

PLASMA PROTEINS

- Total blood volume in adults is about 4.5 - 5 L
- when blood is mixed with an anticoagulant and centrifuged, the all components are precipitated
- The supernatant is called plasma.
55 - 60% of blood - plasma.

Serum

- If blood is withdrawn without anti-coagulant and allowed to clot for about 2 hours, liquid portion is separated from clot. This defibrinated plasma is called serum.
- Serum lacks coagulation factors like prothrombin and fibrinogen.
- The plasma protein consist of several hundreds of proteins.

Total proteins = 6-8 g/dL.

Albumin 3.5 - 5 g/dL.

Globulin 2.5 - 3.5 g/dL.

Fibrinogen 200 - 400 mg/dL.

1 mark the albumin : globulin ratio is usually:
1.2 : 1 - 1.5 : 1.

- Almost all plasma proteins except Igs are synthesized in liver

Plasma contains :

- Water
- Electrolytes
- Metabolites
- Nutrients
- Hormones
- Proteins.

Almost all plasma proteins except immunoglobulins are synthesized in LIVER.

- Functions of plasma proteins are:
 - Nutritional
 - Fluid exchange
 - Buffering action.
 - Transport proteins.
 - Reserve proteins.
 - In blood coagulation
 - Immunological function

PLASMA PROTEINS.

- Anti-chymotrypsin
- Alpha 1-antitrypsin.
- Anti-thrombin.

FUNCTIONS.

Anti-proteases.

- Various coagulation factors and fibrinogen

Blood clotting

- Erythropoietin hormone.
- cholesteryl ester transferase enzymes.
- Immunoglobulin complement proteins. immune defense.
- Acute phase proteins - Reactive proteins. involved in inflammatory response.
- Albumin transport or binding proteins.
- ceruloplasmin
- Haptoglobin
- Lipoproteins
- Hemopexin.
- Retinol binding protein
- Sex Hormone Binding Globulin
- Thyroxine Binding globulin
- Transferrin

Separation of Plasma Proteins

- (1) Precipitation by salting out:
- Globulins - half saturation with ammonium sulphate.
 - Albumins - full saturation.
 - Fibrinogen - $1/5^{\text{th}}$ saturation

- Quantification of total protein

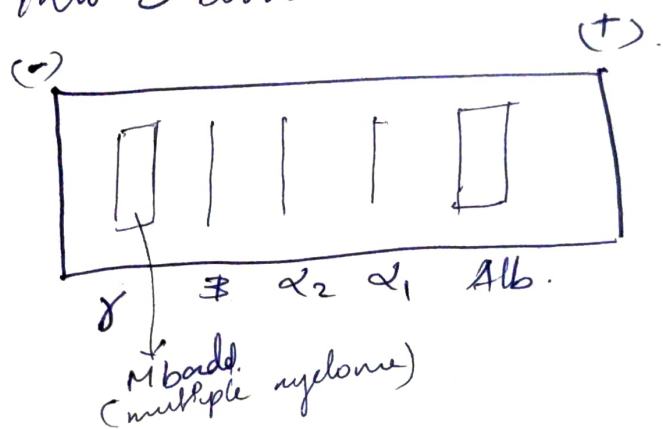
BIURET METHOD.

quantification of serum albumin.

= BCG METHOD.

Bromocresol green.

(2) Electrophoresis.
Electrophoresis refers to the movement of charged particles through an electrolyte when subjected to an electric field.
In clinical laboratory, electrophoresis is employed for separation of serum proteins.
In agar gel electrophoresis, normal serum is separated into 5 bands.



Relative concentrations are:

Albumin : 55-65%.

α_1 globulin : 2-4%.
(α_1 antitrypsin)

α_2 globulin : 6-12%
(α_2 macroglobulin)

β globulin : 8-12%
(low density β)

γ globulin : 12-22%

Albumin has maximum and globulin has minimum mobility in the electric field.

Some features of plasma proteins

Clinically significant condition.

→ HYPOALBUMINEMIA.

Lower than normal level.

Seen in:

1. Cirrhosis of liver.
2. Malnutrition - malabsorption syndrome.
3. Nephrotic syndrome.
4. Albuminuria - pathological condition where albumin is seen in urine.
5. Protein losing enteropathy.

Consequences:

→ Edema.
Albumin level lower than 2 g/dl results in edema.

→ Hypocalcemia (Ca lower by 0.8 mg/dl for a fall of 1 g/dl albumin).

Detection of albumin in urine = Heat and acid acid test

→ A/G ratio is altered in hypoalbuminemia.
compensatory increase in globulin.

→ HYPOPROTEINEMIA.

Hypergamaglobulinemia.
Hypoalbuminemia.

chronic infections
multiple myeloma

Globulins.

α globulin: glycoproteins.

1. α_1 and glycoproteins indicators of acute inflammation.

2. ^(AFP) α_1 fetoprotein: fetal blood
Diagnosis of hepatocellular carcinoma.
tumour marker.

3. Alpha 1 antitrypsin (AAT)

→ Anti protease.

→ Inhibits all serine proteases
chymotrypsin.

cathepsin.

elastase.

trypsin.

AAT is synthesized in liver
Decreased level seen in

→ Emphysema

→ Nephrotic syndrome.

4. α_1 - lipoprotein.

transport cholesterol.

α_1 lipoprotein deficiency leads to Tangier's disease.
accumulation of cholesterol esters in vessels resulting in

→ tonsillar enlargement

→ Hepatomegaly.

2 globulins.

1. ceruloplasmin.
2. Haptoglobin : Increased on inflammatory conditions.

Ceruloplasmin

- Synthesised in liver.
- Contains 6-8 Cu atoms tightly bound per molecule.
- Acute phase protein
- Serum ferroxidase.
- Promotes oxidation of ferrous iron to ferric form which is incorporated into transferrin.

90% of Cu content of plasma is bound to ceruloplasmin and the 10% to ~~Alb~~ Albumin.

- Cu is bound loosely to Albumin hence useful for transport
- Antioxidant.

Increased in :

- all inflammatory conditions
- collagen disorders.
- in malignancies.

Decreased level is :

- Wilson's disease.
- Malnutrition
- Nephrosis.
- Cirrhosis.

Alpha-2-macroglobulin

- Major component of α_2 molecule.
- Synthesized in hepatocytes and macrophages.
- Inactivates all proteases.
(imp: in vivo anticoagulant)
- carrier of many growth factors.
eg: platelet derived growth factor (PDGF)
- Normal serum level $\rightarrow 130-300 \text{ mg/dl}$
- Increased in nephrotic syndrome.

β globulin

- Beta lipoproteins

(1) transferrin

Increased in iron deficiency anemia
decreased in liver diseases, nephrotic syndrome.

(2) C reactive protein (CRP)

(3) Hemopexin

(4) β_2 globulin

C reactive Protein (CRP)

- It reacts with polysaccharide of capsule of pneumococci
- β_2 globulin.
- Synthesized in liver.
- Highest sensitive (hs) CRP is a predictor of risk of CAD.
(coronary artery disease)

Acute phase proteins (short note)

→ Assessment of the presence & degree of inflammation can be obtained from the levels of acute phase proteins.

APP - Increase 50-100 folds during inflammation and neoplastic conditions.

eg. CRP

α_1 - antitrypsin

α_2 - macroglobulin

Negative Acute phase proteins
Decreased during inflammatory responses.

Albumin

Transferrin

RBP

pre-albumin (transthyretin)

γ Globulin

Eg: immunoglobulins having antibody activity.

→ The antibody acts with antigens very specifically.

Albumin (Important)

- Major part of pp (60%)
mol. wt 69,000 D.
- Has one polypeptide chain with 585 aa and 17 interchain disulphide bonds.
- Synthesized in hepatocytes

- Half life is about 20 days
- present in CSF and interstitial fluid
- Albumin level decreased in liver disease

Functions (Question) 8 marks

① Maintenance of blood volume by exerting effective osmotic pressure.

• HYPOALBUMINEMIA ($< 29 \text{ g/dL}$) results in edema.

② Carries of various hydrophobic substances:

Bilirubin, non-essential FA, drugs (sulphonamides, aspirin, salicylate, diazepam), hormones (steroid hormones, thyroid hormones), Ca, Cu, heavy metals

③ Albumin has 16 histidine residues and contributes for buffering action.
Max. buffering capacity.

④ Transport form of excreted ~~ag~~ from liver to extra hepatic tissues - Nutritional function.

⑤ Therapeutic use - useful for treating burns, hemorrhage and shock.

⑥ Albumin - fatty acid complex cannot cross blood brain barrier.

Albumin - bilirubin complex prevent the entry of bilirubin in the brain.

→ Aspirin in new borns can result in kernicterus and mental retardation.
Aspirin replaces bilirubin competitively.

Hypoalbuminemia conditions
(Hypoglobulinemia, reversal of AG ratio).

- ① Liver cirrhosis (liver disease)
- ② Nephrotic syndrome
- ③ Malabsorption
- ④ Protein losing enteropathy
- ⑤ Albuminuria
- ⑥ Burns and hemorrhage

Proteins of clinical importance

① Bence Jones proteins:
Abnormal protein seen in blood of people suffering from paraproteinemias especially multiple myeloma.

② Acute phase proteins:
Increased in inflammatory cond.

③ Cryoglobulins - They are proteins coagulated where temperature is lowered, increased in arthritis.

Immunoglobulins

Immunity: free from burden.
Ability of an organism to recognize and defend itself against specific pathogens or antigens.

- Features of Immunological α^n
 - (A) Recognition of self from non self or foreign substance.
 - (B) Specificity of the reaction.
 - (C) Memory of the response.

→ Immune response: third line of defence. Involves production of antibodies and generation of specialised lymphocytes against specific antigens.

Cells of Immunoglobulins constitute a heterogeneous family of serum proteins which either function as antibodies or are chemically related to antibodies.

→ produced by B lymphocytes.

→ On electrophoresis mainly occupy the γ region.

Five types: IgG, IgM, IgA, IgD, IgE.

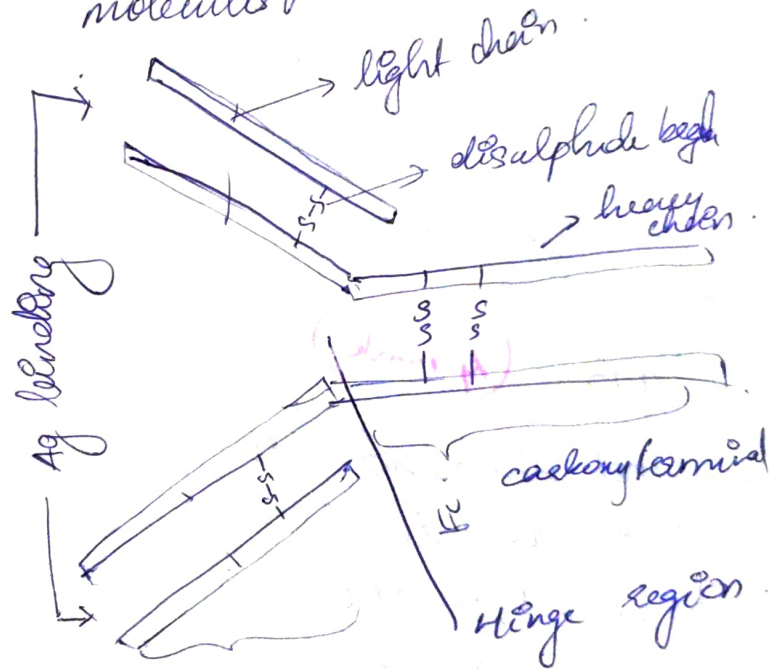
Structure of I G's (Essay)

→ Basic unit consists of 4 polypeptide chains linked by disulphide bonds.

→ Each unit consists of two identical heavy chains and two light chains.

H: 440 AA, L: 214 AA.
H-Mut: 50,000, L-Mut: 25,000.

→ The major disulphide bond is between the 'L' and 'H' chains and also between cysteine residues of two half of the molecule.



5 classes: depending on the heavy chain:

IgD: $\delta^{H.C.}$

IgG: $\gamma^{H.C.}$

IgM: $\mu^{H.C.}$

IgE: $\epsilon^{H.C.}$

IgA: $\alpha^{H.C.}$

Light chains can be either K or λ

→ Both heavy and light chains contain variable and constant regions.

→ V_L , C_L , V_H , C_H .

First 108 aa in K chain,
109 aa in λ chain and
first 118 aa in γ heavy
chain are variable.

→ can be cleaved to two
fragments by enzyme papain.

→ This occurs at hinge region.

→ This produces Fab (fraction
of antibody) and F_c (fraction
of crystalline) fragments.

→ Fab is the antigen
binding part.

→ Neutralise bacterial toxins
and virus.

→ Cross placenta.
- leads to Rh 180 immunisation

→ Major protective antibody in
new born

Ig M

→ Macroglobulin - Pentamer.
5 units joined together by
J chain

1200 ng/dl (50-200).

Sedimentation coefficient = 19S.

Primary antibody against
bacteria.

• Antibody response in primary immune

→ Can bind with 5 antigens
simultaneously.

Ig G

(4 mark)

→ dearly diagen
(V) diagen

→ Most abundant 75-80%.

1000 ng/dl (800-1200)

Mut: 1,50,000

Can be $\gamma_2 K_2$ or $\gamma_2 \lambda_2$.

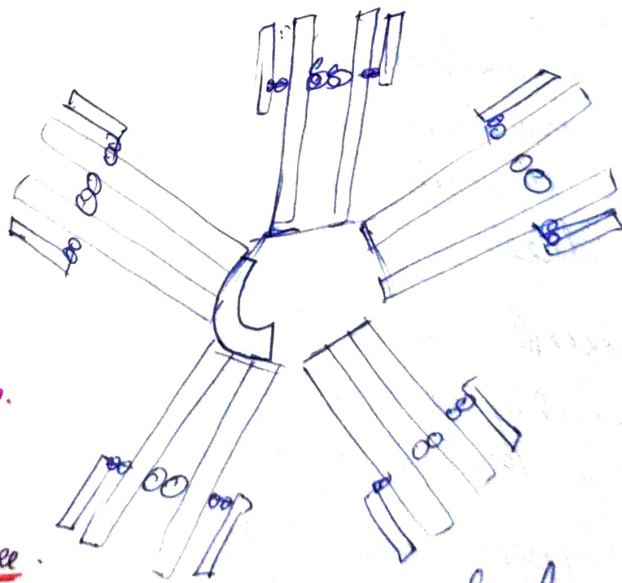
Also called 75 Immunglobulin.

Half life: 20 days

mark → Antibody in secondary response.

→ Opsonise bacteria - for
phagocytes etc.

→ Fixes complement - for
bacterial killing

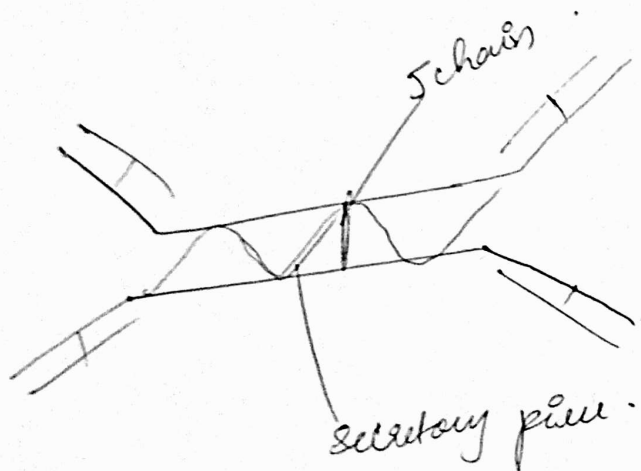


→ Can fix complement
→ Can't come out of blood vessel
→ Doesn't cross placenta
→ called natural antibody

as it is produced without any known antigenic stimulus of blood group antibodies.

Ig A

- second most abundant class.
- 10-15% of Ig.
- 200 mg/dl (150-300).
- Half life 5-8 days.
- ~~two forms~~ Monomeric.
- ~~is a dimer~~.



- Mol wt. 170,000 or 4,00,000.
- 7S or 11S.
- secretory IgA formed by joining two monomeric units at their carboxy terminal by S chains.
- Also contains secretory piece produced in liver.
- Secretory IgA present

Ig A

- usually dimers connected by S chains
- secretory antibodies.
- Ig A is the second most common serum Ig.
- 4 domains
- 11 S immunoglobulin.
- contains additional secretory piece.
- conc. in serum: 50-200 mg/dl
- functions are complement fixation, agglutination.

Ig D

- Delta chain
- 7S immunoglobulin.
- Mol wt 1,85,000 D.
- conc. in serum 1-10 mg/dl
- functions as B cell receptor.

Ig E

- Epsilon chain.
- 8S.
- Mol. wt. 1,90,000 D.
- fixation to mast cells and basophils.
- they mediate allergy and hypersensitivity.