this protects from the toxic side effects of unconjugated bilirubin.**
- **d. *Bile acid:* albumin carries the bile acids that are recycled from the intestine to the liver in the hepatic portal vein.**
- **e. *Hormones:* such as thyroid hormones and the steroid hormones.**
- **f. Drugs – sulfonamides, barbiturates**

3. Buffering action

- All plasma proteins act as buffers.

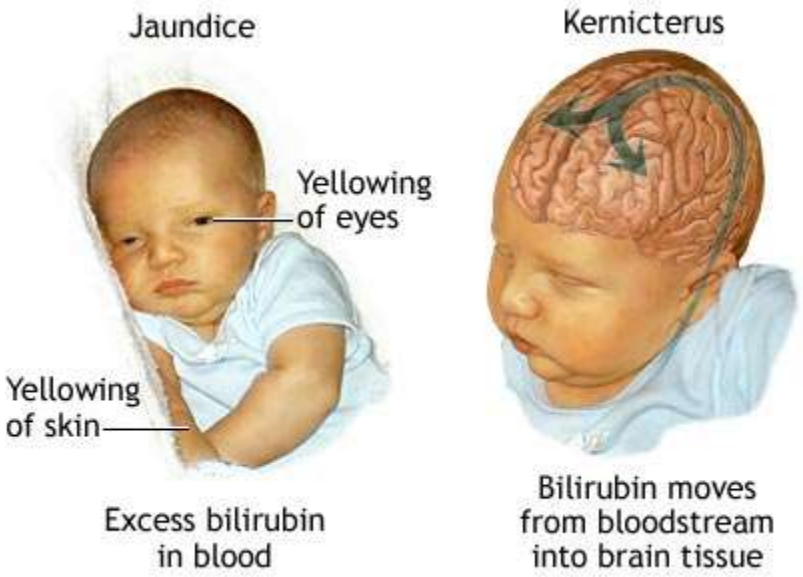
- Albumin contributes maximum to the buffering capacity of plasma.

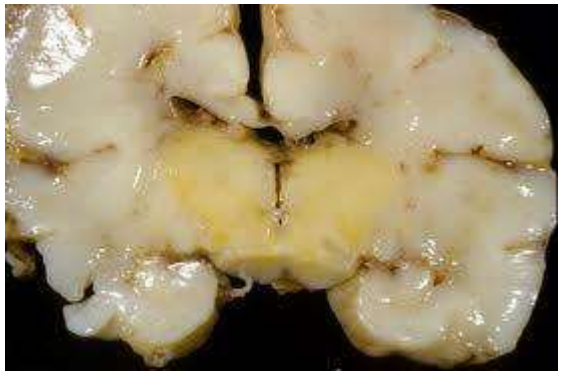
4. Nutritive function

- Albumin being a complete protein, serves as an amino acid reserve during starvation.
- Can assess nutritional status.

Clinical aspects

1. **Albumin binds different drugs and strongly affects the pharmacokinetics of these drugs.**
   - **For example, *sulfonamides* can cause the release of unconjugated bilirubin from albumin by competitive binding. If given to *infants*, sulfonamides may lead to *kernicterus*.**
2. **In cases of liver disease or starvation, albumin synthesis decreases.**
   - **This lead to *edema*.**





Clinical aspects

3. Hyperalbuminemia

   dehydration

4. Hypoalbuminemia
   - Renal causes- nephrotic syndrome
   - GI disorders, protein losing enteropathy, malnutrition.
   - Hepatic causes
   - Other causes – severe hemorrhage.

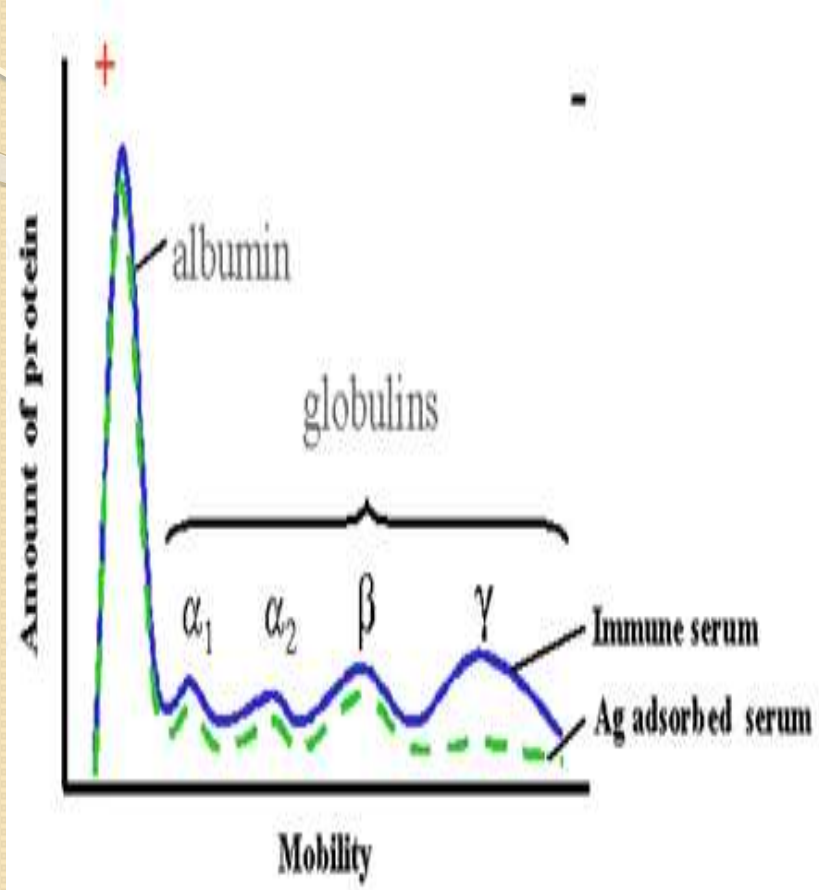
4. Albuminuria
   - albumin is excreted into urine
   - in nephrotic syndrome and certain inflammatory conditions of urinary tract.

5. Albumin is therapeutically useful for the treatment of burns and hemorrhage.

6. Analbuminemia- genetic

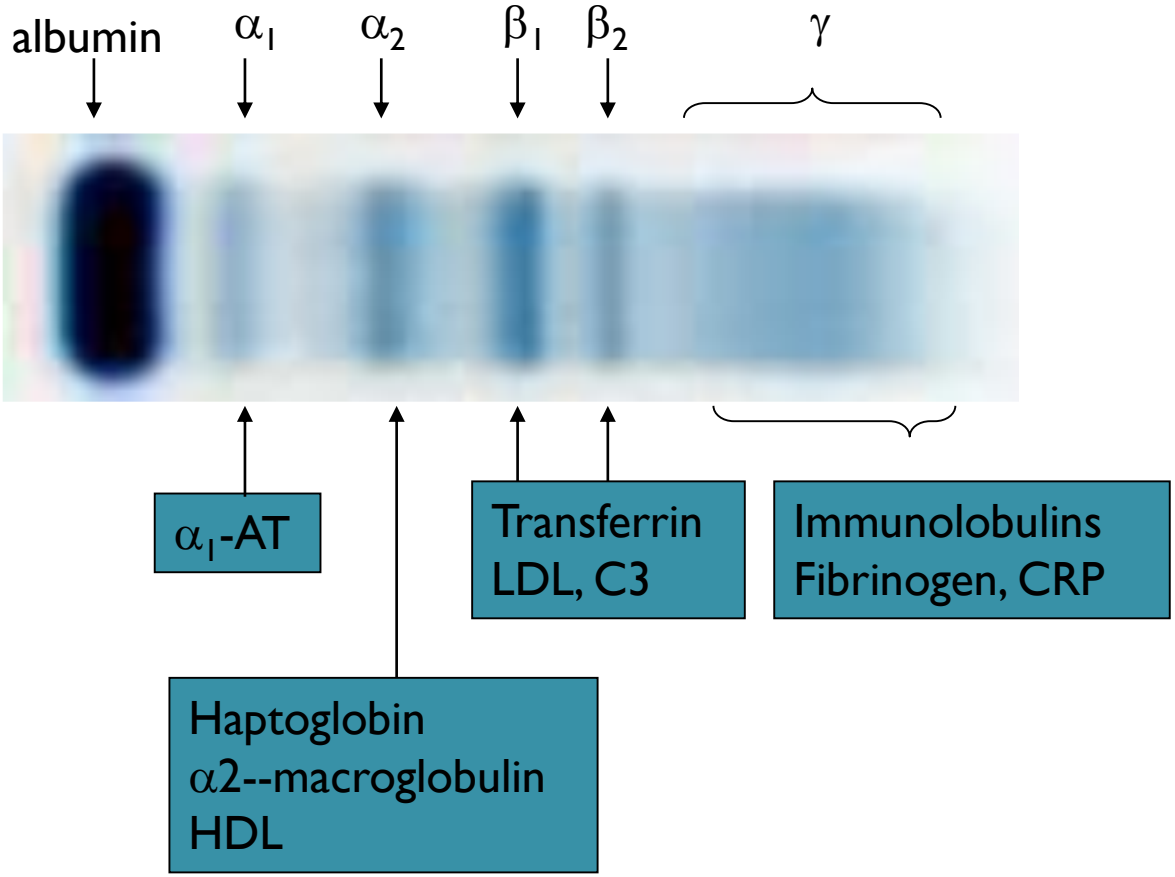
TRANSTHYRETIN – Prealbumin

Globulins



α_1 glob- α_1 antitrypsin
TBG
orosomucoid
transcortin
α_2 glob- α_2 macroglobulin
ceruloplasmin
haptoglobulin
ß glob. – hemopexin
transferrin
plasminogen
prothrombin
LDL
γglob. – Immunoglob.

The main components of the globulins

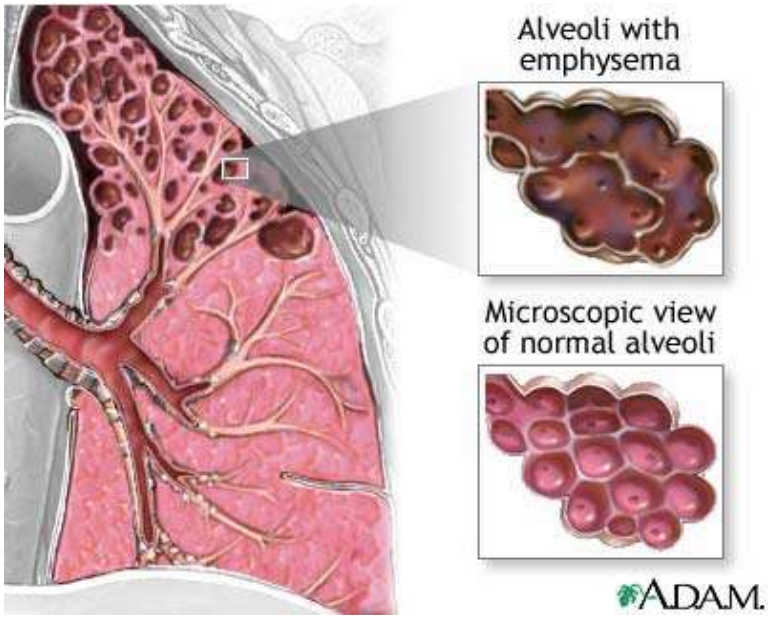


α_1–Antitrypsin
α_1–Antiproteinase(α_1–AT or AAT)

- It (52 kDa) is a glycoprotein with 394 aa.
- It is a major constituent of α_1 globulin fraction of plasma protein, normal concentration about 200mg/dl.
- It is a *serine protease inhibitor* and can combines with trypsin, elastase and other protease and inhibits them.

Clinical significance

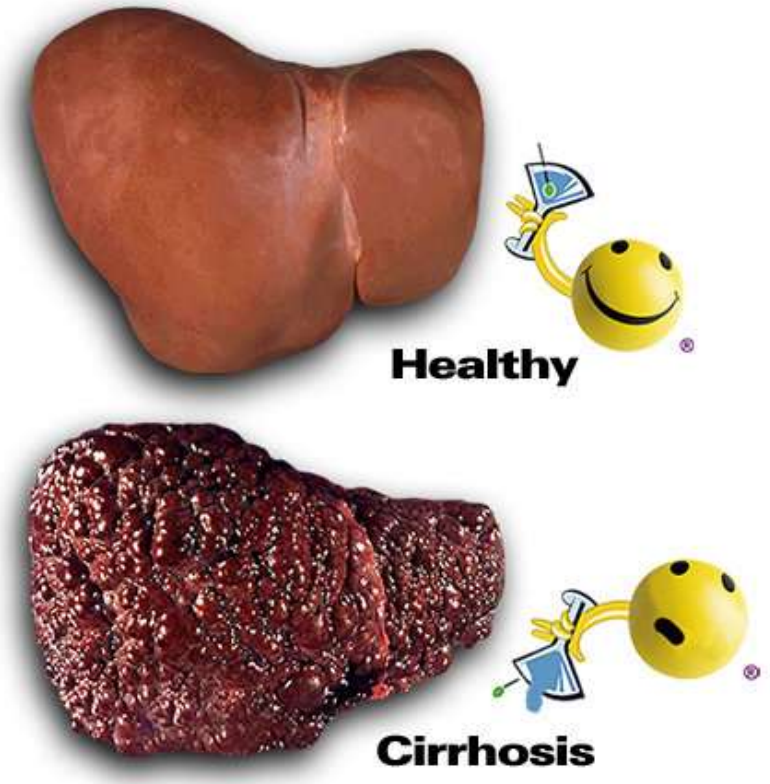
1. ***Emphysema:*** **used to represent the abnormal distension of lungs.**
   - **About 5% is due to the deficiency of α_1–AT.**
   - **This is associated with *lung infection and increase the activity of macrophage to release elastase* that damage lungs tissue.**



- Smoking can cause oxidation of Met_{358} to methionine sulfoxide and inactivate α_1–AT.

2. α_1 –antitrypsin deficiency liver disease

- due to mutant α_1 -antitrypsin accumulates and aggregates to form polymers, by unknown mechanism, cause liver damage followed by accumulation of collagen resulting in fibrosis (cirrhosis).



α_1 globulins

- HDL
- Orosomucoid
- Retinol binding protein
- Thyroxine binding globulin
- Transcortin

α_2 globulins

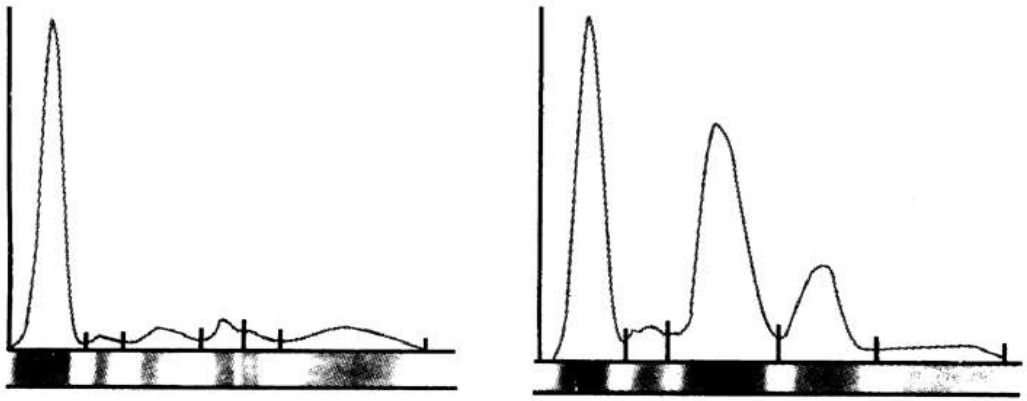
- α_{2}macroglobulin
- Haptoglobin
- Prothrombin
- Ceruloplasmin

α_2 –Macroglobulin (α_2 –MG)

- Major constituent of α2 globulin.
- It is a *pan protease inhibitor* and can combine and inhibit many protease.
- Concentration raised in Nephrotic syndrome..

Clinical significance

- *α2* -MG levels are increased in *nephrotic syndrome*
  - a condition wherein the kidneys start to leak out some of the smaller blood proteins. Because of its size, *α2* -MG is retained in the bloodstream.
- This increase has little adverse effect on the health, but is used as a diagnostic clue.



normal nephrotic syndrome

Haptoglobin (Hp)

- It (90 kDa) is a glycoprotein.
- It can bind with the free hemoglobin (extra-corpuscular Hb) in a tight noncovalent complex *Hp-Hb* during hemolysis.
- Hp-Hb(155 kDa) cannot pass through glomeruli of kidney while free Hb(65kDa) can and Hp *prevent the loss of free Hb into urine*.

※Low levels of plasma concentration of Hp can diagnose *hemolytic anemia*.

Prothrombin

- Component of coagulation system.
- transformed to thrombin by a clotting factorX.
- Def. leads to prolonged bleeding.
- Most commonly seen in obstructive jaundice.

Ceruloplasmin(CER)

- It (160 kDa) is a *blue-coloured, copper-containing α_2 fraction*.
- It can carry 90% of plasma copper tightly so that copper is not readily exchangeable.
- It possesses copper-dependent oxidase activity so also called ferroxidase. Helps in incorporation of fe in transferrin.
- Albumin carries the other 10% , which is the major supplier of copper to tissue.

Clinical significance

- Low level of ceruloplasmin is associated with Wilson's disease (hepatolenticular degeneration)
  - Wilson's disease is an inherited disorder in which there is too much copper in the body's tissues. The excess copper *damages the liver and nervous system* .
- Treatment: penicillamine is the first treatment used.
  - This binds copper (chelation) and leads to excretion of copper in the urine.

β Globulins

- LDL
- Transferrin
- Hemopexin
- Plasminogen

Transferrin (Tf)

- It (76 kDa) is a glycoprotein, part of *β fraction*.
- It can transport iron in plasma as ferric ions (Fe^{3+}) and protect the body against the toxic effects of free iron.

Fibrinogen

- Syn in liver
- Converted into fibrin by thrombin
- Plays a role in inflammatory response

Acute Phase Proteins, APP

- The levels of certain plasma proteins change during inflammation, infection, injury, cancer etc. These proteins are "*Acute Phase Proteins, APP* "
  - Include C-reactive protein,*CRP*, α_1-acid glycoprotein, fibrinogen, haptoglobin ,α_1-antitrypsin, albumin and transferrin.
- APP are believed to play a role in the body's response to inflammation, changes in their plasma concentrations are generally regarded as being sensitive, although non-specific, indicators of inflammation.

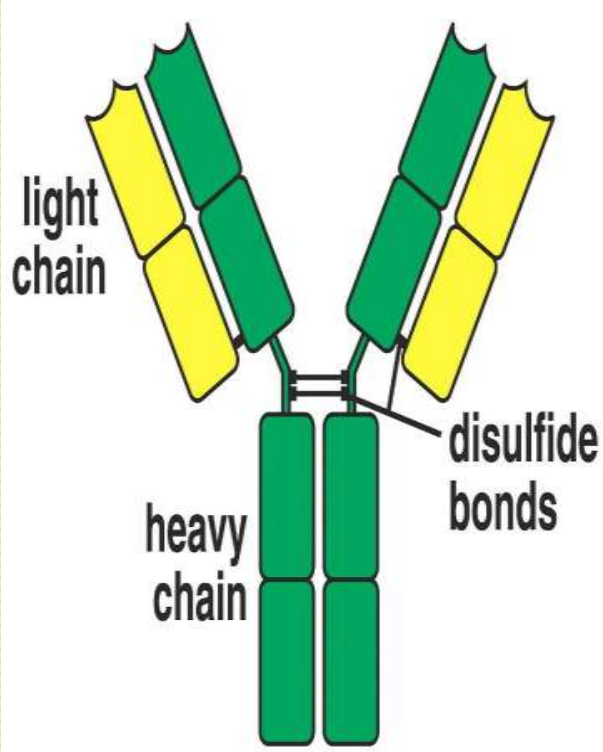
Acute phase reactants

- Positive
  - alpha1 antitrypsin
  - Orosomucoid
  - Ceruloplasmin
  - Haptoglobin
  - Complement-C3,C4
- Negative
  - Albumin
  - Transferrin
  - prealbumin

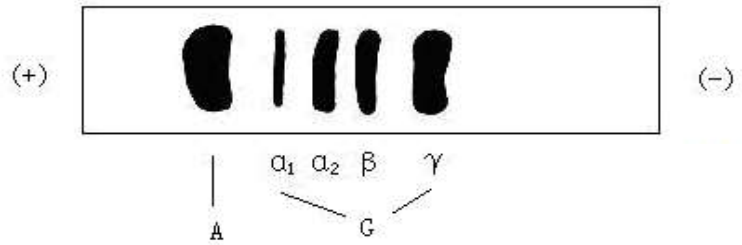
C-reactive protein, *CRP*

- **A major component of acute phase protein.**
- ***It reacts with the C polysaccharide of pneumococci*.**
- **Involved in the promotion of immune system through the activation of complement cascade.**
- Estimation of CRP in serum is important for the evaluation of acute phase response***.***
  - **CRP rises up to *50,000-fold* in acute inflammation, such as infection. It rises above normal limits *within 6 hours*, and peaks at 48 hours.**

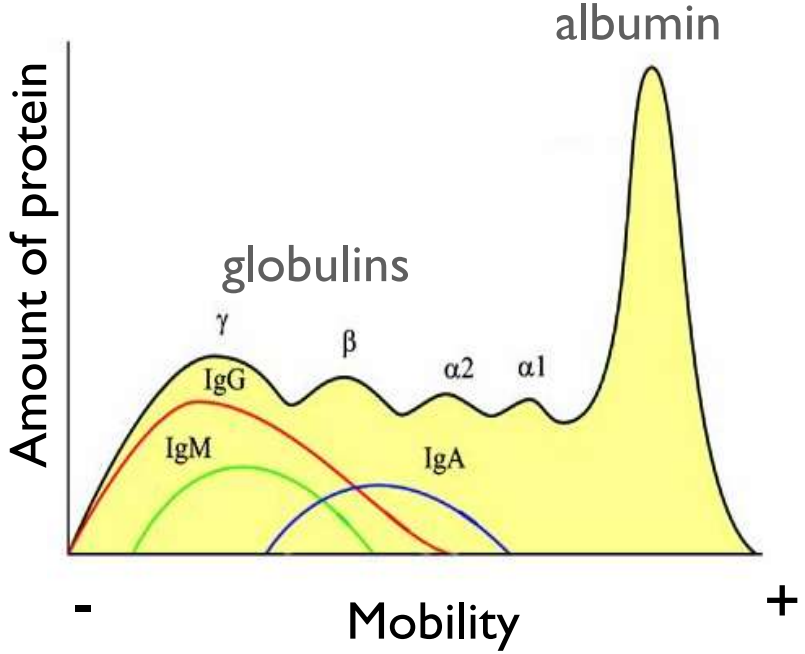
Immunoglobulins



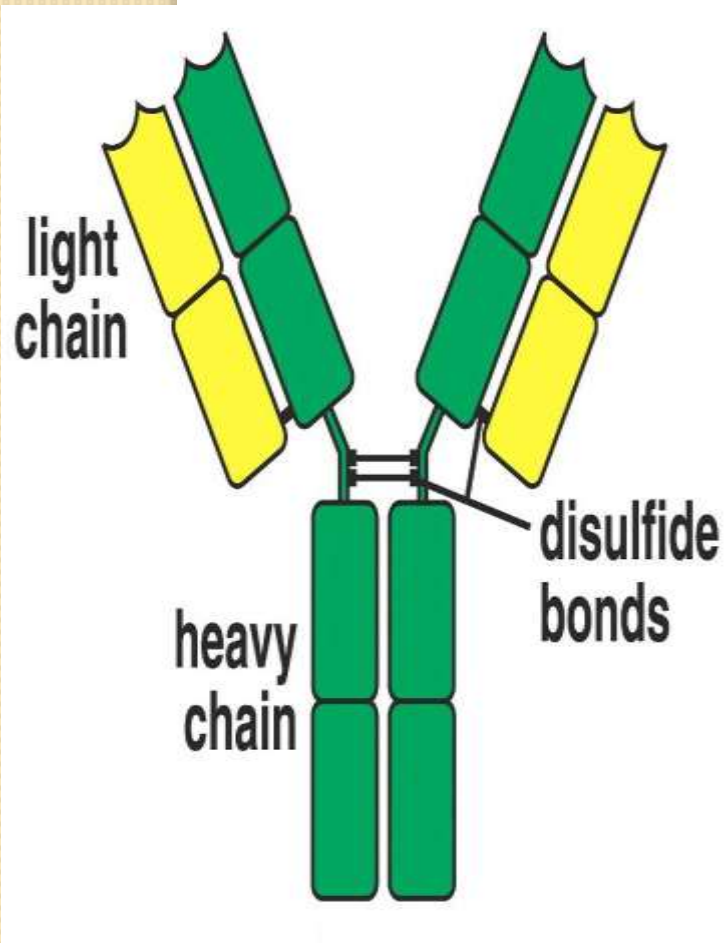
Immunoglobulin(Ig)/antibody(Ab):



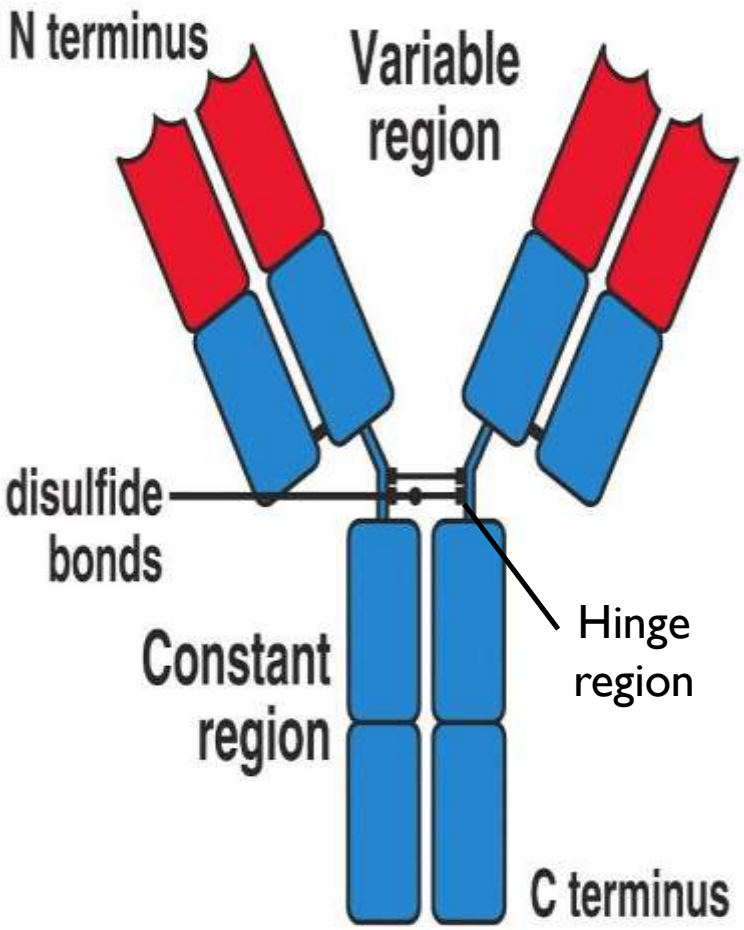
- Glycoprotein molecules that are produced by *plasma cells* in response to an immunogen and which function as antibodies, mostly associated with γ fraction.
- But γ-globulin and Ig are not synonymous.
  - *Ig is a functional term*
  - *γ-globulin is physical term*.



Basic Structure



1. four chains (H_2L_2): Y shape
   two identical light chains (L):
   23 kDa
   two identical heavy chains (H):
   53-75 kDa
2. Disulfide bonds and such noncovalent interactions as salt linkages, hydrogen bonds and hydrophobic bonds to form heterodimer (H-L).



1. Variable region (V): V_L&V_H
2. Constant region (C): C_L&C_H
3. Hinge region: flexibility

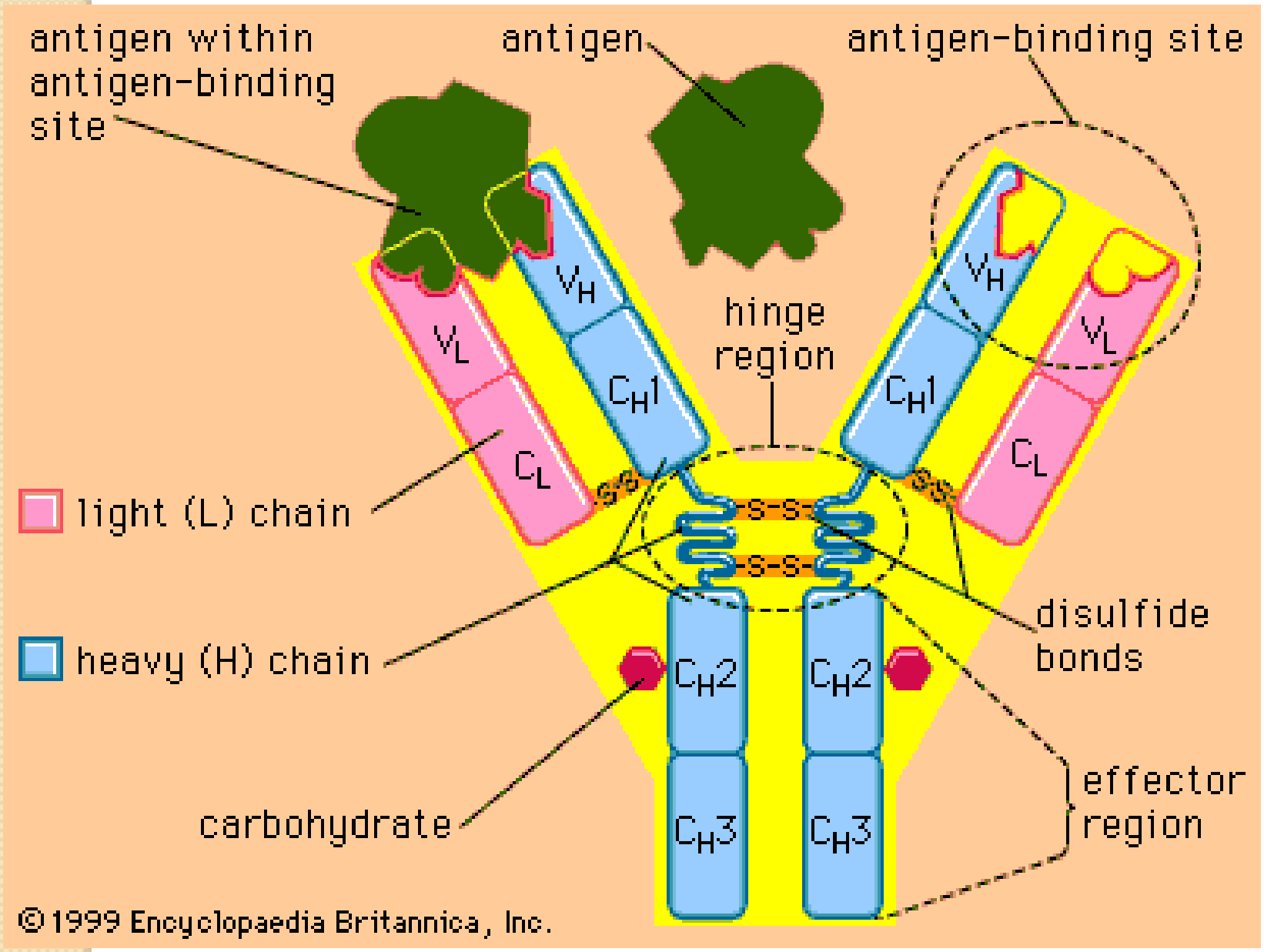
Light Chain:

- V_L + C_L

Heavy Chain:

- V_H + $C_{H1\text{-}CH3}$ (or C_{H4})

Structural Regions



Immunoglobulin Classes and Subclasses

In terms of the differences in amino acid sequence of *constant region of heavy chain*, immunglobulin molecules are divided into 5 classes:

- IgG, IgA, IgM, IgD and IgE

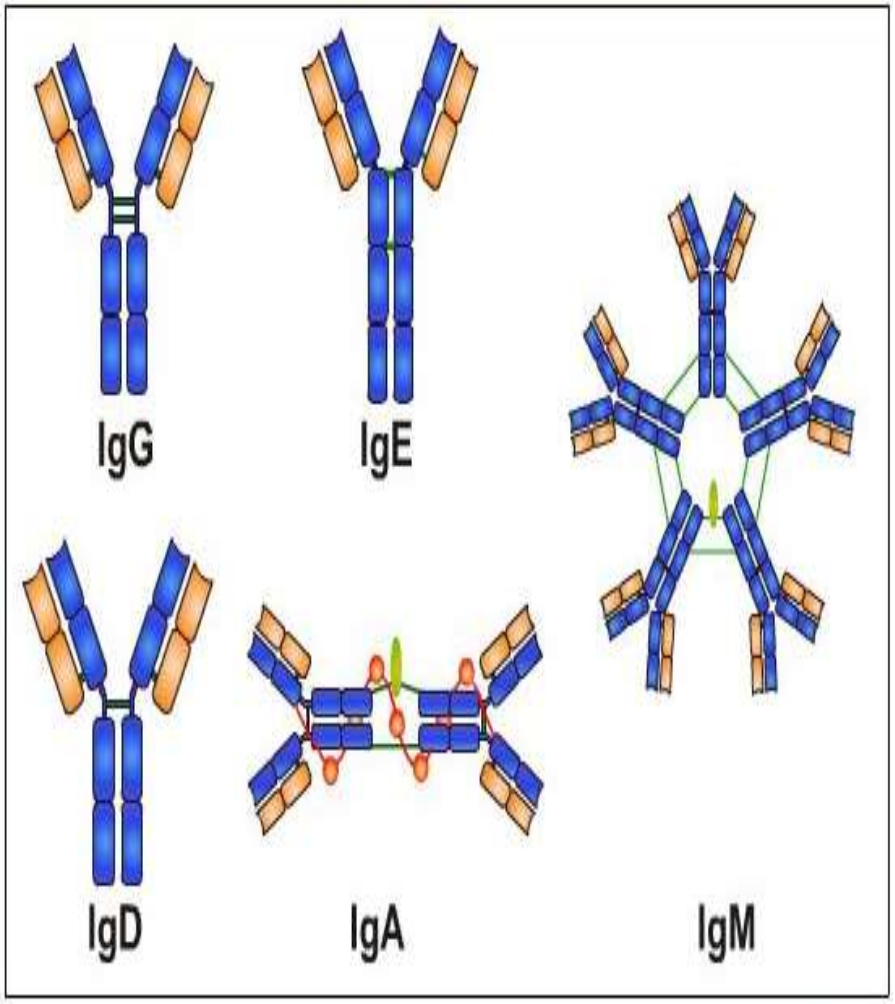
Heavy chain:

- 5 types: γ,α,μ,δ and ε.

Light chains

- 2 types: κ and λ.

Immunoglobulin Classes of Mammals

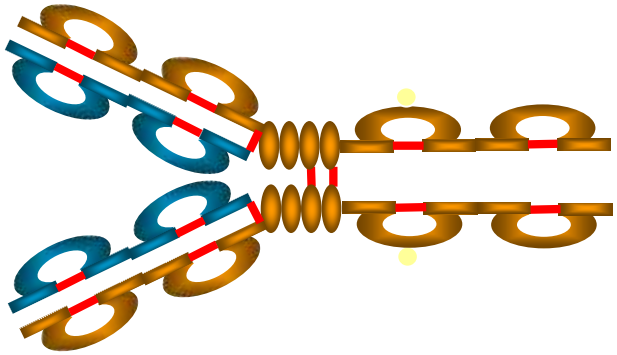


1. IgG - γ heavy chains
2. IgM - μ heavy chains
   pentamer
3. IgA - α heavy chains
   dimer
4. IgD - δ heavy chains
5. IgE - ε heavy chains

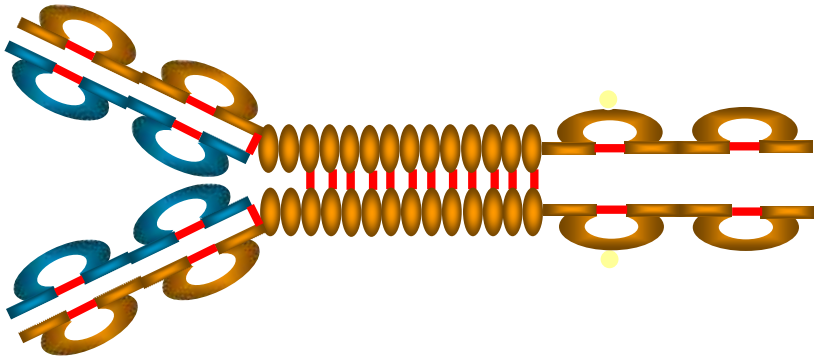
TABLE 9.2 Characteristics of human immunoglobulins

Type	H-Chain	L-Chains	Molecular formula	Molecular weight	Percentage carbohydrate	Serum conc. mg/dl	Major function(s)
IgG	γ	κ or λ	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	~150,000	3	800–1,500	Mostly responsible for humoral immunity
IgA	α	κ or λ	$(\alpha_2\kappa_2)_{1-3}$ or $(\alpha_2\lambda_2)_{1-3}$	~$(160,000)_{1-3}$	8	150–400	Protects the body surfaces
IgM	μ	κ or λ	$(\mu_2\kappa_2)_5$ or $(\mu_2\lambda_2)_5$	~900,000	12	50–200	Humoral immunity, serves as first line of defense
IgD	δ	κ or λ	($\delta_2\kappa_2$ or $\delta_2\lambda_2$)	~180,000	13	1–10	B-cell receptor?
IgE	ε	κ or λ	$\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$	~190,000	12	0.02–0.05	Humoral sensitivity and histamine release.

IgG



IgG1, IgG2, IgG4



IgG3

- **It is the most abundant class in serum, constitutes about 80% of the total serum Ig.**

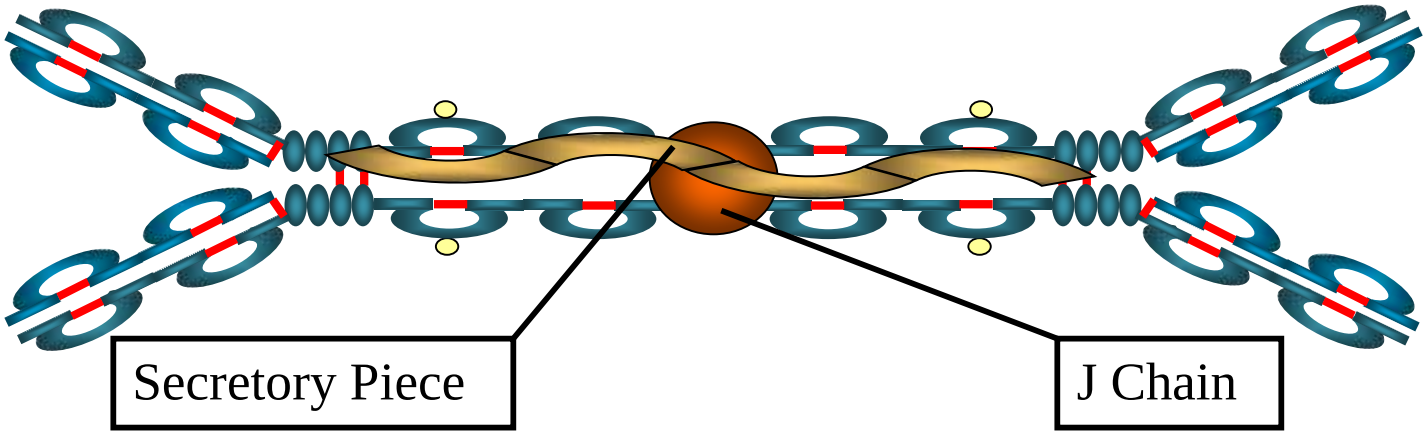
- **All IgG's are monomers. The subclasses differ in the number of disulfide bonds and length of the hinge region.**

Functions of IgG

1. **Major Ig in extravascular spaces.**
2. ***Placental transfer*: IgG is the *only class of Ig that crosses the placenta*.**
3. **Complement activation.**
4. **Binding to cells - Macrophages, monocytes, PMNs (polymorphonuclear leukocyte), and some lymphocytes have Fc receptors for the Fc region of IgG.**

IgA

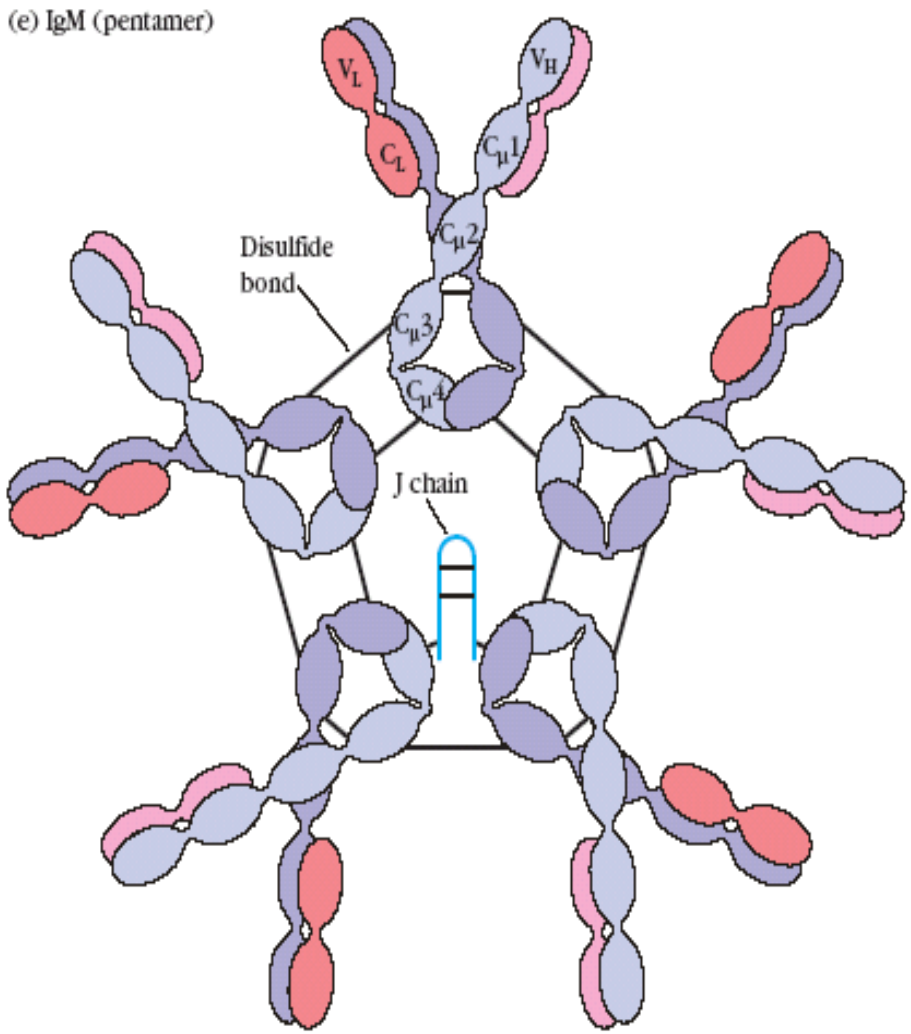
- **Structure**
  - **Serum - dimer**
  - **Secretions (sIgA)**
    - **Dimer**
    - **J chain**
    - **Secretory component**



IgA

- **Function**
  - **2nd highest serum Ig**
  - **Major secretory Ig (Mucosal or Local Immunity)**
    - **Found in the body secretions: tears, breast milk, saliva, mucus of the bronchial, genitourinary, and digestive tract**
    - **IgA is the most predominant antibody in the colostrum, the initial secretion from the mother' breast after a baby is born.**
  - **Does not activate complement (*unless aggregated*)**
  - **Binds to Fc receptors on some cells**

IgM



Structure

The largest Ig composed of 5 Y-shaped units held together by a J polypeptide chain.

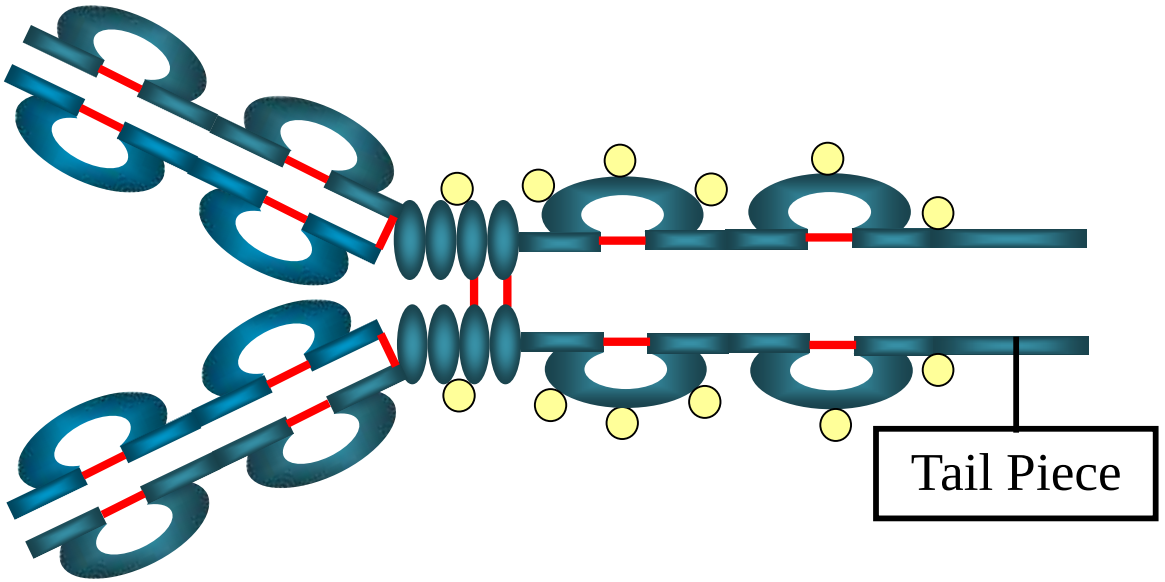
1. Pentamer
2. Extra domain (C_{H4})
3. J chain

Functions of IgM

1. 3rd highest serum Ig.
2. IgM cannot traverse blood vessels, hence *it is restricted to the blood stream*.
3. ***1st Ig*** produced in a primary response to an antigen and ***serve as first line of defense***.
4. ***Natural antibodies***
5. a **good complement activation** Ig. Thus, IgM is the **most effective** in leading to the lysis of microorganisms.
6. Binds to Fc receptors.

IgD

- **Structure**
  - **Monomer**
  - **Tail piece**

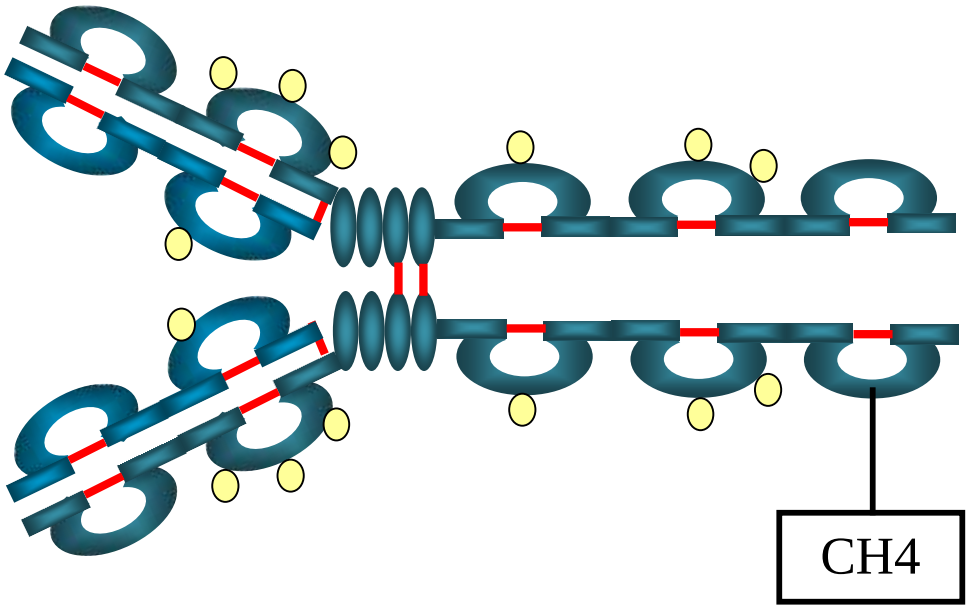


IgD

- **Properties**
  - **4th highest serum Ig, its role in serum uncertain.**
  - **B cell surface Ig.**
  - **Does not bind complement.**

IgE

- **Structure**
  - **Monomer**
  - **Extra domain (C_{H4})**



IgE

- **Function**
  - **Least common serum Ig**
  - ***Allergic reactions, hypersensitivity, anaphylaxis***

    Binds to basophils and mast cells (Does not require Ag binding)
  - ***Parasitic infections*** **(Helminths)**
    - **Binds to Fc receptor on eosinophils**
  - **no complement activation.**

Multiple Myeloma

- Uncontrolled proliferation of plasma cells.
- Paraproteinemia,anemia,bone resorption and proteinuria
- Bradshaw test
- Heat Test
- Bence jones proteins - 20%
- M band on electrophoresis