Chapter 13

Plasma Proteins and Enzymes

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Clinical Chemistry

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Introduction

Functions of plasma proteins

Function	Example
transport	thyroxine-binding globulin (thyroid hormones) apolipoproteins (cholesterol, triglyceride) transferrin (iron)
humoral immunity	immunoglobulins
maintenance of oncotic pressure	all proteins, particularly albumin
enzymes	renin coagulation factors complement proteins
protease inhibitors	α_1-antitrypsin (acts on proteases)
buffering	all proteins

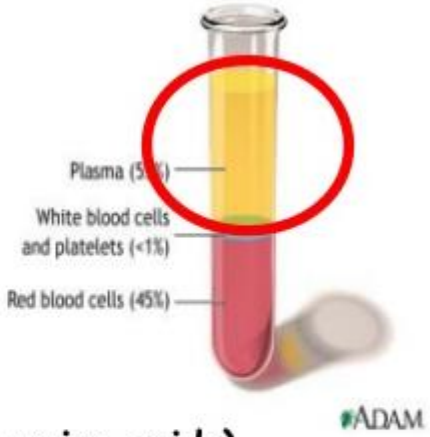
Figure 13.1 Functions of plasma proteins.

Measurement of Plasma Proteins

- Total Plasma Protein
- **concentration of total protein in human plasma is approximately 6.0–8.0 g/dL**

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)

Causes of changes in total plasma protein concentration			
Increase		**Decrease**	
hypergammaglobulinaemia paraproteinaemia	↑ protein synthesis	malnutrition and malabsorption liver disease humoral immunodeficiency	↓ protein synthesis
artefactual	haemoconcentration due to stasis of blood during venepuncture	over-hydration increased capillary permeability	↑ volume of distribution
dehydration	↓ volume of distribution	protein-losing states catabolic states	↑ excretion/catabolism

Figure 13.2 Causes of changes in total plasma protein concentration.

Art efactual

posture: an increase in concentration of 10–20% occurs within 30 min of becoming upright after a period of recumbency.

Tourniquet is applied before venepuncture, a significant rise in protein concentration can occur within a few minutes. In both cases, the change in protein concentration is caused by increased diffusion of fluid from the vascular into the interstitial compartment. These effects must be borne in mind when blood is being drawn for the determination of protein concentration.

Protein electrophoresis

Principal plasma proteins		
Class	**Protein**	**Approximate mean serum concentration (g/L)**
	prealbumin	0.25
	albumin	40
α_1-globulin	α_1-antitrypsin	2.9
	α_1-acid glycoprotein	1.0
α_2-globulin	haptoglobins	2.0
	α_2-macroglobulin	2.6
	caeruloplasmin	0.35
β-globulin	transferrin	3.0
	low density lipoprotein	1.0
	complement components (C3)	1.0
γ-globulins	IgG	14.0
	IgA	3.5
	IgM	1.5
	IgD	0.03
	IgE	trace

Figure 13.3 Principal plasma proteins. Many other important proteins are present in only very low concentrations, for example thyroxine-binding globulin, transcortin and vitamin-D-binding globulin.

Electrophoresis in diagnosis

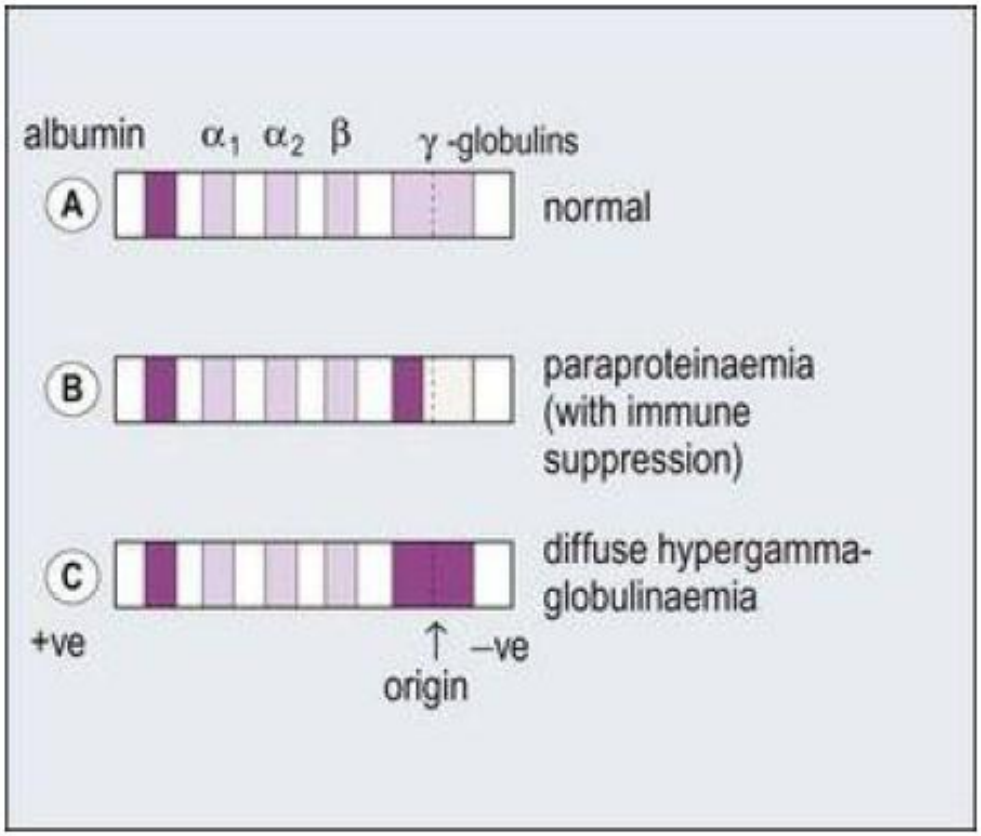


Figure 13.4 Some typical serum electrophoretic abnormalities.

Plasma proteins

Transthyretin **(prealbumin)**

- Synthesis in liver
- Transthyretin **(prealbumin)** (half life 2 days).
- Migrate before albumin in electrophoresis
- Function: transport protein in serum and CSF that carry thyroxin and retinol-binding protein bound to retinol (vitamin A).

Clinical significance:

Prealbumin increased in:

- ✓ Patients receiving steroids
- ✓ Chronic renal failure
- ✓ Alcoholism

Prealbumin decreased in:

- ✓ Poor protein nutrition or increase catabolism.
- ✓ Acute phase inflammatory response
- ✓ Hepatic damage

Specific plasma proteins

- **Albumin**
- Major plasma proteins
- **Synthesis** and secreted by the liver.
- Biological **half life** in plasma is about 20 days.
- **Function**: contributing to **plasma oncotic pressure** in both vascular and extravascular spaces.
- **Albumin** have ability to **bind** and **transport** a large number of compounds such as fatty acids, phospholipids, metal ion Ca^{+2}, Mg^{+2}), amino acids, drugs, hormones (ex; **cortisol**) and bilirubin.

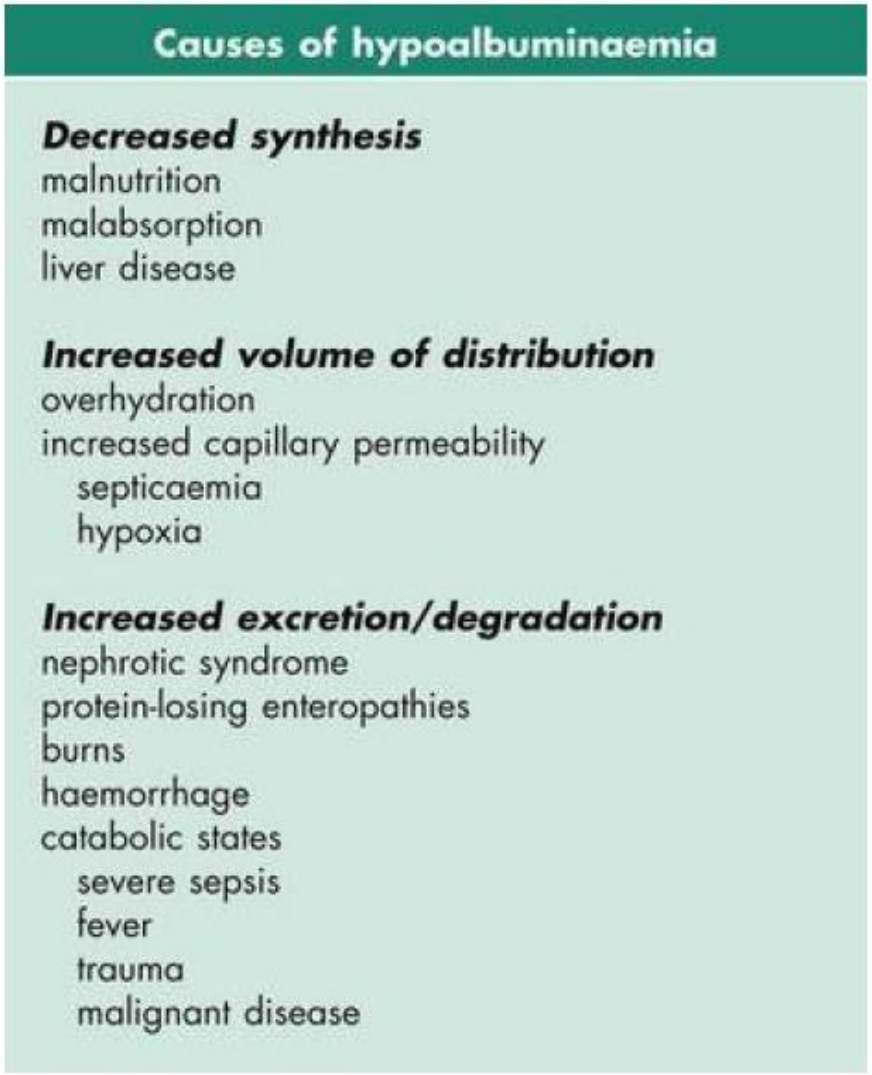


Figure 13.6 Causes of hypoalbuminaemia.

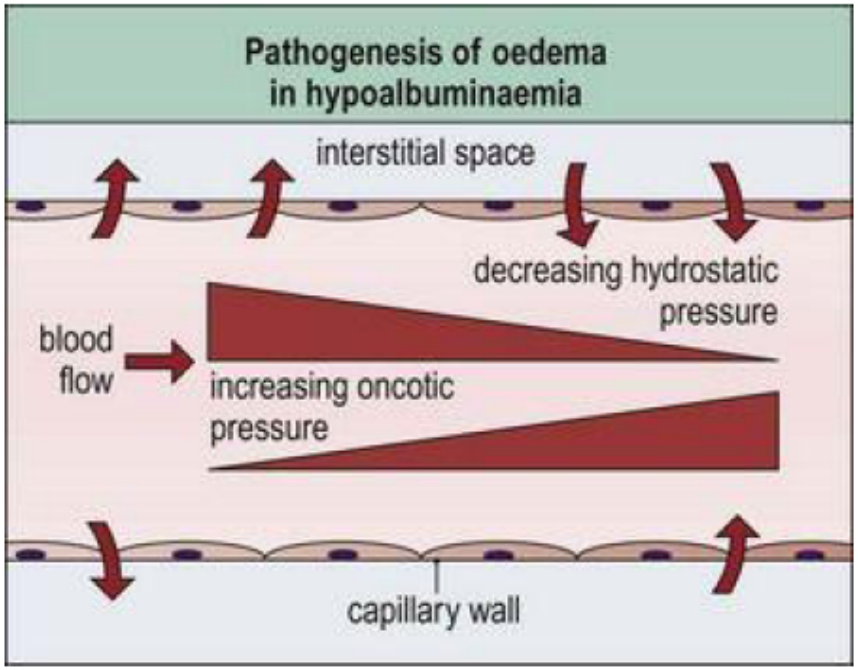


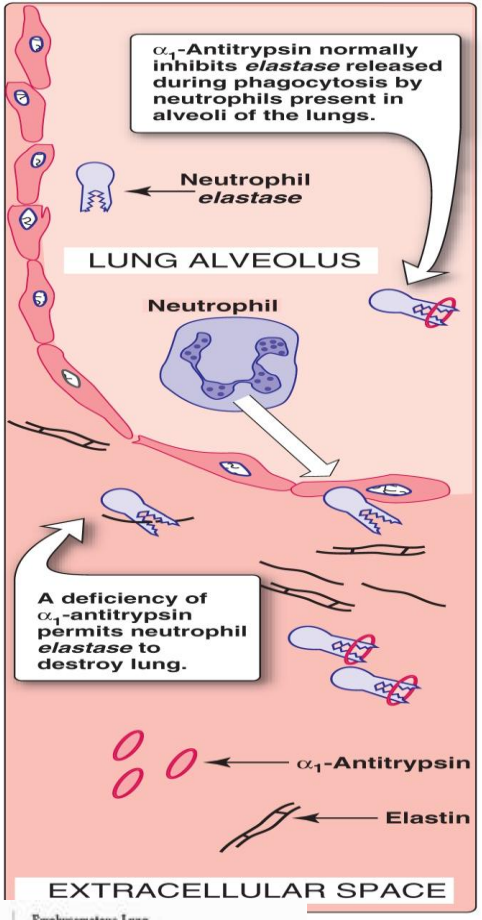
Figure 13.5 Pathogenesis of oedema in hypoalbuminaemia. The normal balance of hydrostatic and oncotic pressures is such that there is net movement of fluid out of the capillaries at their arteriolar ends and net movement in at their venular ends (indicated here by arrows). Oedema can thus be due to an increase in capillary hydrostatic pressure, a decrease in plasma oncotic pressure or an increase in capillary permeability.

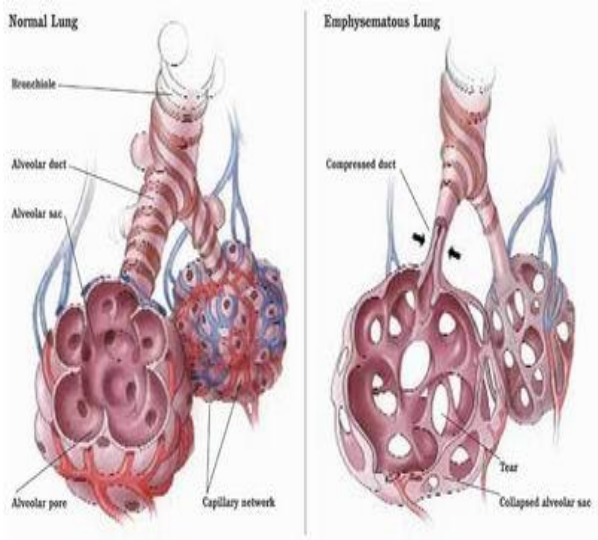
Plasma proteins

α1-antitrypsin or α1-globulins:(110-200 mg/dL)

- Inhibitor of serine proteases enzyme such as elastase and trypsin.
- It is synthesized by hepatocytes and macrophages
- Inherited disorder (decreased) of α1-antitrypsin can cause
  - ✓**Emphysema**
  - ✓**Cirrhosis**

- *Smoking oxidizes a thiol group in methionine at the active site of α1-antitrypsin result decreasing the activity*
- *Pizz homozygotes plasma α1-antitrypsin is reduced to 10%-15%*
- *Pizz heterozygotes plasma α1-antitrypsin concentration that are about 60% of normal.*





Plasma proteins

Haptoglobin (HP) or α2-globulins: (40-180 mg/dL)

- **Synthesized** mostly by hepatocytes but also by other tissues such as skin, lung and kidney.
- **Function:** 1. preventing loss of iron through the kidneys and protecting the kidneys from damage by hemoglobin (suicide protein).
- HP is an acute phase protein
- Because Hb-HP complex is too large to pass through the glomerulus of kidneys. Hb-HP complexes are removed by macrophage system.

Clinical significance:

***increased plasma HP level in patients**

- ✓ **increase** in any **inflammatory** process such as infection, burns, injury and allergy.
- ✓ HP **increase** in nephrotic syndrome.

***Decreased plasma HP level in patients**

- ✓ In **Hemolytic anemia**
- ✓ **Decrease** HP and increase reticulocyte indicate
  - •Spleen damage
  - •Liver damage
  - •drug-induce hemolysis.
- ✓ **Decrease** (Hp) without hemolytic anemia indicate **liver damage** or sever sepsis.

Plasma proteins

α2-macroglobulin (α2M):

High M.Wt protein (820 kDa)

- Synthesis by hepatocytes and macrophages.
- Function: inhibitor of protease enzymes.
- Serum level of **α2M** increase in the nephrotic syndrome (characterized by large proteinuria).

Why?

Plasma proteins

Caeruloplasmin (α2-globulin) (22.9-43.1 g/dL)

- It is copper carrying protein in the blood.
- Acute phase protein.
- Synthesized in liver.
- Caeruloplasmin functions as ferroxidase and superoxidase scavenger.

Clinical significance:

Elevated level of Caeruloplasmin in:

- ✓ Oestrogen-related effect
- ✓ pregnancy

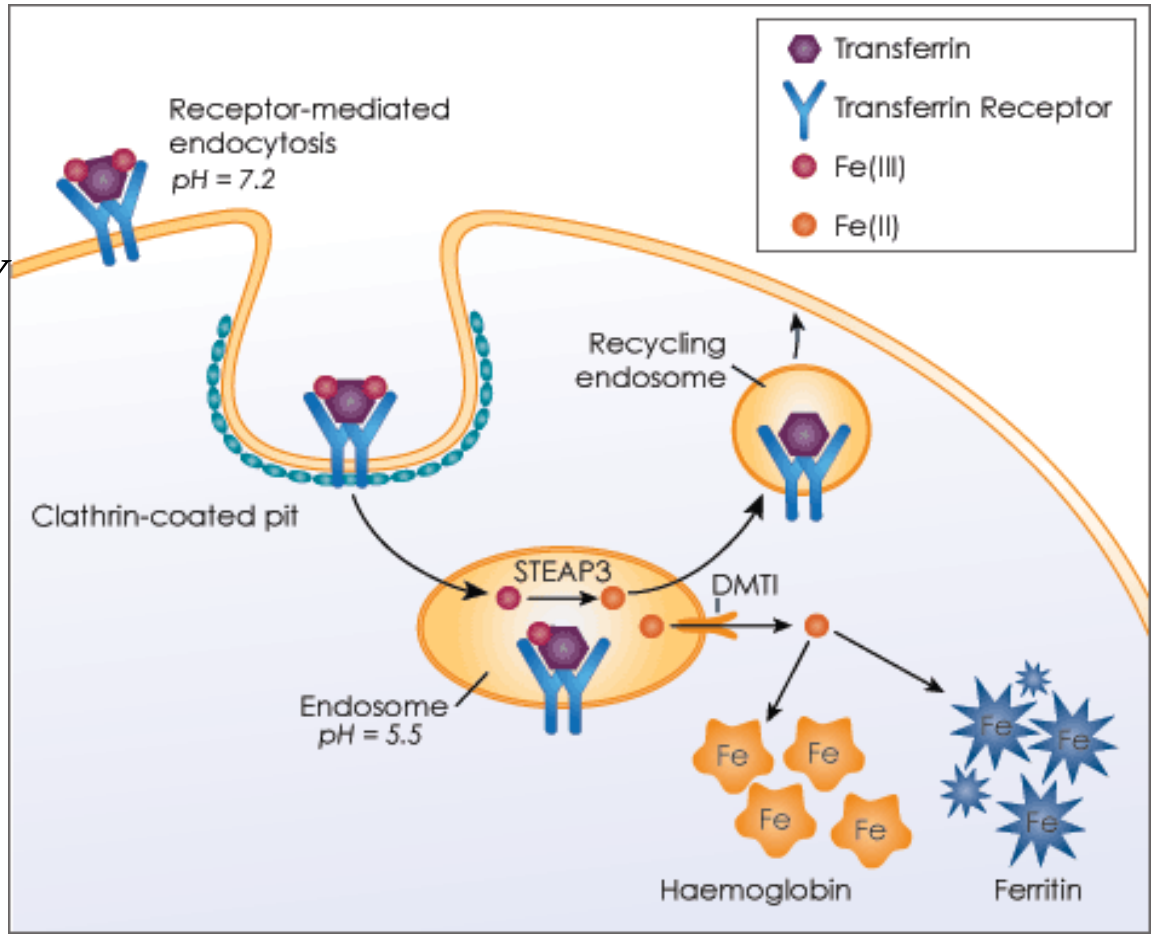
Low level of Caeruloplasmin in:

- ✓ **Wilson disease**
- ✓ Nephrotic syndrome

Transferrin (TF) (adults: 250-425 mg/Dl, Children: 203-360 mg/Dl)

- TF is a β globulin and TF is synthesized in the liver.
- TF are iron-transporting glycoproteins in the plasma that control the level of free iron in biological fluids
- **TF** is a serum protein that carries iron (2 mole of Fe^{+3} per mole of TF) through the blood stream to the bone marrow and other organs.

- ***TF** binds to **Fe**$^{+3}$ by two receptor and transported into the cell in a vesicle by receptor-mediated endocytosis.*
- *Normally, the iron bound to TF turns over 10-20 times a day.*



Clinical significance of Transferrin (TF)

- **Transferrin iron-binding capacity** (**TIBC**) is a medical laboratory test that measures the blood's capacity to bind iron with transferrin.

- **NOTE: Transferrin level related to TIBC level.**

Transferrin and **TIBC increased** in :

- ✓ Iron deficiency anemia.
- ✓ During pregnancy.

Transferrin and **TIBC decreased** in :

- ✓ Liver disease.
- ✓ Nephrosis
- ✓ Hemochromatosis. (Inherited disease cause accumulate of iron in tissue)

Ferritin

Men : 20-300 ng/ml; Women: 20-120 ng/ml

- Ferritin serves to store iron in a non-toxic form and to transport it to areas where it is required.

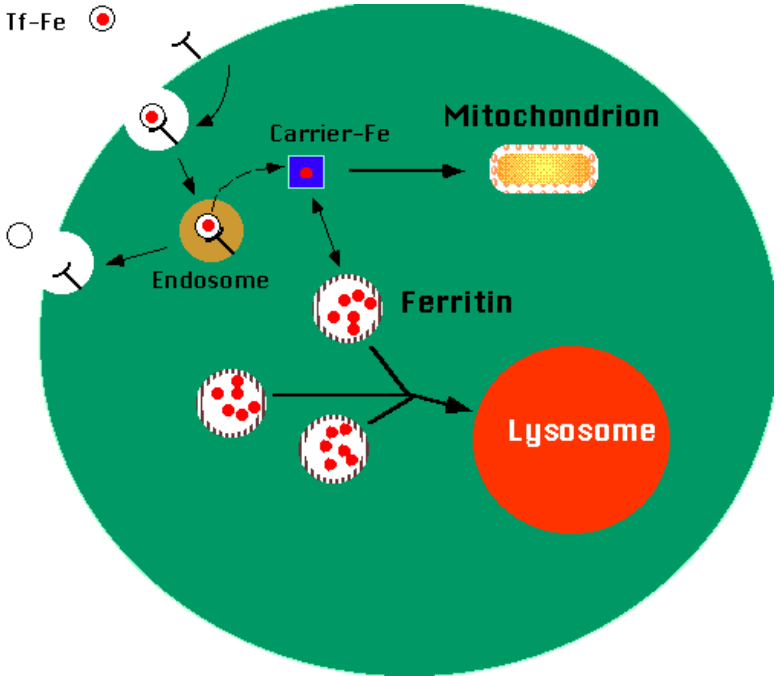
Clinical significance of Ferritin:

***An increased plasma Ferritin level in patients**

- iron overload disorders, such as hemochromatosis or hemosiderosis.
- Leukemia

***Decreased plasma Ferritin level in patients**

- iron deficiency anemia



Acute Phase Proteins

- The term ‘acute phase response’ encompasses a complex range of physiological changes that occur following trauma and in burns, infection, inflammation and other related conditions.
- It comprises **haemodynamic changes, increases in the activity of the coagulation and fibrinolytic systems, leukocytosis, changes in the concentration of many plasma proteins and systemic effects, particularly pyrexia.**
- **It is mediated by a host of cytokines, tumour necrosis** factor and vasoactive substances.

Acute phase proteins and the acute phase response

Acute-phase proteins are a class of proteins whose plasma concentration increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation or other related condition. This response is called the *acute-phase reaction* (also called acute-phase response).

Clinical significance of acute-phase proteins:

Positive acute-phase proteins (increase); increase synthesis by interlokin-6

- C-reactive protein
- Fibrinogen, prothrombin
- Alpha 2-macroglobulin
- Ferritin
- Ceruloplasmin
- Haptoglobin
- Alpha 1-antitrypsin

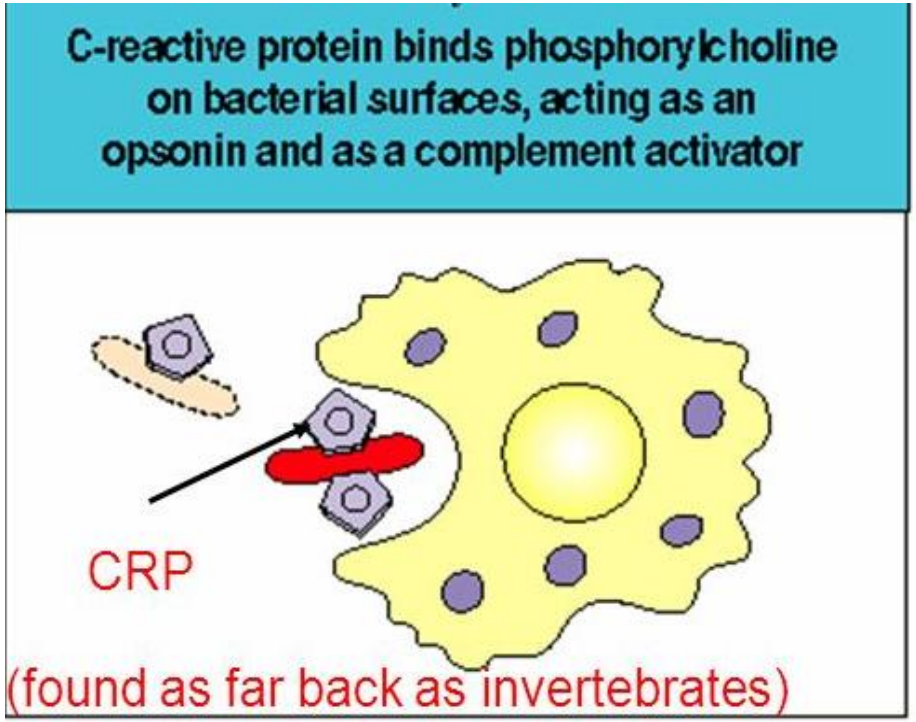
Negative acute-phase proteins (decrease)

albumin, transferrin, retinol-binding protein, antithrombin

C-reactive protein (CRP) (<5mg/dL)

- Named because reacted with the somatic 'C' polysaccharide antigen of Pneumococcus.
- Inflammation release of interleukin-6 and other cytokines that trigger the synthesis of CRP by the liver.
- CRP binds to phosphocholine on the surface of dead and some bacteria. This **activates** the **complement system** (C1q), promoting **phagocytosis** by macrophages, which clears necrotic, apoptotic cell and bacteria.
- half-life of 18 hours.

- **CRP** rises within **6 hours** of the onset of inflammation and peaks at **48 hours before beginning to fall**.



C-reactive protein (CRP)

Clinical significance of CRP:

CRP increased in:

- ✓ Viral and bacterial infections
- ✓ Myocardial infarction (MI) or cardiovascular disease.
- ✓ rheumatoid arthritis

Note: can be used to **screen** for inflammation.

Note: Interferon alpha inhibits CRP production from liver cells which may explain the relatively low levels of CRP found during viral infections compared to bacterial infections.

D-Dimer

- What is D-DIMER????

Fibrinogen (1.5-3 g/L)

- **Fibrinogen (Factor I)** is a glycoprotein in vertebrates that helps in the formation of blood clots.
- Synthesis in the liver.
- fibrinogen molecule is a soluble, large, and complex 340 kDa.
- **Fibrinogen** converted by thrombin into fibrin gel during blood clot formation.
- fibrinogen use in the investigation of some bleeding disorders.

Increase fibrinogen levels include:

- ✓Acute or chronic inflammatory
- ✓Nephrotic syndrome
- ✓Liver disease and cirrhosis
- ✓Pregnancy or estrogen therapy
- ✓Compensated intravascular coagulation

decreased fibrinogen levels include:

- ✓disseminated intravascular coagulation [DIC]).
- ✓ advanced liver disease

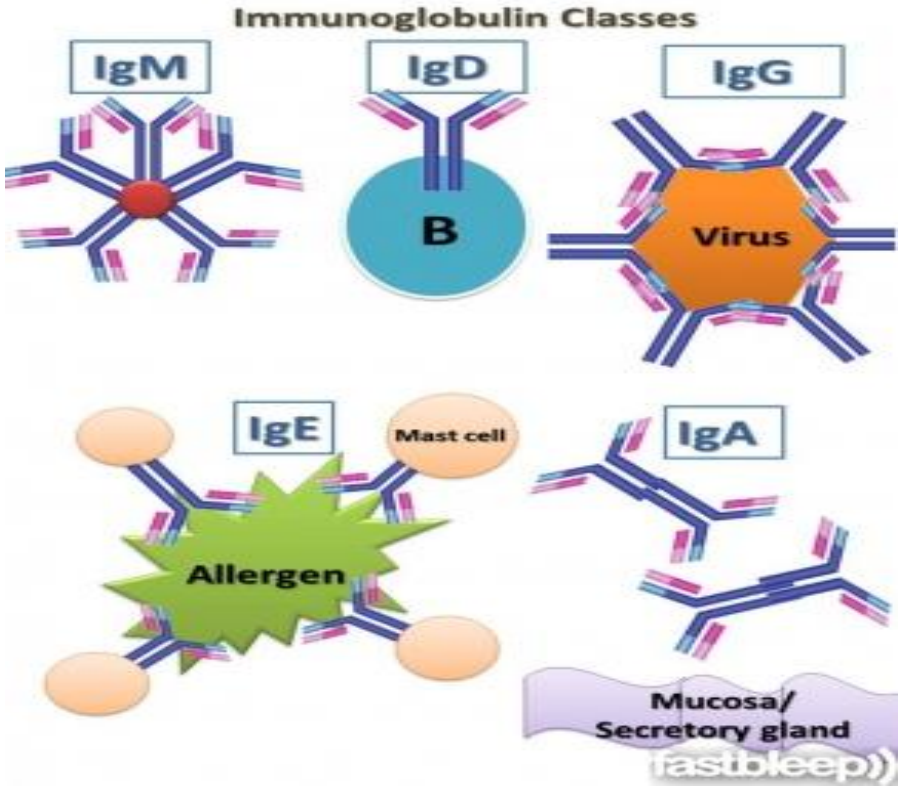
Other plasma proteins

- Proteins of Complement system investigate immunological disease and high levels are found in chronic infections.

- **β_2 microglobulin** is a component of **MHC class I** molecules, which are present on all nucleated cells can be elevated in multiple **myeloma and lymphoma.**

Immunoglobulins (Ig)

- An antibody (Ab), also known as an **immunoglobulin** (Ig).
- Ig behave mainly as **γ-globulins**.
- Ig play a key role in the defense mechanisms of the body.
- Types of heavy chain : α (alpha), γ (gamma), δ (delta), ε (epsilon), and μ (mu). This gives 5 types of immunoglobulins **IgA, IgG, IgD, IgE,** and **IgM**.
- Two type of light chain (κ,λ)



Immunoglobulins (Ig)

Structure of immunoglobulins

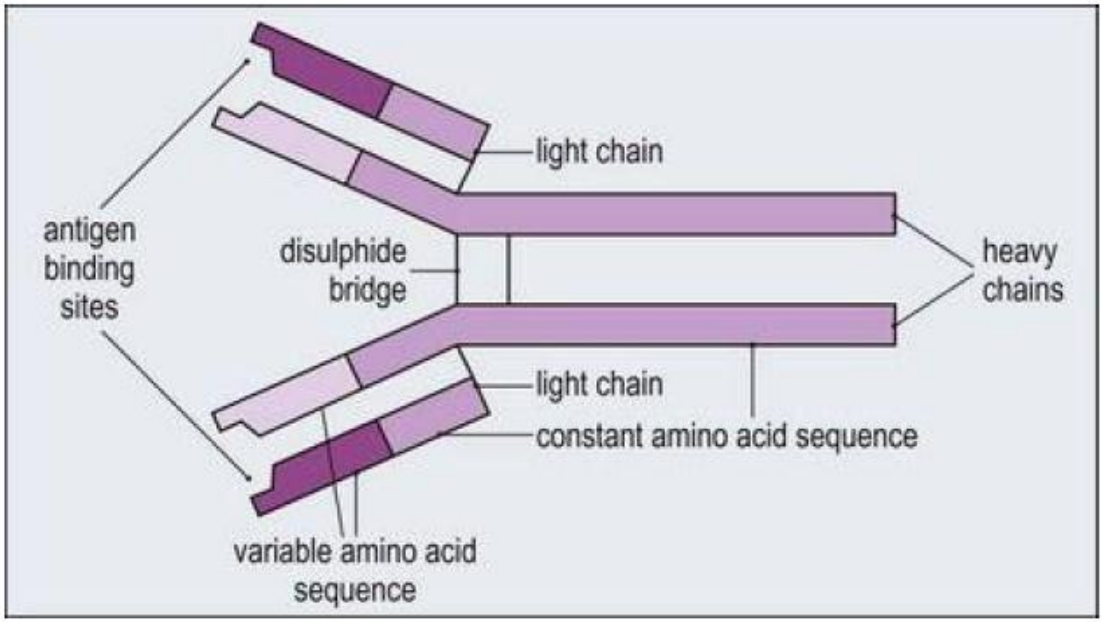


Figure 13.7 Structure of immunoglobulins. All immunoglobulins have the same basic structure. IgM consists of a pentamer of the basic structure. IgA is secreted as a dimer.

Class	Heavy chain	Mean plasma concentration (g/L)	Molecular weight (kDa)	Function
IgG	γ	14.0	146	the major antibody of secondary immune responses
IgA	α	3.5	160	secreted as a dimer (molecular weight 385 kDa) the major antibody in seromucous secretions, e.g. saliva, intestine, bronchial mucus
IgM	μ	1.5	970	a pentamer, confined to the vascular spaces the major antibody of the primary immune response
IgD	δ	0.03	184	present on the surface of B-lymphocytes, involved in antigen recognition
IgE	ε	trace	188	present on surface of mast cells and basophils probable role in immunity to helminths and associated with immediate hypersensitivity reactions

Figure 13.8 Characteristics of the immunoglobulins. Immunoglobulins of each class conta
either κ or γ light chains. In IgA and IgG, slight variations in the structure of the constant regions

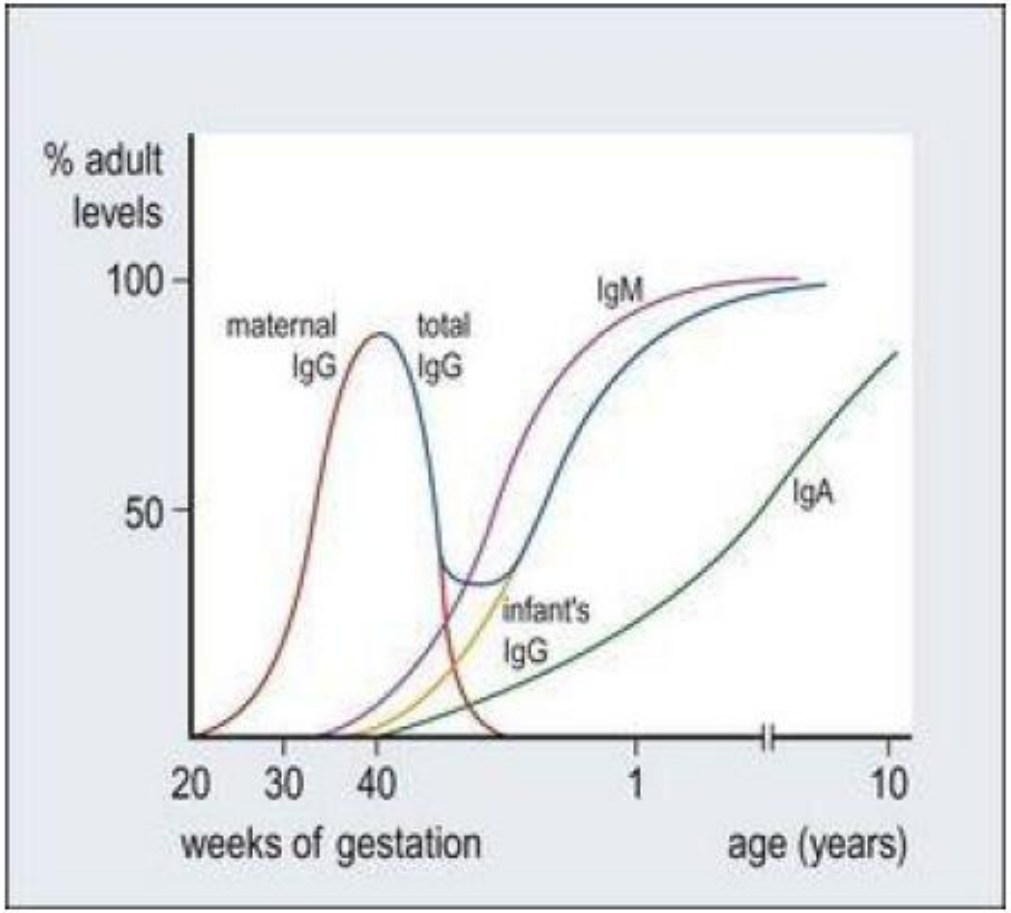


Figure 13.9 Changes in plasma immunoglobulin concentrations with age.

Immunoglobulins (Ig)

Increase in concentration of Ig (polyclonal antibodies) in:

- ✓ acute and chronic **infection**
- ✓ **autoimmune disease** such as rheumatoid disease, Systemic lupus erythematosus.
- ✓ chronic liver disease.

Hypergammaglobulinemia

1)Polyclonal-

- ❑ Chronic infections
- ❑ Chronic liver diseases
- ❑ Sarcoidosis
- ❑ Auto immune diseases

Hypogammaglobulinemia

- **Losses from body-** same as albumin- through urine, GIT or skin
- Decreased synthesis
- Transient neonatal
- Primary genetic deficiency
- **Secondary –** drug induced (Corticosteroid therapy), uremia, hematological disorders
- AIDS(Acquired Immuno deficiency syndrome)

- **Monoclonal increases in :**

- ❑ Multiple myeloma
- ❑ Macroglobulinaemia
- ❑ Lymphosarcoma
- ❑ Leukemia
- ❑ Hodgkin’s disease

Immunoglobulins (Ig)

Paraproteins

- A paraproteins is an Ig produced by a single clone of cells of the B-lymphocyte (monoclonal gammaglobulin).
- **Decrease or normal** concentration of Ig in patients with **paraproteinaemia** due to **myeloma**. (note: paraproteinaemia is excessive amounts of paraprotein)
- Immunoglobulins light chain found in urine known as bence Jones Protein and present in 75% of myeloma cases.
- paraproteins increase in 3% in people over the age of 70.

Incidence of paraprotein types	
Protein	**Incidence (%)**
IgG	52
IgA	21
IgD	1.5
IgM (predominantly WM)	12
Bence Jones only	11

Figure 13.10 Types of paraproteins in myeloma and Waldenström's macroglobulinaemia (WM). Up to 75% of patients have detectable free light chains (Bence Jones protein) in urine. IgE and IgM myelomas occur, but are very rare. In about 1% of all cases, no paraprotein can be detected.

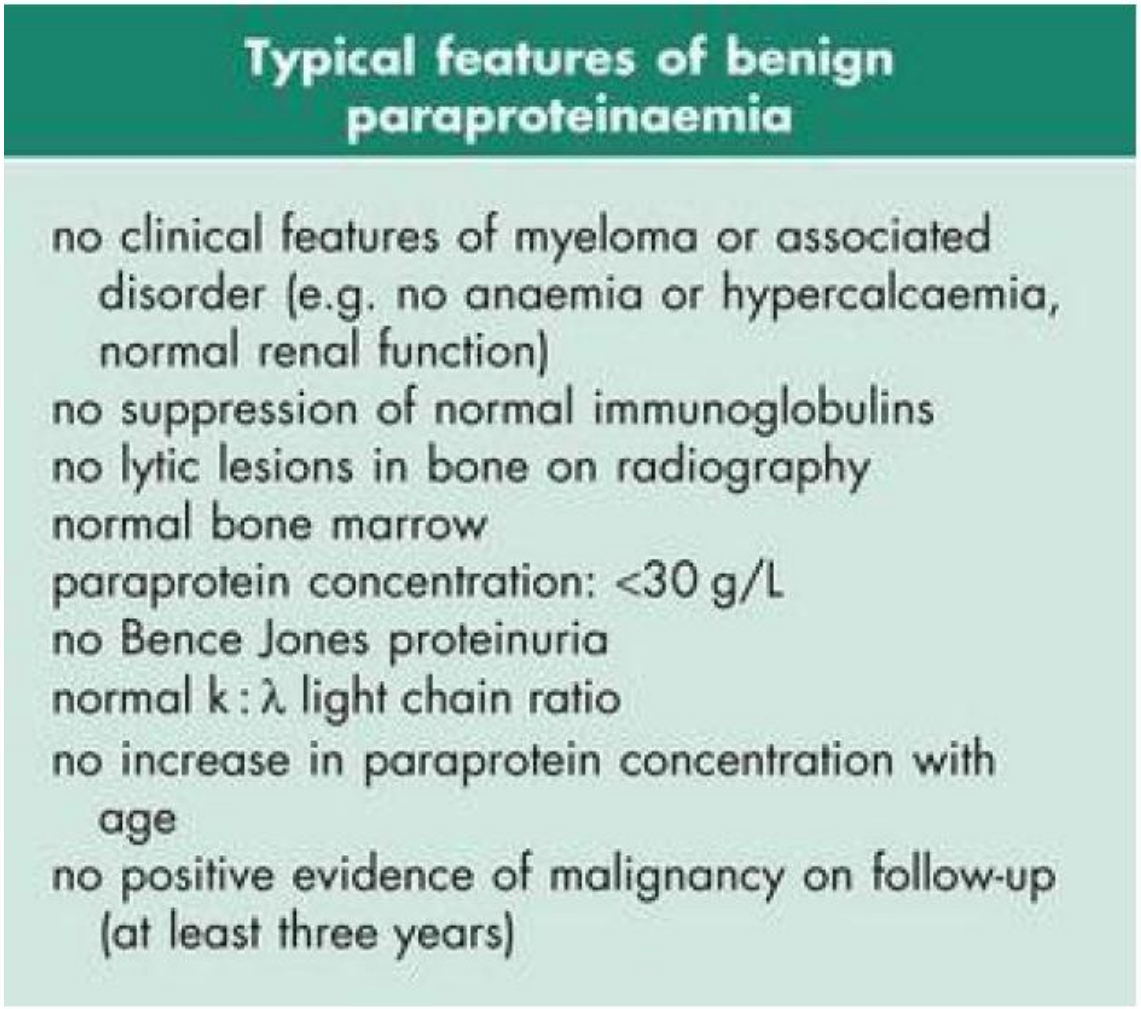


Figure 13.11 Typical features of benign paraproteinaemia.

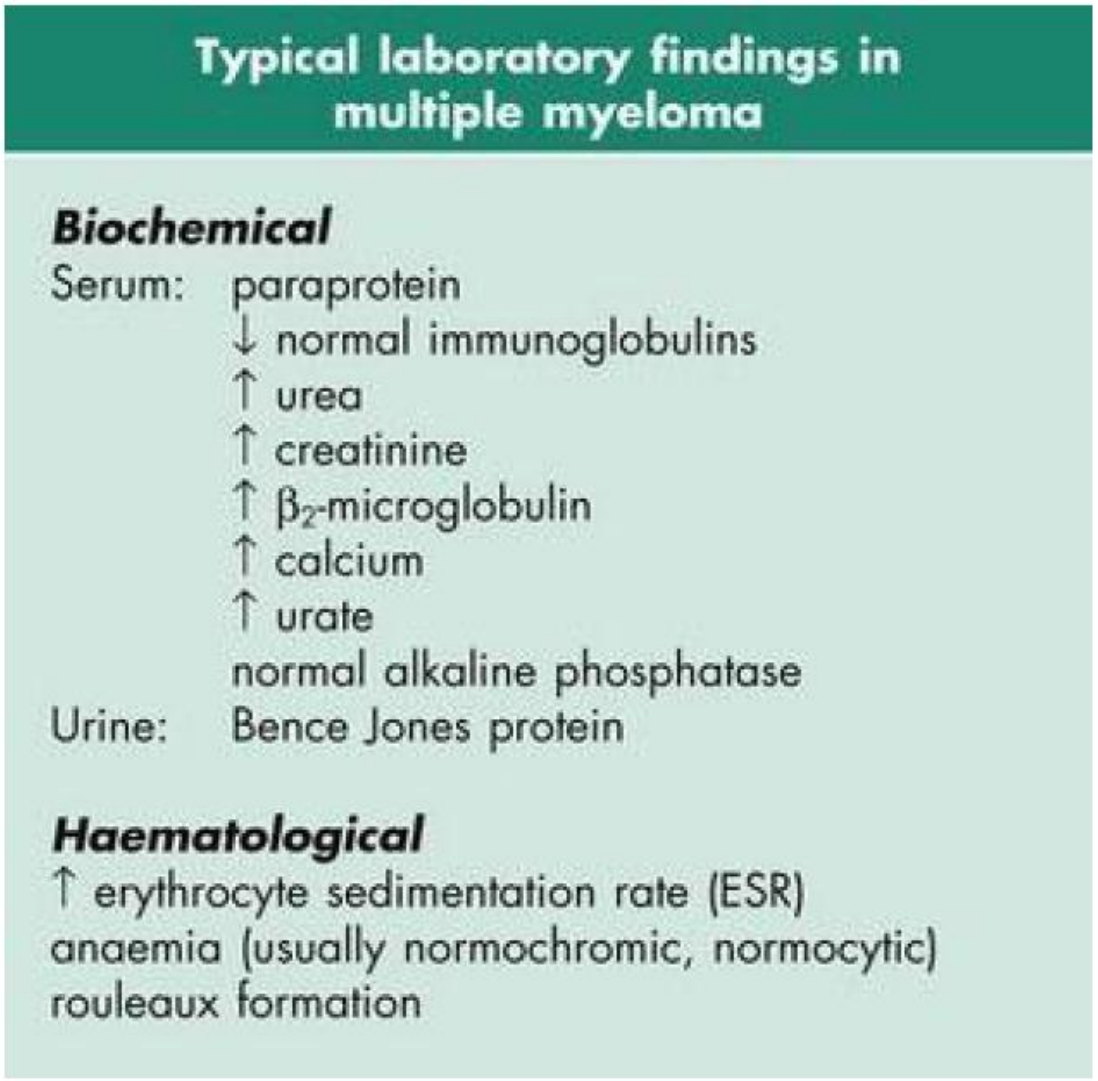


Figure 13.12 Typical laboratory findings in multiple myeloma.

Cytokines

- **Cytokines** are large group of autocrine (secrete by cell=chemical messenger) and paracrine (cell-cell communication) regulatory peptides, which modulate the activity of the immune system and are involved in the coordination of acute inflammation and the immune response.

Four major of cytokines are recognized:

- Interleukins (IL), which are regulators of inflammation.
- Interferones (IF), antiviral agents, and inhibitory effect on cell growth.
- Colony-stimulating factors (CF), stimulate the growth of macrophages and white blood cells.
- Tumor necrosis factors (TNF), stimulate the proliferation of many cells, including cytolytic T-cells.

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