PLASMA PROTEINS

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Objectives

- Outline the different components of plasma proteins.
- Describe the separation of plasma proteins using electrophoresis.
- Explain the functions of the different plasma proteins.
- Discuss the changes in plasma proteins in relation to different clinical disorders.

Learning outcomes

At the end of the lecture, the students should be able to

- Distinguish the different proteins present in plasma and explain how they can be separated using electrophoresis.
- Discuss the functions of each of the plasma proteins.
- Explain the clinical disorders related to plasma proteins.

General concepts

- The concentration of total protein in human plasma is about 6-8.5 g/dl.
- It is a complex mixture of simple proteins, glycoproteins, lipoproteins, and other conjugated proteins.
- The proteins can be separated into 3 major groups :
  - Albumins (3.5 – 5 g dl)
  - Globulins (2.5 – 3.5 g/dl)
  - Fibrinogen (200 – 400 mg/dl)

Albumin : Globulin ratio = 1.2 : 1 – 1.5 : 1

Serum vs. Plasma

- After blood is mixed with an anticoagulant and the cell components centrifuged down, the supernatant is called PLASMA.
- When the blood is allowed to clot & then centrifuged to precipitate the cell components, the supernatant is called SERUM.

General characteristics

Most pp are synthesized in the liver and macrophages except Ig

which are synthesized only by the B cells of the immune system.

PP are synthesized as pre-proteins and are post-translationally

modified before secretion into plasma.

Almost all pp are glycoproteins except albumin.

The concentration of certain pp increase in disease states such as

inflammation & tissue damage. E.g. acute phase proteins

Each pp has a characteristic half life.

Albumin – 20 days , Haptoglobin – 5 days

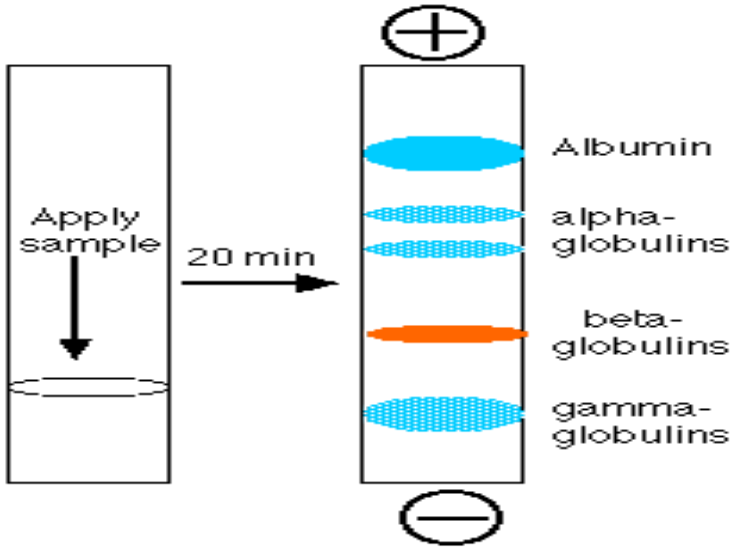
In certain diseases half life of the pp could get altered.

Half life may varies in certain disease conditions e.g. Crohn's

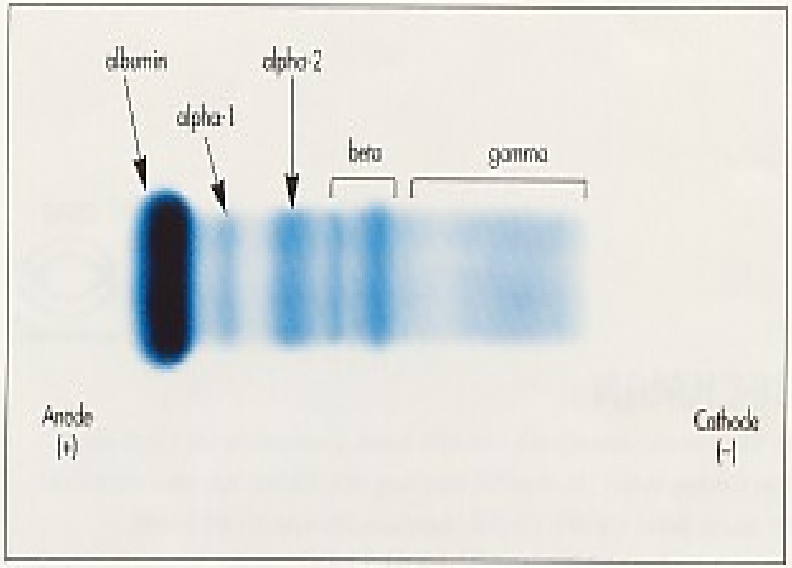
Separation of plasma proteins

- Based on differential solubility, size, or charge.
- Salting out (differential solubility)
  - First step in purification
  - Usually uses increasing concentrations of ammonium sulfate to compete with the protein groups for the available water.
  - Crude method but fast.
- Electrophoresis (size and charge)
  - Refers to the movement of charged particles through an electrolyte when subjected to an electric field.
  - In an electric field, the plasma proteins will move with a velocity that depends directly on the charge and inversely on the size and shape of the molecule.
  - pH is important in determining the net charge. At pH greater than the pI of the protein is negatively charged and migrate to the anode.

Plasma protein electrophoresis



Separating serum proteins by electrophoresis



A normal serum protein electrophoresis (Beckman SPE)

Usually performed on serum rather than

on plasma using barbital buffer pH 8.6 (no interference from fibrinogen).

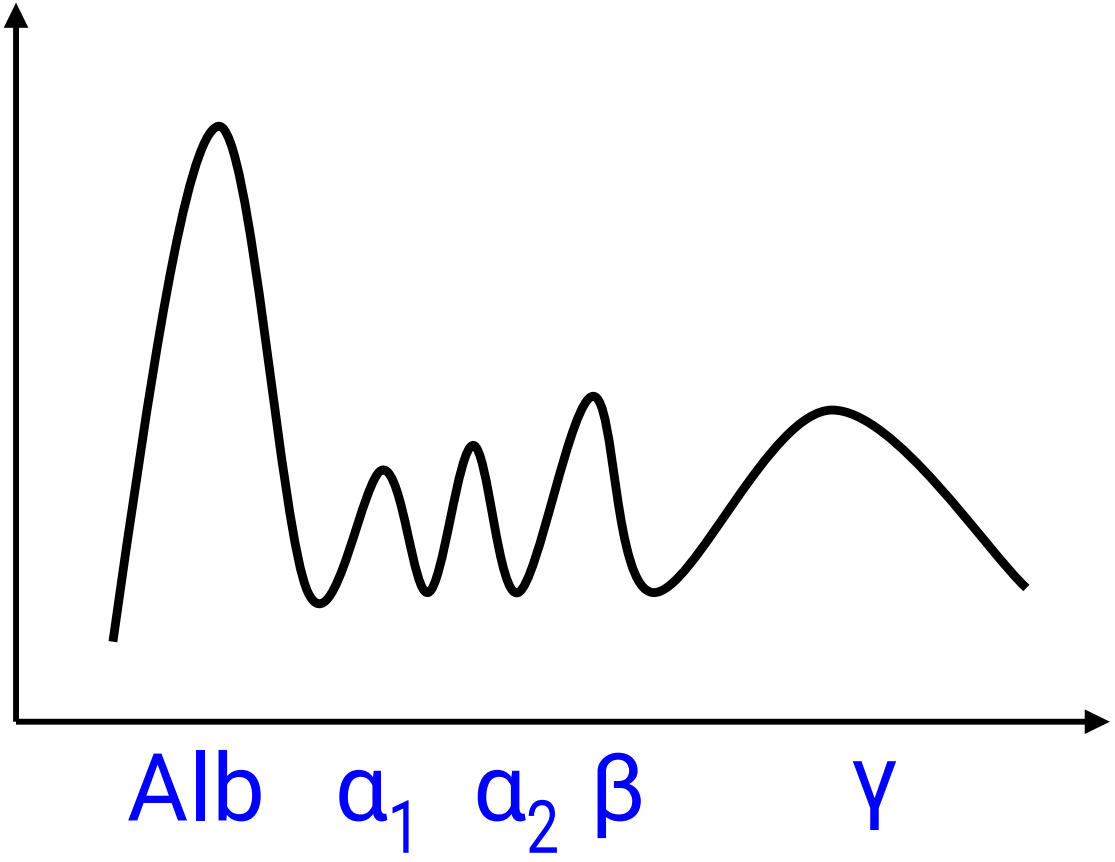
At this pH all the major proteins are negatively charged.

Separates into 5 bands on electrophoretic

field; albumin, $\alpha_1$globulin, $\alpha_2$globulins, β

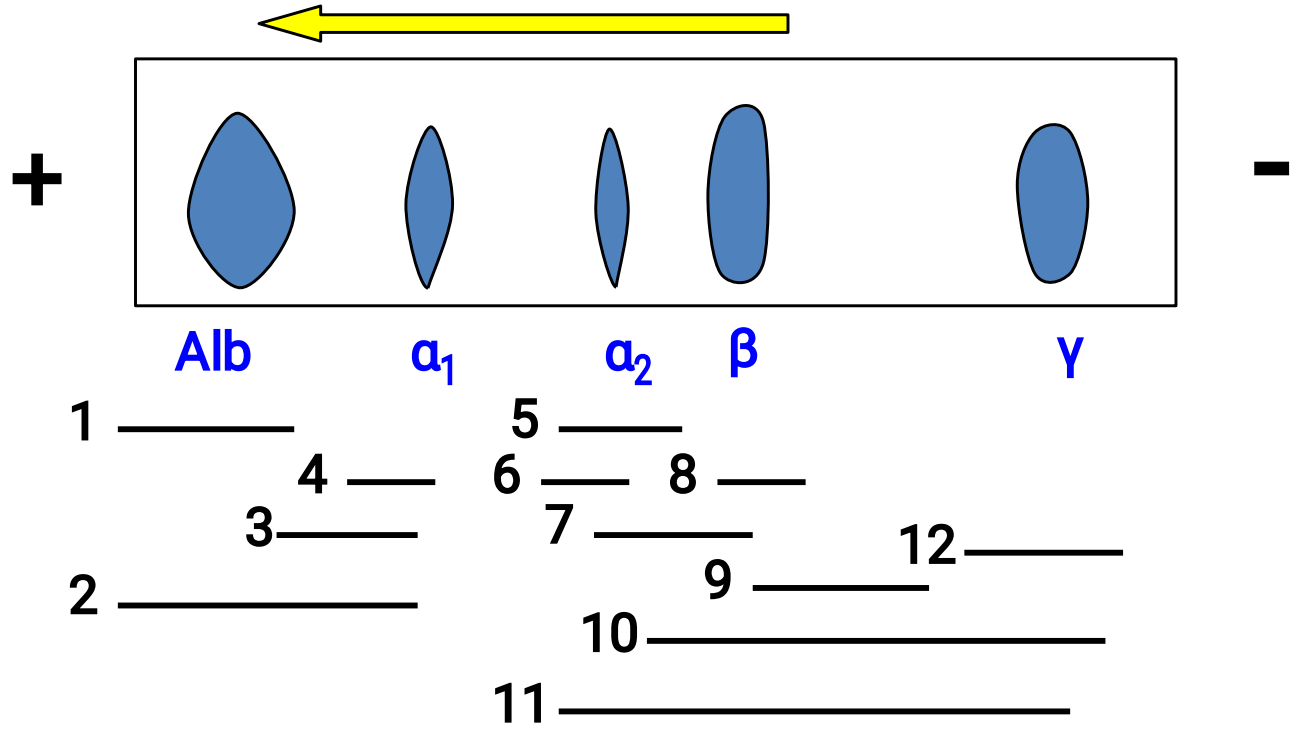
globulins, globulins.

Densitometer scanning



The bands of the electrophoretogram can be quantified by densitometer scanning.
Characteristic changes in one or more of the five bands are found in many diseases.

Separation of different plasma protein



1. Albumin
2. α lipoprotein (HDL)
3. α_1 – acid glycoprotein
4. α_1 – antitrypsin
5. α_2 – macroglobulin
6. Haptoglobin
7. β – lipoprotein (LDL)
8. Transferrin
9. C_3 fraction of complements
10. IgG
11. IgA
12. IgM

Functions of plasma proteins

i) Maintenance of plasma osmotic pressure (oncotic pressure) and therefore the control of ECF distribution.

ii) Transport function

Many hormones, metal ions , FFA , drugs and other substances are transported by specific pp (carrier proteins). Such binding makes them water soluable (lipids, bilirubin) or physiologically inactive (drugs, calcium, hormones) or non filterable by the renal glomeruli (Hb bound to haptoglobullin).

iii) Nutritive function – in protein mal-nurishment serve as a source of protein e.g.:- albumin, pre-albumin

iv) Defense function – by Ig and the complement system .

v) Coagulation and fibrinolysis.

vi) Buffering action –

1/8th of total buffer capacity of blood is due to pp.

vii) Maintenance of blood pressure- Due to presence of proteins in plasma ,it is a viscous fluid and provides resistance to the flow of blood in blood vessels and helps to maintain blood pressure.

Specific plasma proteins

Pre-albumin

Helps in the transport of retinol (Vit. A) and thyroid hormones (T_3&T_4).

Retinol binding proteins (RBP) forms a 1:1 complex with PA and transport retinol to target cells.

Because of short half life of PA ,estimation of this protein is a most sensitive assessment of protein malnourishment.

Albumin

Synthesis is dependent on protein intake ,but subject to feed back regulation by plasma.

Clinical significance

Plasma concentration of albumin, because it depends on dietary protein intake are frequently used to

1) Asses nutritional status
2) Plasma total Ca^{++} concentration
3) Severity of liver disease
4) Contribution to development of oedema

AFP (alpha feto protein)

- Synthesized in foetal yolk sac and liver
- ↑ Level at 30th week of gestation ,then begins to decline
- Production supressed in adults
- Low MW – appear in urine and amniotic fluid

*** * Clinical significance at 16-18th wks**

(i) ↑↑ AFP in amniotic fluid/ maternal plasma

Eg:- multiple pregnancy

↓ neural tube defect (spinabifida)

(ii) AFP in ↑↑ Downs syndrome

(iii) In adults AFP- eg:- hepatocellular carcinoma, germcell tumors

Haptoglobulins

- A group of proteins in the α_2 region
- Binds extracorpuscular Hb in a tight complex and prevents loss of free Hb through kidneys
- Low Haptoglobulin level in haemolytic anaemia

  t1/2 of Haptoglobulin- 5 days

  t1/2 of (Haptoglobulin-Hb) complex - 9 min

- High Haptoglobulin level in inflammatory states

Acute phase proteins (complement system)

- A group of 20 or more plasma proteins (excluding Ig) whose concentration significantly alter in a characteristic fashion following tissue injury (eg:- infection, surgery, trauma, tumors, necrosis, myocardial infarction) Eg. Haptoglobulin, C-reactive protein, cerruloplasmin

-These proteins limit further tissue damage and initiate and maintain repair

-The acute phase response is a general reaction to inflammation e.g.: C-reactive proteins stimulates classical complimentary pathway.

-The presence of these APP is responsible for the rise in ESR and increased plasma viscosity

Members of acute phase proteins

- α_1-antitrypsin.
- α_1-antichymotrypsin.
- α_1-acid glycoprotein.
- Ceruloplasmin.
- Haptoglobin.
- Complement component C3 and C4.
- Anti-thrombin III.
- Ferritin

Negative proteins: albumin, transferrin

Transferrin – shuttles the iron

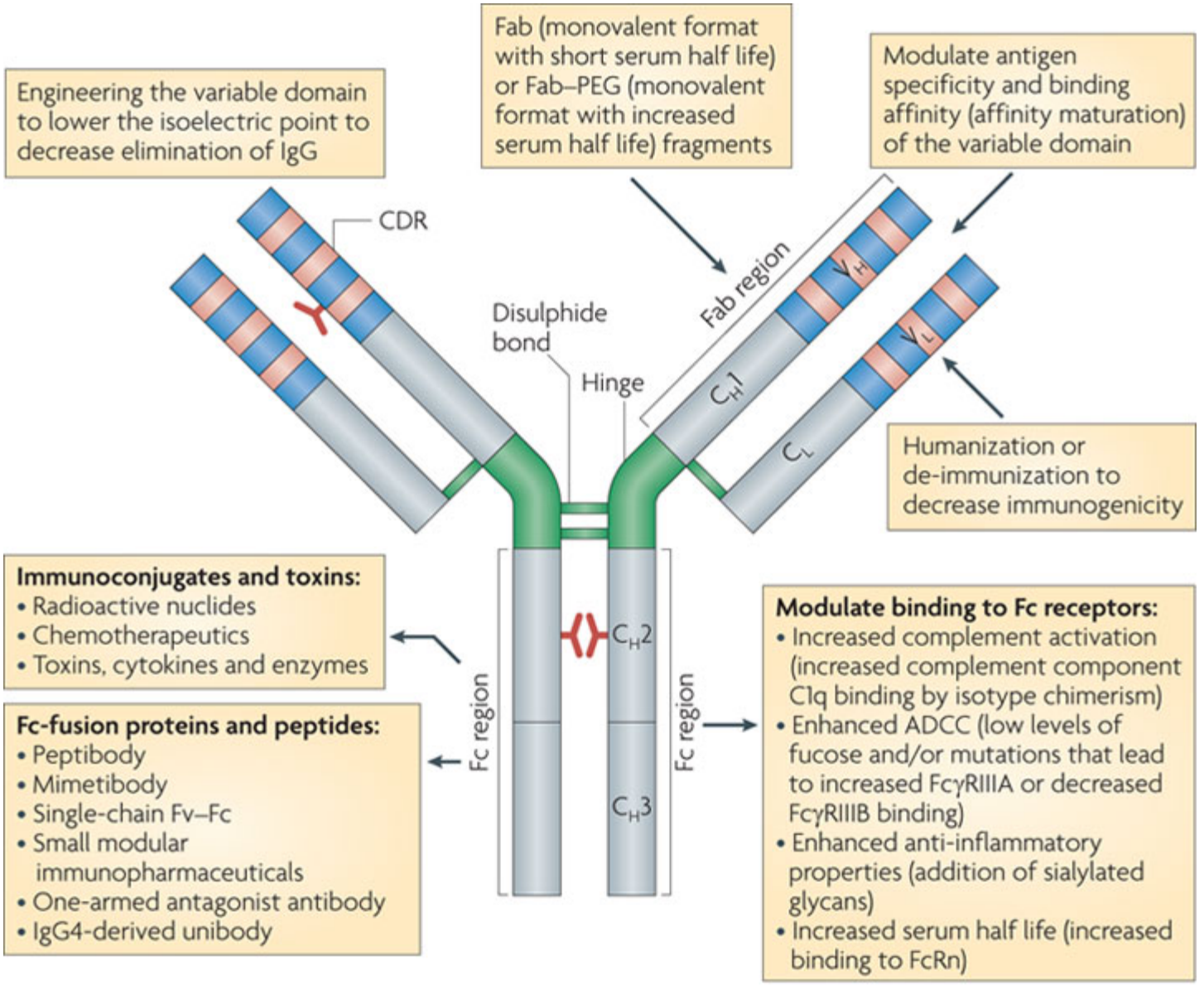
- Glycoprotein synthesized in liver belongs to β_1 - globulin.
- Assists in serum iron transport.
- Iron transport by transferrin is supported by ceruloplasmin; α_2 globulin which transport serum copper iron.
- Ferritin is another protein important to store iron in cells. In excess iron; hemochromatosis, serum ferritin is significantly elevated.

Immunoglobulins (γ globulins)

- Chemically called Antibodies
- Produced in the plasma cells.
- 5 types of γ-globulins.

1. IgG
   - 70 – 80% of total immunoglobulins in circulation.
   - Major antibody of secondary immune response
   - 4 subunits
   - Subdivided into IgG1, IgG2, IgG3 and IgG4 depending on the amino acid sequence of the heavy chain constant region..
2. IgM
   - Macroglobulins, natural antibodies
   - 5 subunits
   - Predominant class of antibodies in primary response.
3. IgA
   - Present as monomer in serum
   - Dimeric forms are the secretary antibodies from secretions of GIT, Urinary tract, saliva, tears, sweat, nasal discharge.
4. IgE
   - cytophilic antibodies- mediate allergy, hypersensitivity & anaphylaxis
5. IgD

How does the immunoglobulin appear



Nature Reviews | Immunology

Plasma proteins in disease diagnosis

- Levels of different blood proteins rise or fall in response to disorders as cancer, intestinal or kidney protein-wasting syndromes, disorders of the immune system, liver dysfunction, impaired nutrition, and chronic fluid-retaining conditions.

- Electrophoretic analysis of the plasma proteins is commonly used to evaluate, diagnose, and monitor a variety of diseases and conditions.

TOTAL PROTEIN ABNORMALITIES

- Normal protein level: 6 – 8 gm/ dl
  albumin level: 4 – 5 gm / dl

- HYPOPROTEINEMIA
  - occurs where a negative nitrogen balance exists

- HYPERPROTEINEMIA
  - Elevated concentration of total serum proteins.

Abnormal results

- Albumin
  - Levels are increased in dehydration.
  - Decreased in malnutrition, pregnancy, liver disease, inflammatory diseases, and such protein-losing syndromes as malabsorption syndrome and certain kidney disorders.
- α1 globulins
  - Increased in inflammatory diseases.
  - Decreased or absent in juvenile pulmonary emphysema, which is a genetic disease.
- α2 globulins
  - Increased in a kidney disorder called nephrotic syndrome.
  - Decreased in patients with an overactive thyroid gland (hyperthyroidism) or severe liver dysfunction.
- β-globulins
  - Levels are increased in conditions of high cholesterol levels (hypercholesterolemia) and iron deficiency anemia.
  - Decreased in malnutrition.
- γ-globulins
  - Levels are increased in chronic inflammatory disease (for example, rheumatoid arthritis, systemic lupus erythematosus); cirrhosis; acute and chronic infection; and a cancerous disease characterized by uncontrolled multiplication of plasma cells in the bone marrow (multiple myeloma).
  - Decreased in a variety of genetic immune disorders, and in secondary immune deficiency related to steroid use, leukemia, or severe infection.

TABLE 2

Characteristic Patterns of Acute-Reaction Proteins Found on Serum Protein Electrophoresis and Associated Conditions or Disorders

Increased albumin

Dehydration

Decreased albumin

Chronic cachectic or wasting diseases

Chronic infections

Hemorrhage, burns, or protein-losing enteropathies

Impaired liver function resulting from decreased synthesis of albumin

Malnutrition

Nephrotic syndrome

Pregnancy

Increased $alpha_1$ globulins

Pregnancy

Decreased $alpha_1$ globulins

Alpha$_1$-antitrypsin deficiency

Increased $alpha_2$ globulins

Adrenal insufficiency

Adrenocorticosteroid therapy

Advanced diabetes mellitus

Nephrotic syndrome

Decreased $alpha_2$ globulins

Malnutrition

Megaloblastic anemia

Protein-losing enteropathies

Severe liver disease

Wilson's disease

Increased $beta_1$ or $beta_2$ globulins

Biliary cirrhosis

Carcinoma (sometimes)

Cushing's disease

Diabetes mellitus (some cases)

Hypothyroidism

Iron deficiency anemia

Malignant hypertension

Nephrosis

Polyarteritis nodosa

Obstructive jaundice

Third-trimester pregnancy

Decreased $beta_1$ or $beta_2$ globulins

Protein malnutrition

Increased gamma globulins

Amyloidosis

Chronic infections (granulomatous diseases)

Chronic lymphocytic leukemia

Cirrhosis

Hodgkin's disease

Malignant lymphoma

Multiple myeloma

Rheumatoid and collagen diseases (connective tissue disorders)

Waldenström's macroglobulinemia

Decreased gamma globulins

Agammaglobulinemia

Hypogammaglobulinemia

Serum protein electrophoresis

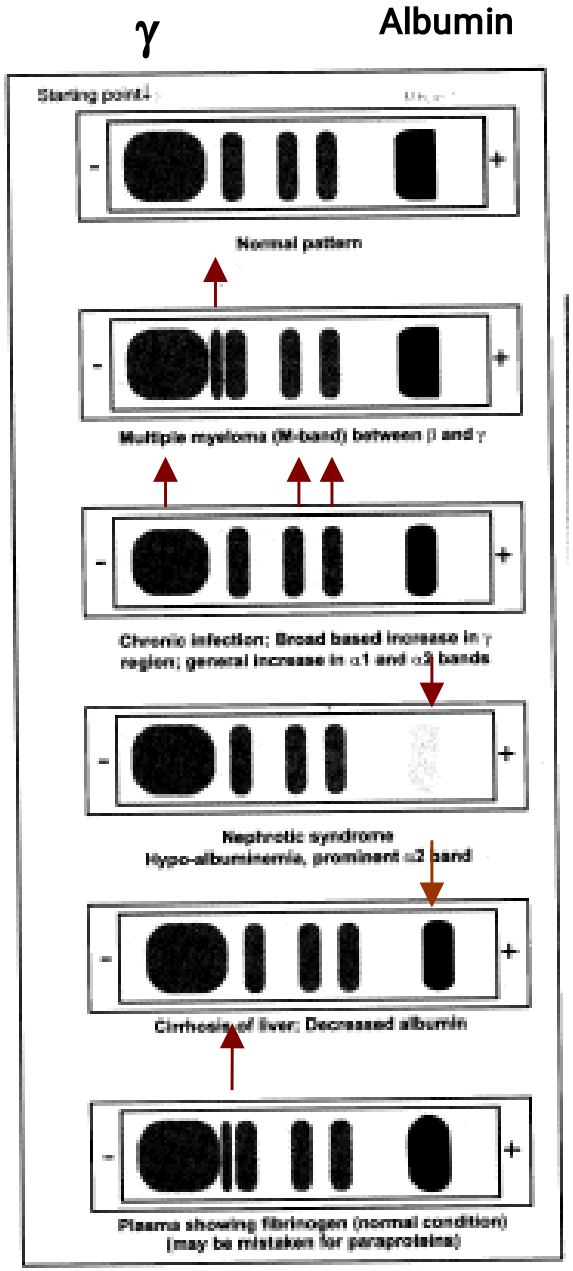


Fig. 21.1. Serum electrophoretic patterns

Normal pattern

Multiple myeloma : (M band) between β and γ

Chronic infection: Increase in γ region, general increase in α1 and α2 bands.

Nephrotic syndrome: hypo-albuminaemia, prominent α2 band.

Cirrhosis of liver: decreased albumin

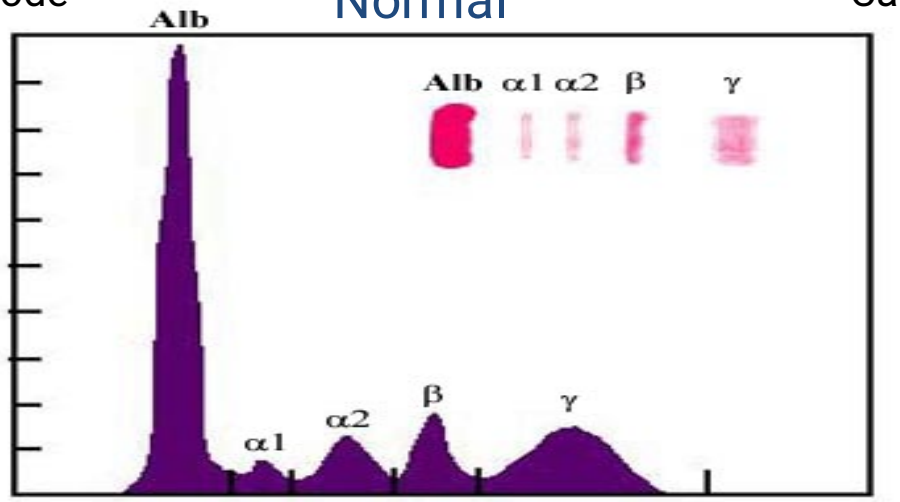
Plasma showing fibrinogen

Understanding & interpreting serum protein electrophoresis

Anode

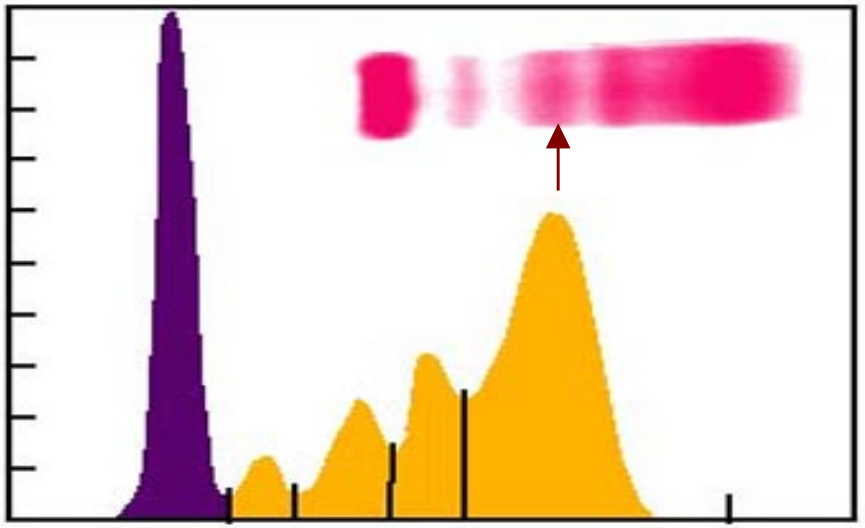
Cathode

Normal



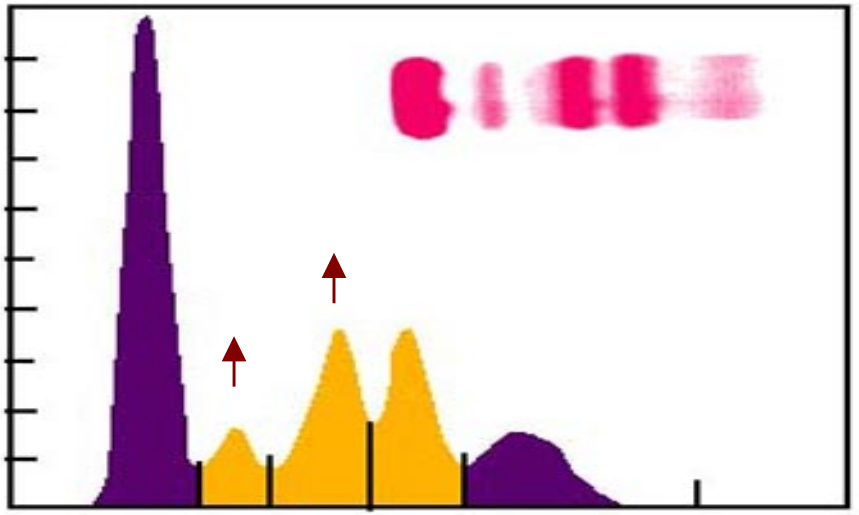
Electrophoretic pattern of a healthy individual indicates five distinct bands depending on their mobility

Polyclonal Gammopathy



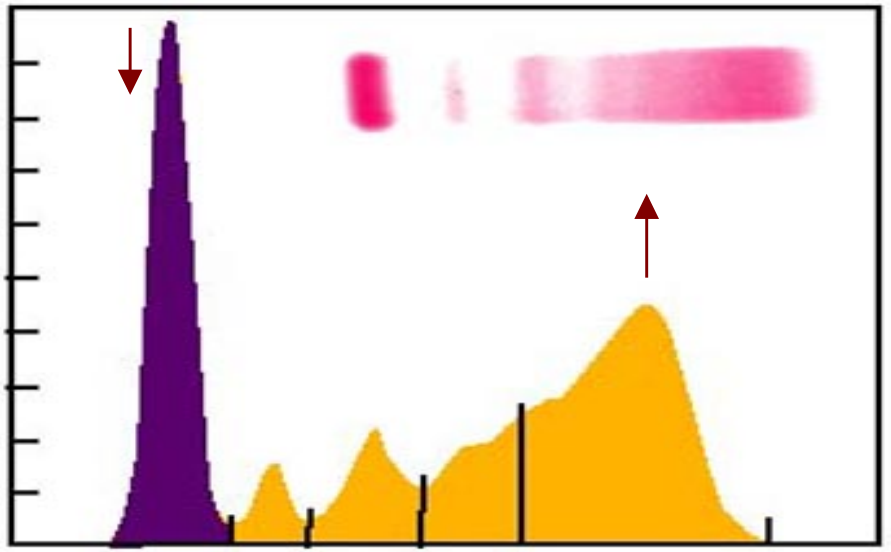
Gammopathy is a disturbance in immunoglobulin synthesis where there is a heterogeneous increase in immunoglobulins involving more than one cell line.

Acute inflammatory pattern



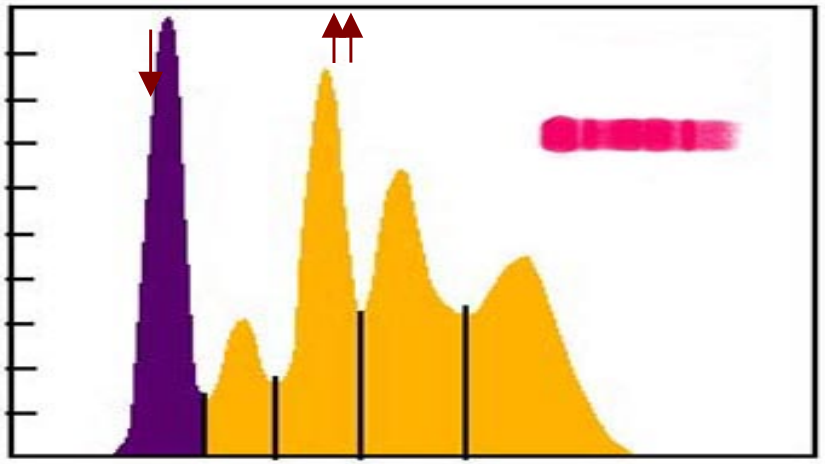
α1 globulins are increased in inflammatory diseases

Hepatic Cirrhosis



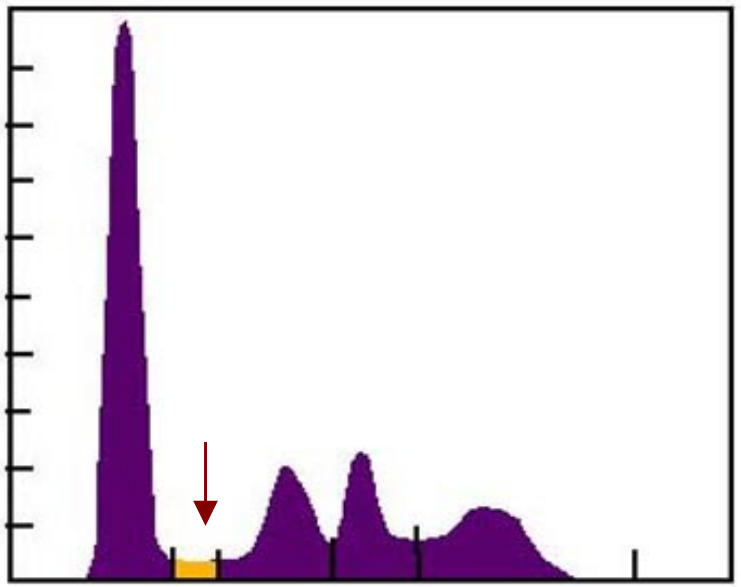
A slight decrease in albumin, slight increase in γ-globulins & normal α2 globulins.

Nephrotic syndrome



α2 globulins are increased in nephrotic syndrome. Selective loss of lower molecular weight proteins.

Alpha 1 anti-trypsin deficiency



Low levels or no α-1 antitrypsin.

- α-1 antitrypsin is a protein made in the liver which releases it into the bloodstream.
- α-1 antitrypsin protects the lungs so they can work normally.
- Without enough α-1 antitrypsin, the lungs can be damaged and may make breathing difficult.
- In addition, liver damage (hepatitis, cirrhosis) can occur.
- α-1 antitrypsin deficiency is an inherited disorder with no or little α-1 antitrypsin in the blood.

Paraproteins

Plasma cells → Immunoglobulins (Ig)

Each type of Ig is synthesized by a specific group of plasma cells (clone).

Polyclonal hyper-gammaglobulinaemia

Proliferation of several clones of plasma cells leading to increased production of several types of Ig.

Eg. chronic infection

This shows as a diffuse rise in the gamma globulin band.

Monoclonal hyper-gammaglobulinaemia

Proliferation of a single clone of plasma cells leading to over production of a single type of Ig (paraprotein)

Ig produced by monoclonal proliferation is referred to as a paraprotein.

This shows as a discrete (sharp) gamma globulin band.

Monoclonal proliferation is often (but not always) malignant.

Paraproteinaemia –

(1) Myelomatosis (multiple myeloma) abnormal proliferation of plasma cells in bone marrow. Increased is IgG.

(2) Waldenstrom`s macroglobulinaemia malignancy of B cells. Increased IgM.

Bence Jones Proteins (BJP)

Special proteins which appear in urine of patients with malignancy of B cells.

BJP consists of free monoclonal light chains or fragments of them which have been synthesised in excess of heavy chains.

Due to low MW they are filtered through the glomerulus and appear in urine.

Detected in urine by heating the sample: precipitates at boiling temp. and dissolve upon further heating.


THANK YOU