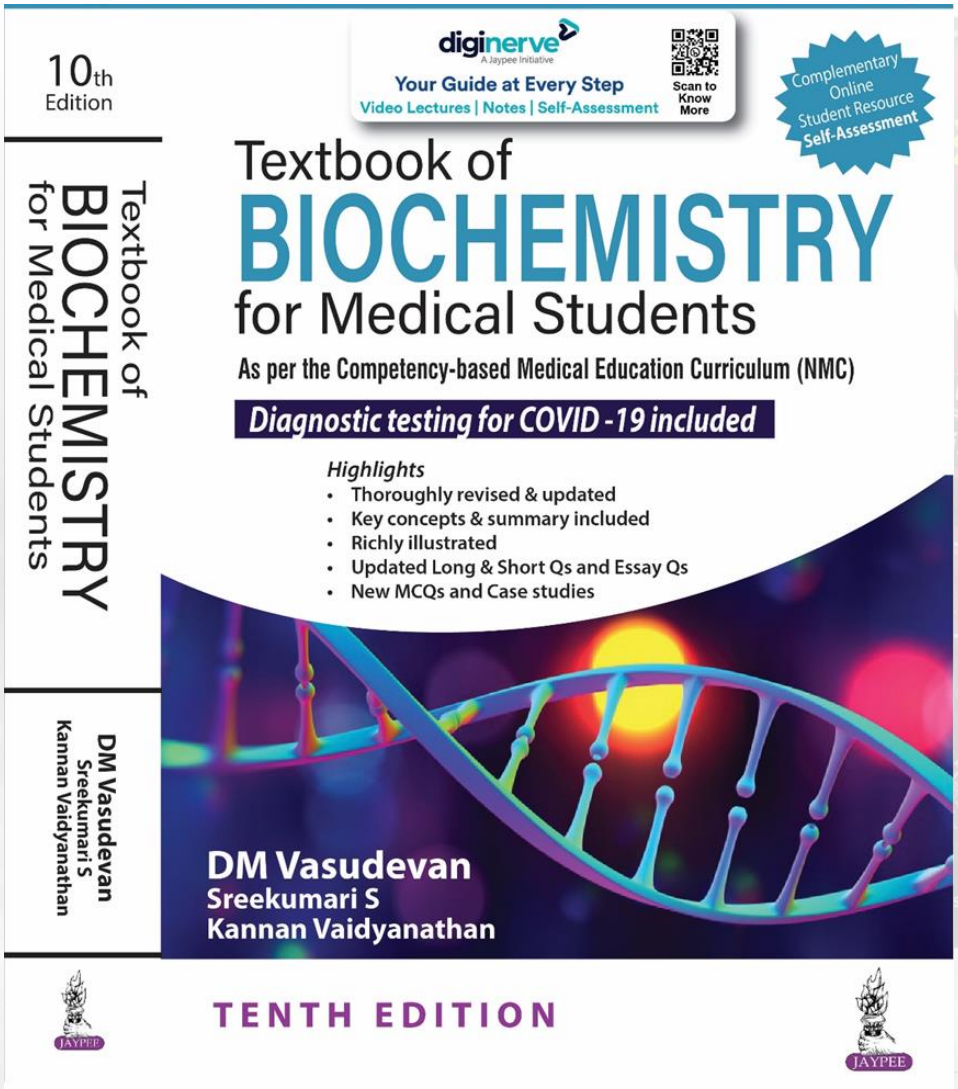
Chapter 23:

Plasma Proteins



Textbook of

BIOCHEMISTRY

for Medical Students

By DM Vasudevan, *et al.*

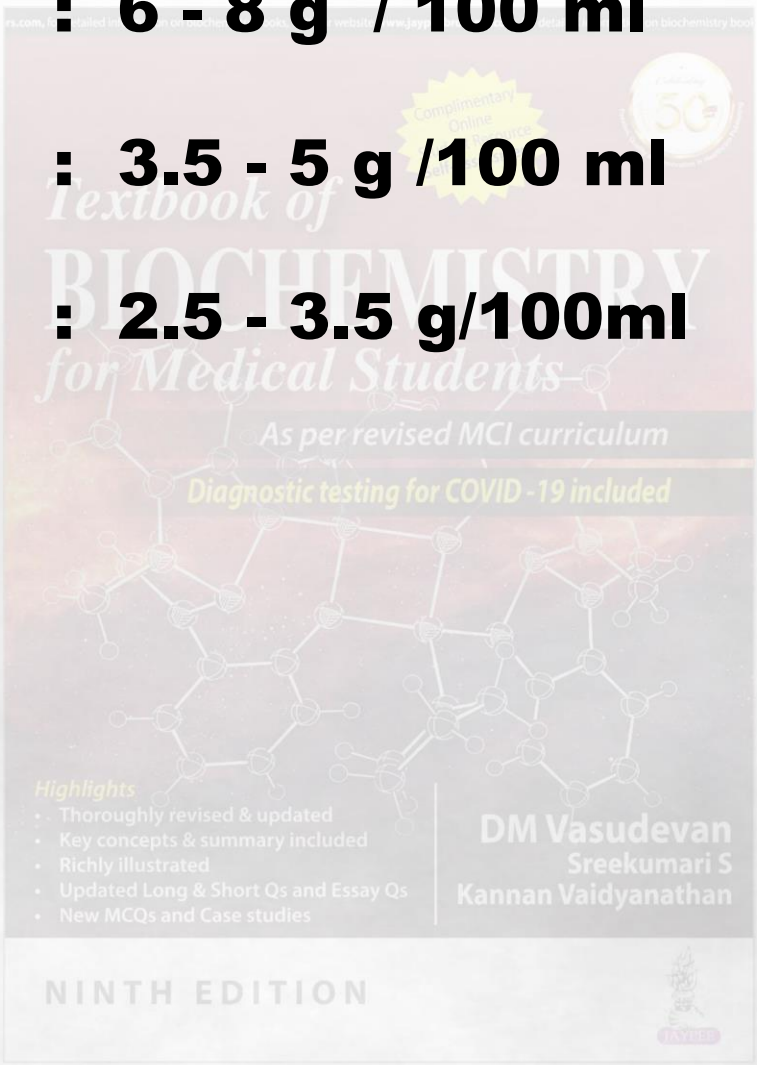
TENTH EDITION

Plasma Proteins

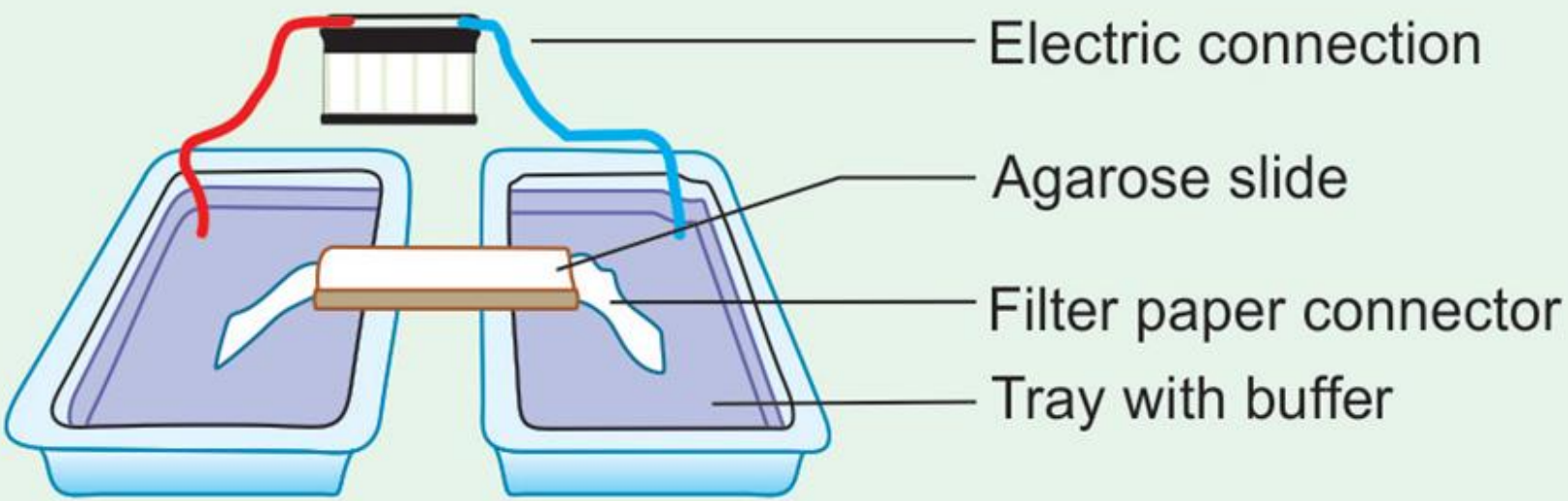
Total Protein : 6 - 8 g / 100 ml

A L B U M I N : 3.5 - 5 g /100 ml

GLOBULIN : 2.5 - 3.5 g/100ml



Electrophoresis



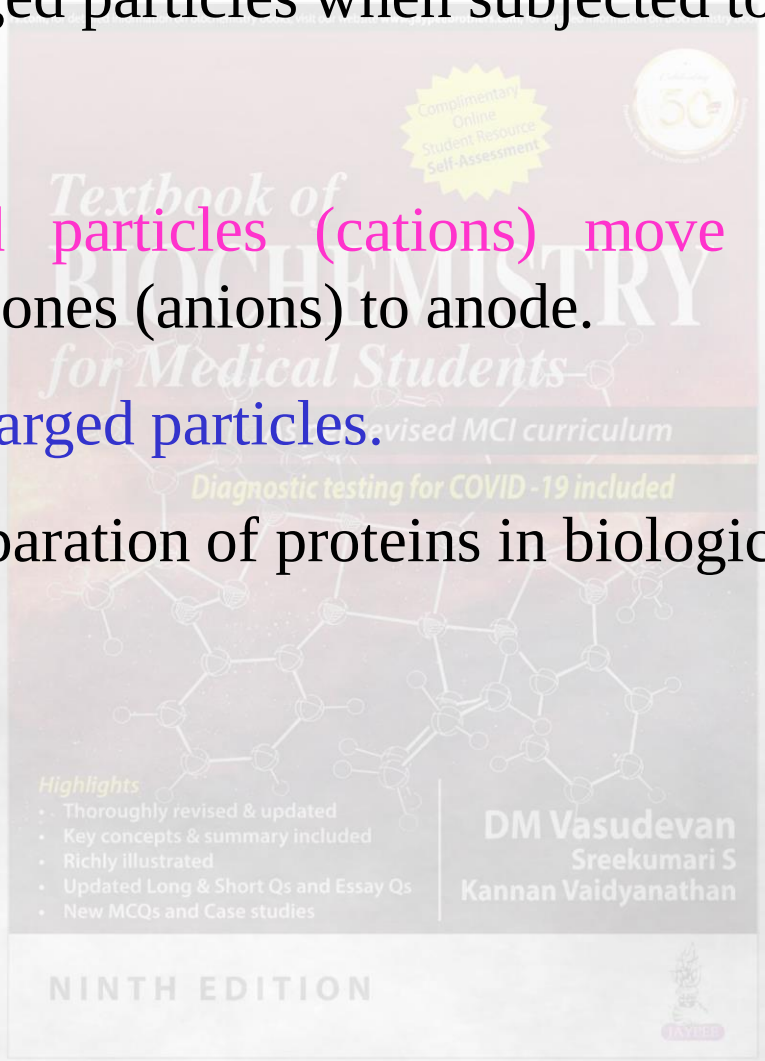
Electrophoresis

Movement of charged particles when subjected to an electric field.

Positively charged particles (cations) move to cathode and negatively charged ones (anions) to anode.

Proteins exist as charged particles.

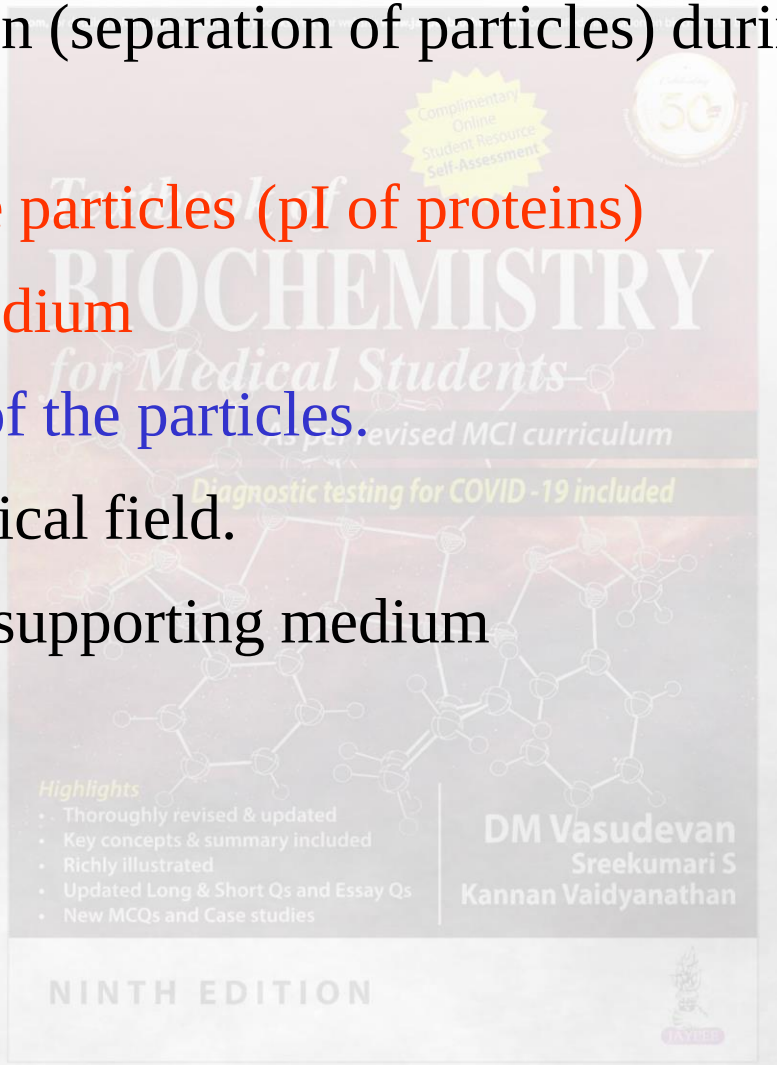
Widely used for separation of proteins in biological fluids.



Factors Affecting Electrophoresis

The rate of migration (separation of particles) during electrophoresis will depend upon :

1. Net charge on the particles (pI of proteins)
2. The pH of the medium
3. Mass and shape of the particles.
4. Strength of electrical field.
5. Properties of the supporting medium
6. Temperature.



Support Medium for Electrophoresis

Filter paper electro-phoresis for 16–18 hours at a low voltage.

Disadvantages

Long time

Diffusion of particles leading to blurring of margins are the of paper.

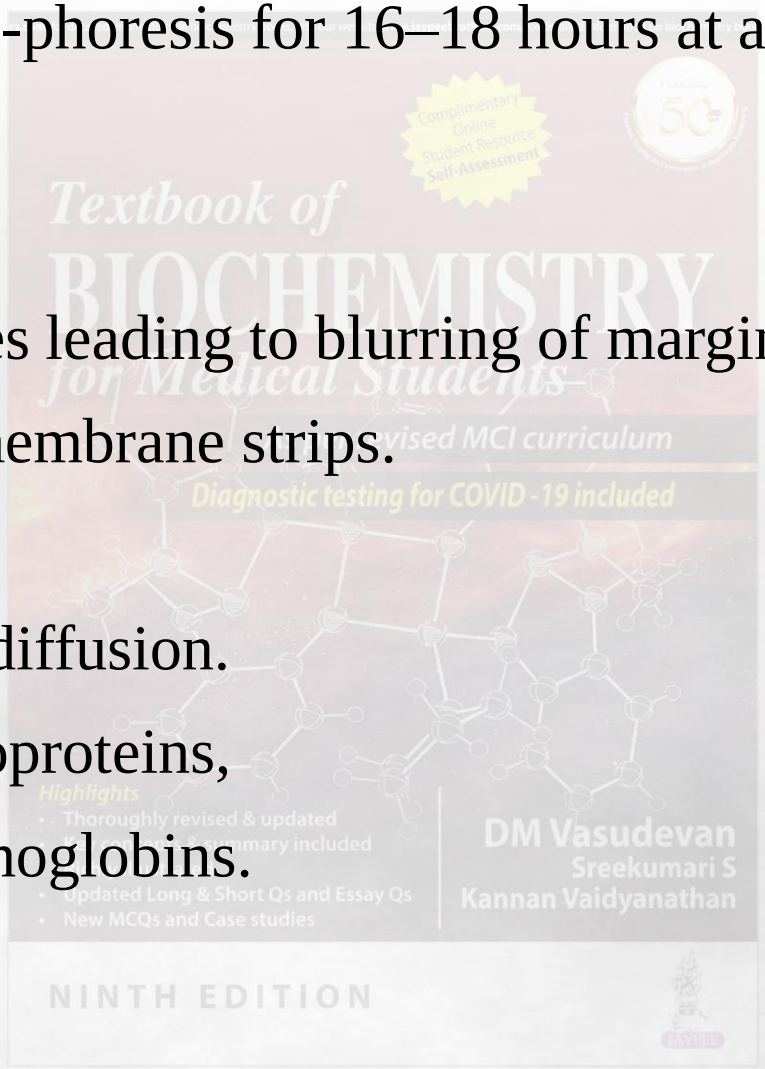
Cellulose acetate membrane strips.

Only one hour

Separation without diffusion.

identification of lipoproteins,

isoenzymes and hemoglobins.



Agarose Gels

heterogeneous polysaccharides viscous liquid when hot but solidify to a gel on cooling.

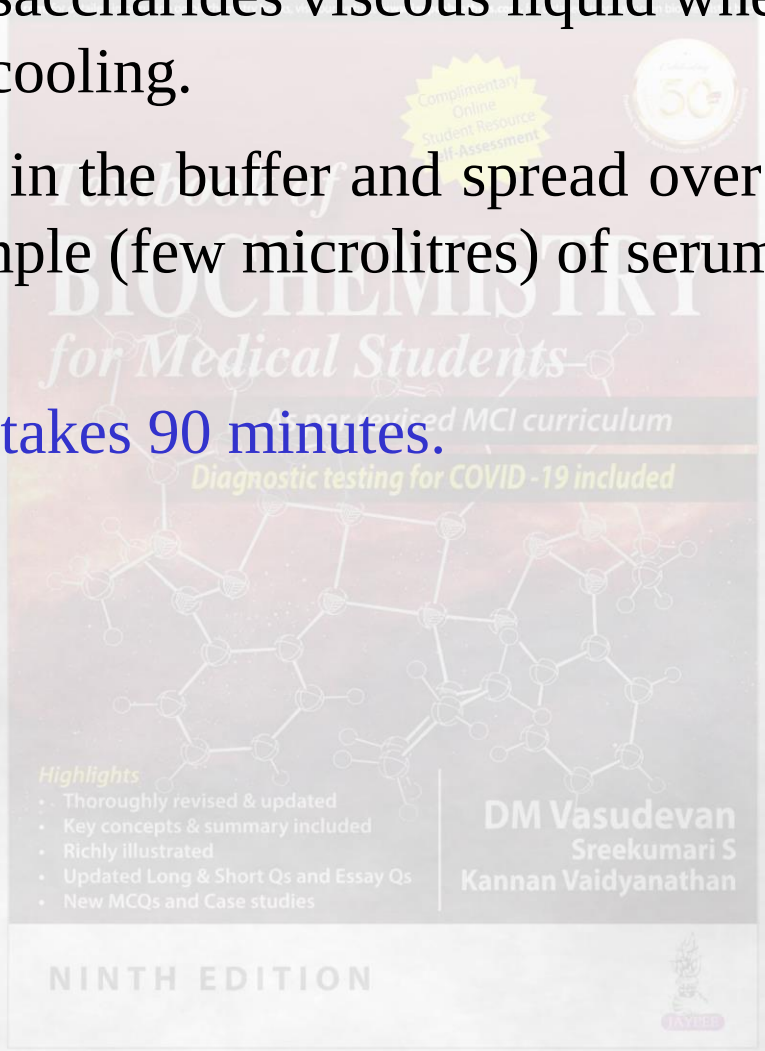
The gel is prepared in the buffer and spread over slides and allowed to cool. A small sample (few microlitres) of serum or biological fluid is applied.

Electrophoretic run takes 90 minutes.

Serum proteins

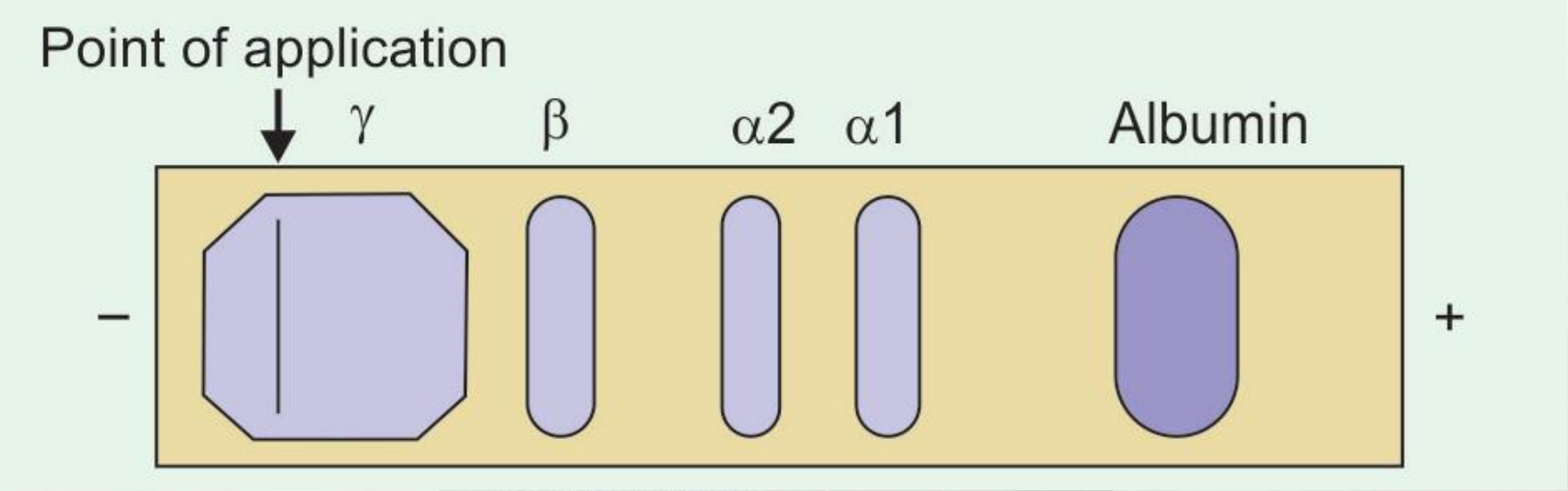
protein mixtures

nucleic acids.



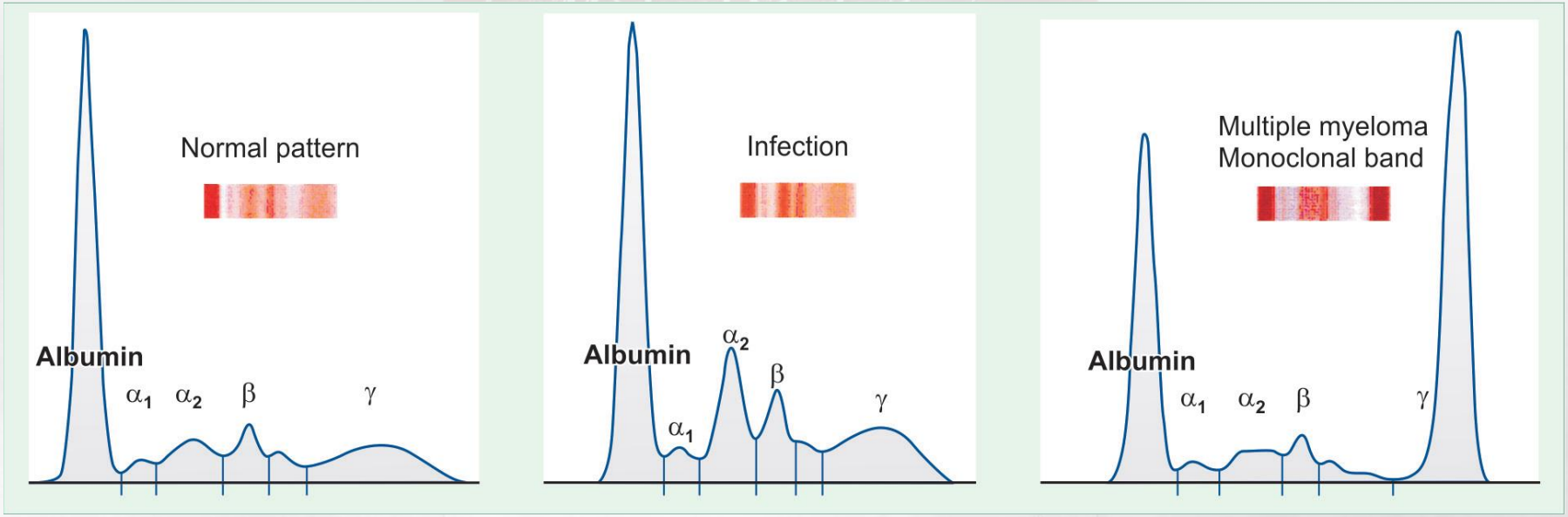
Visualisation of Protein Bands

After the run, the proteins are fixed to the solid support using acetone or methanol. Then stained by (Amido Schwarz, Ponceau S or Coomassie Blue).

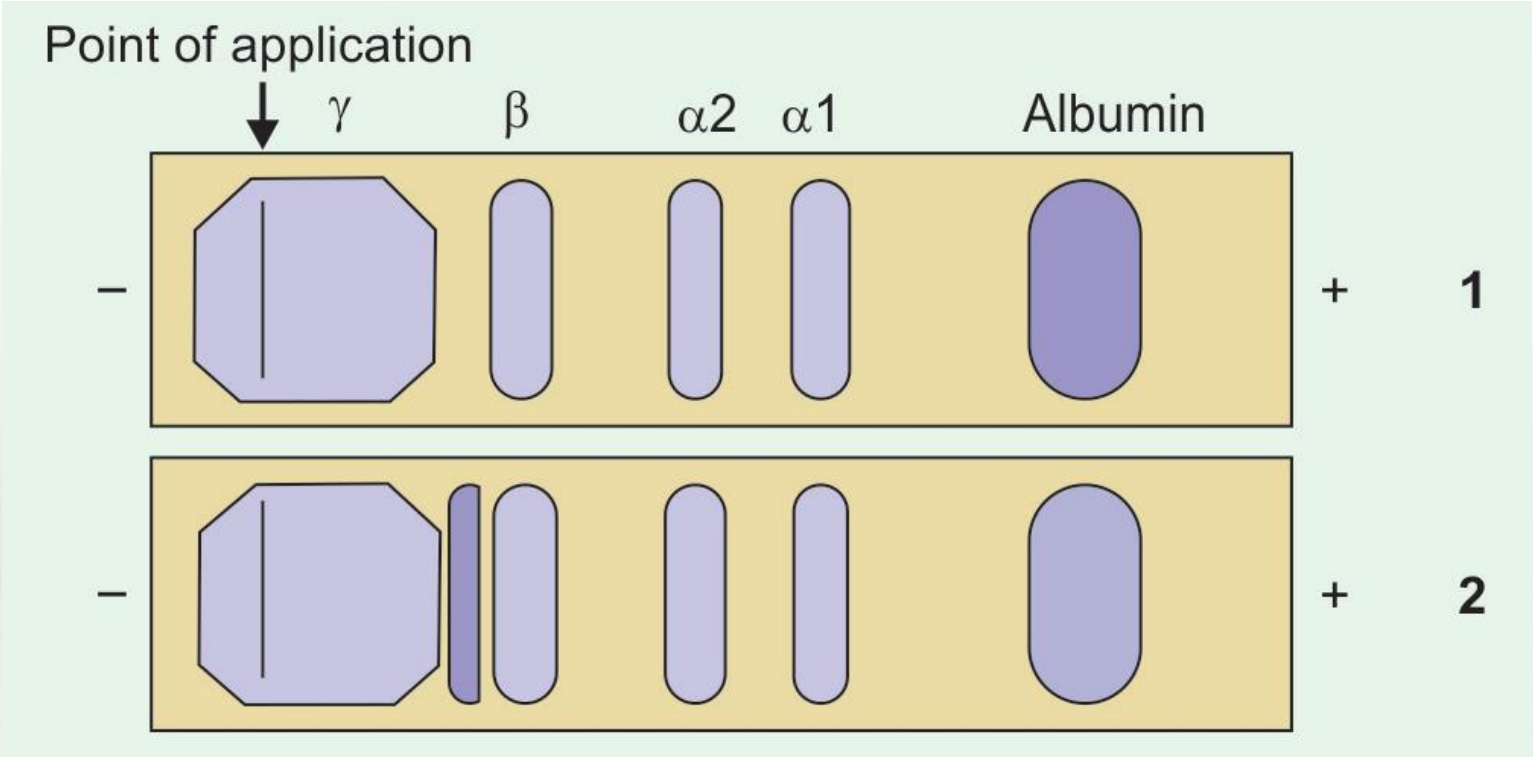


The Electrophoretogram Scanned Using a Densitometer

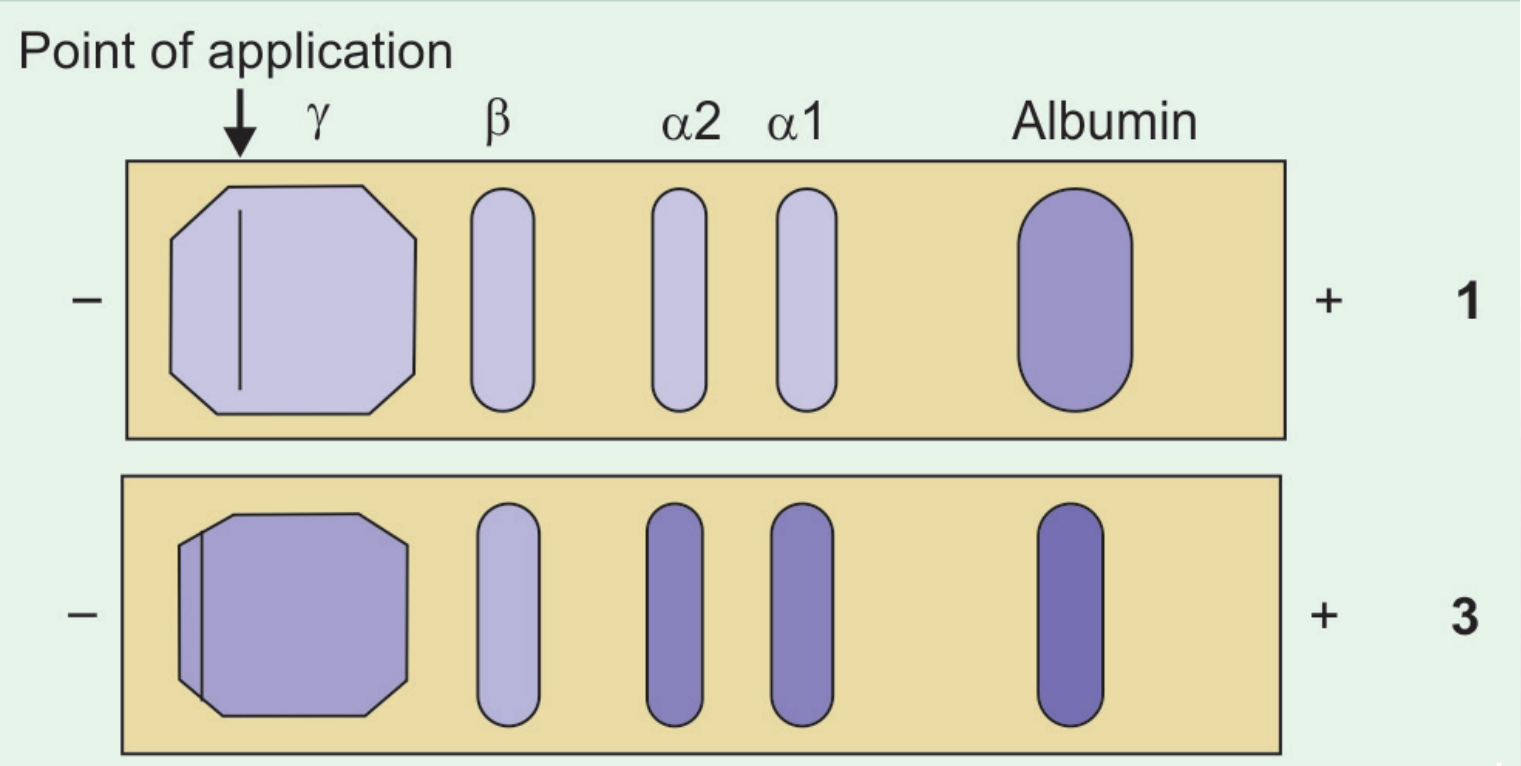
Light is passed through agar gel plate; absorption of light will be proportional to the quantity of protein present on a band.



Normal and abnormal electrophoretic patterns.

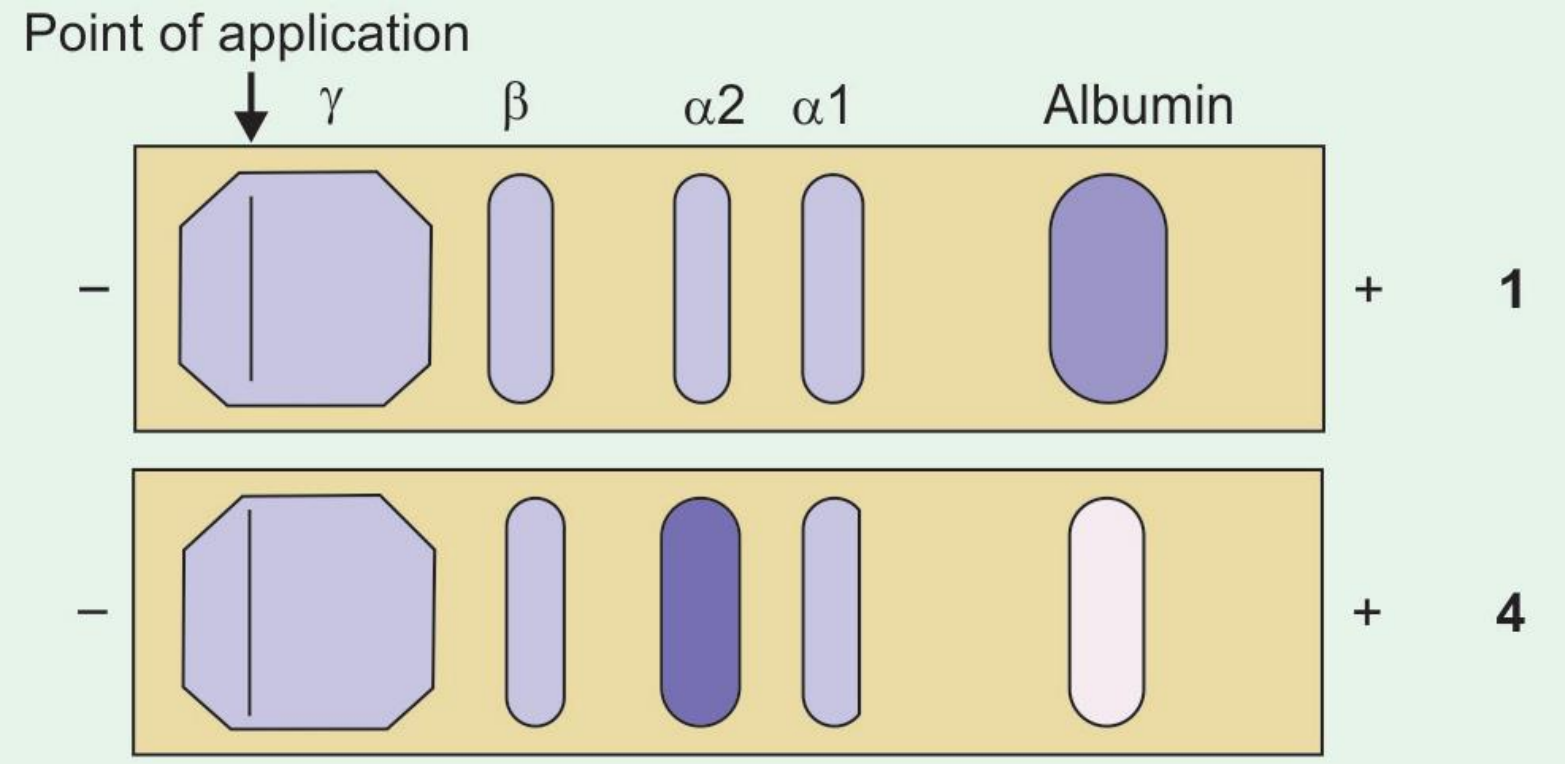


1 = Normal pattern;
2 = **Multiple myeloma**
(M-band) between beta and gamma region.

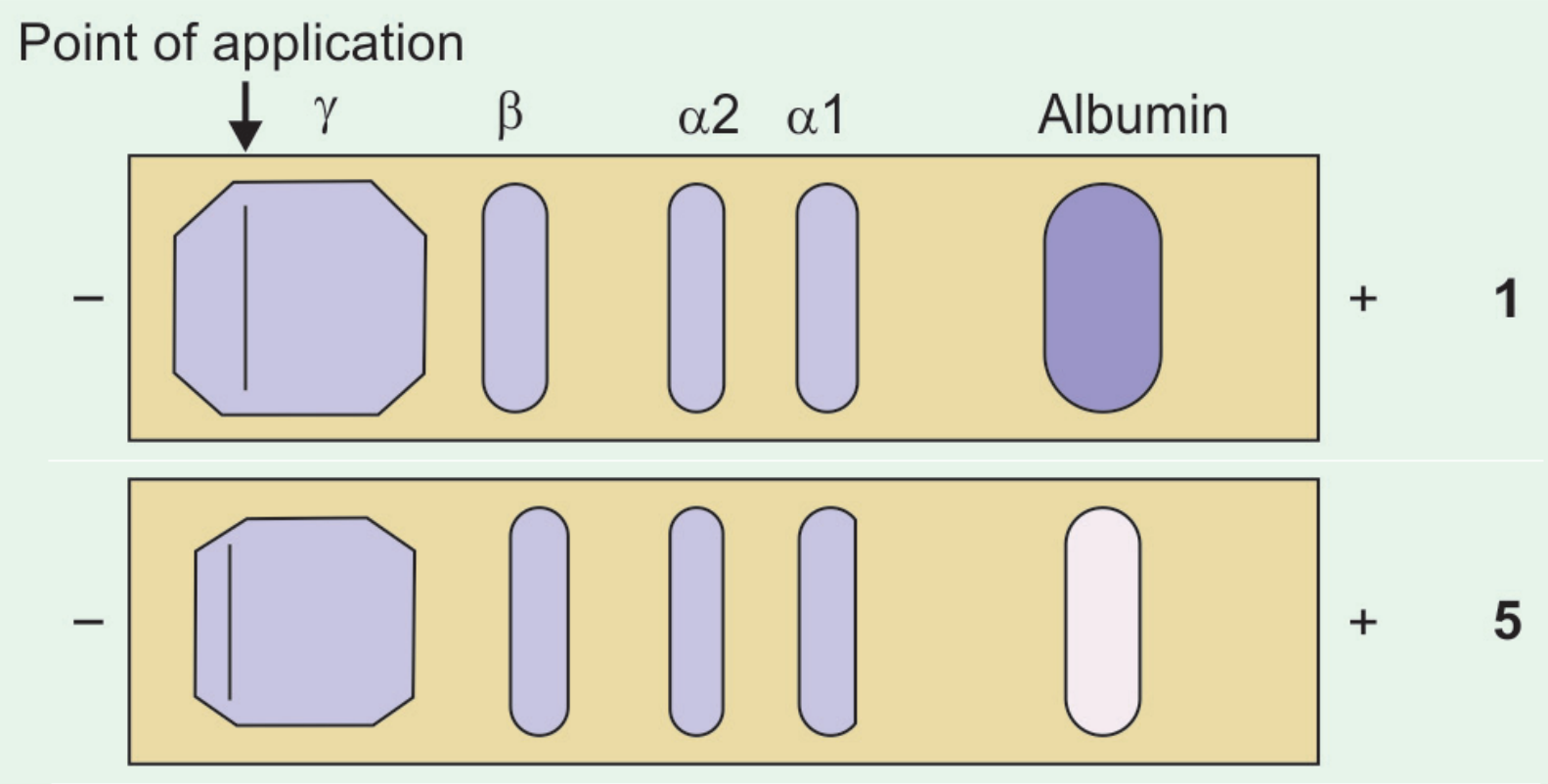


1 = Normal pattern;

3 = **Chronic infection**, broad based increase in gamma region; general increase in alpha1 and alpha2 bands;

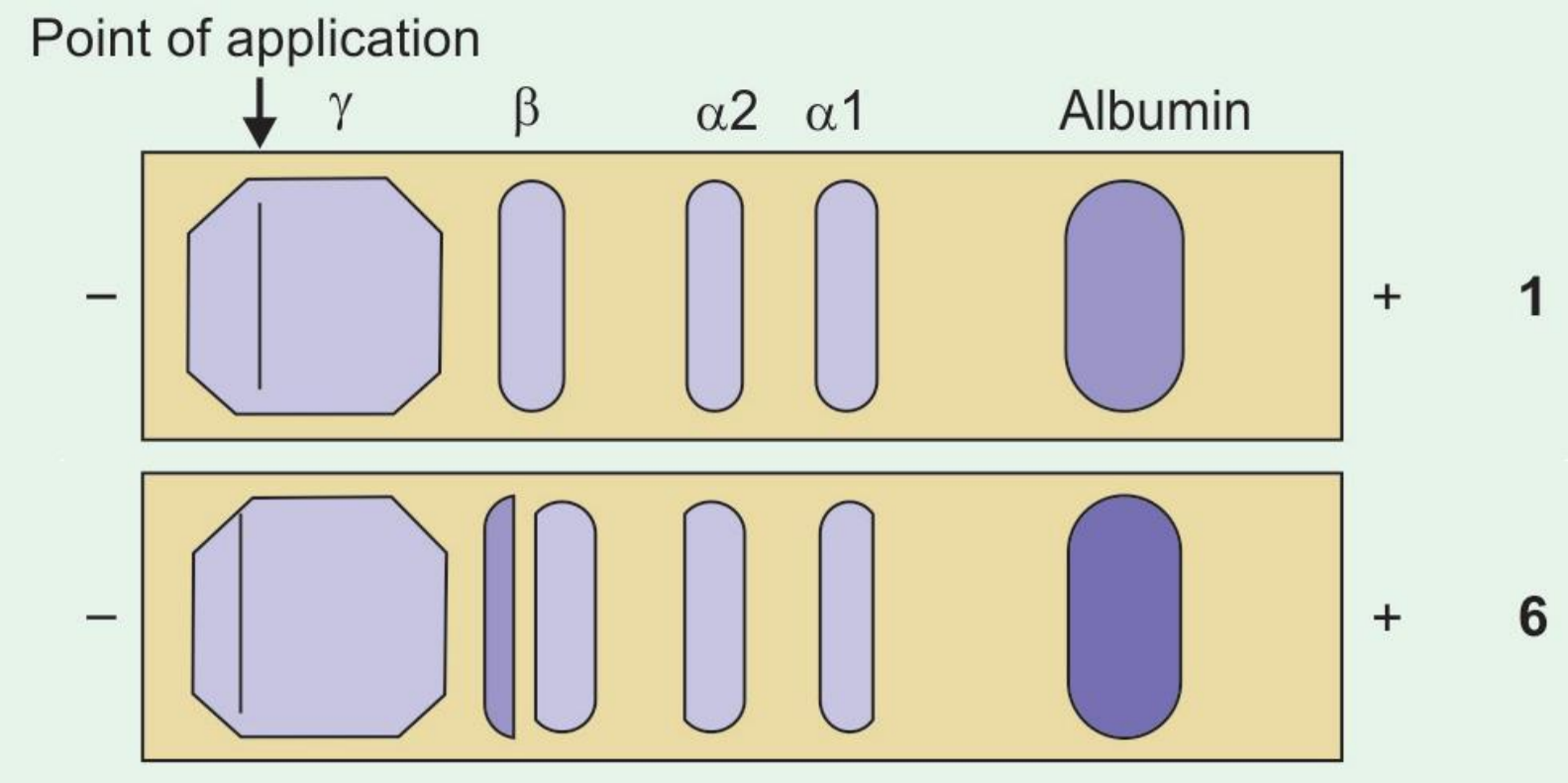


1 = Normal pattern;
4 = **Nephrotic syndrome**; hypoalbuminemia; prominent alpha-2 band

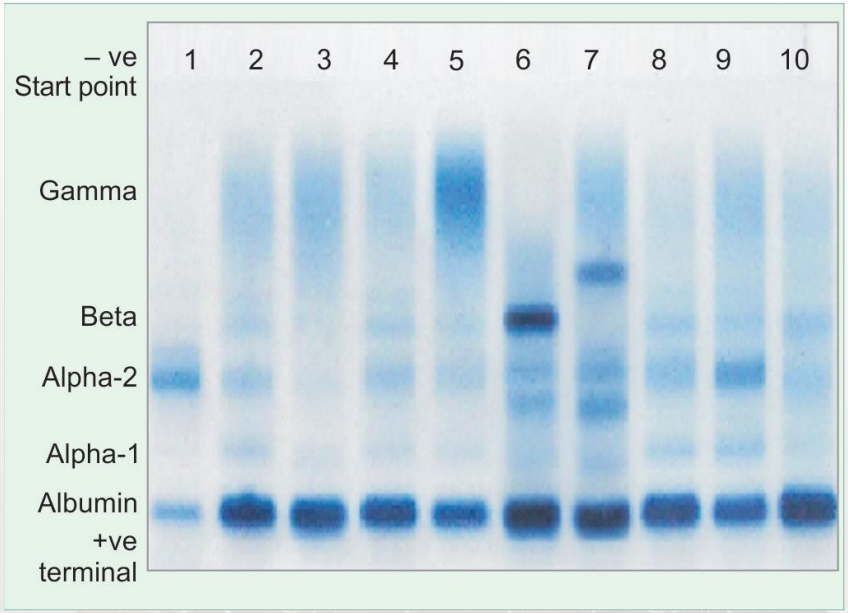


1 = Normal pattern;

5 = **Cirrhosis of liver**; decreased albumin band



1 = Normal pattern;
6 = Plasma showing **fibrinogen** (normal condition) which may be mistaken for paraproteins.



Serum electrophoretic pattern, as seen in agar gel. Lanes 2, 4, 8, 10 = Normal pattern. Lane 1 = Nephrotic syndrome, hypoalbuminemia, prominent alpha-2 band. Lane 3 = Cirrhoris, hypoalbuminemia with beta-gamma bridging. Lane 5 = Chronic infection, broad based increase in gamma region, general increase in alpha-1 and alpha-2 bands, comparative reduction albumin band. Lanes 6, 7 = Multiple myeloma, monoclonal band (M band) between beta and gamma. Lane 9 = Acute inflammation, reduced albumin and increased alpha-2.

Albumin

Latin, albus = white

Mol. Wt. 69,000

Synthesised by hepatocytes 25% of total hepatic protein synthesis

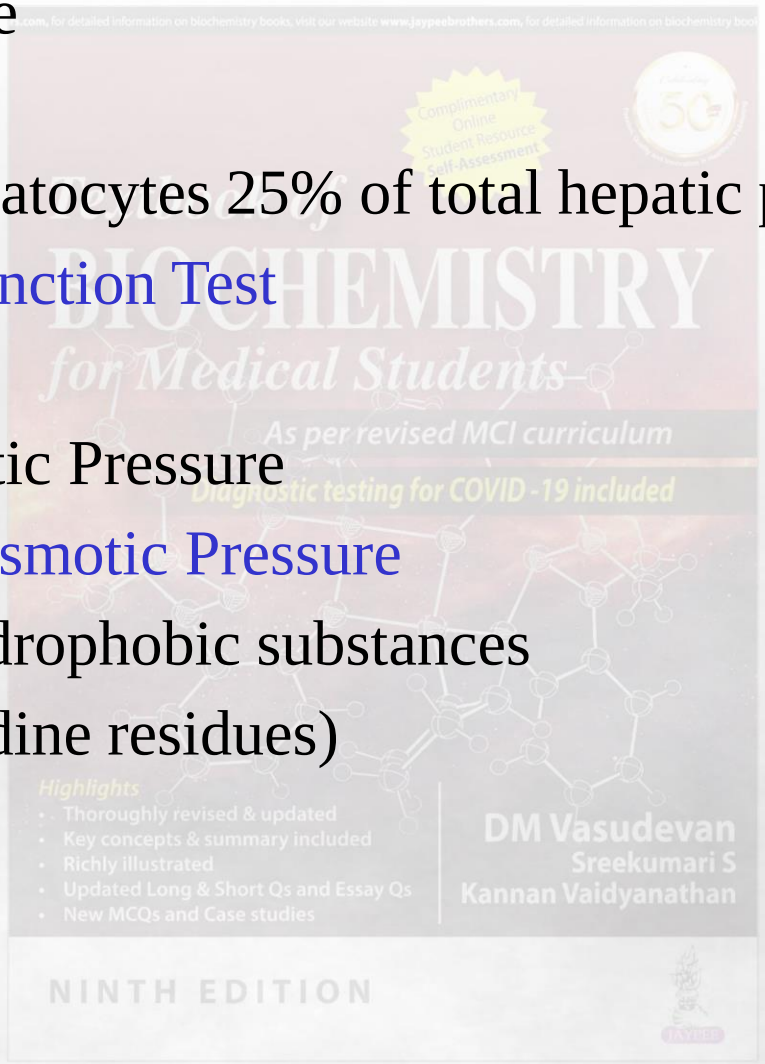
Important Liver Function Test

Functions:

1. Effective Osmotic Pressure

   Colloidal Osmotic Pressure

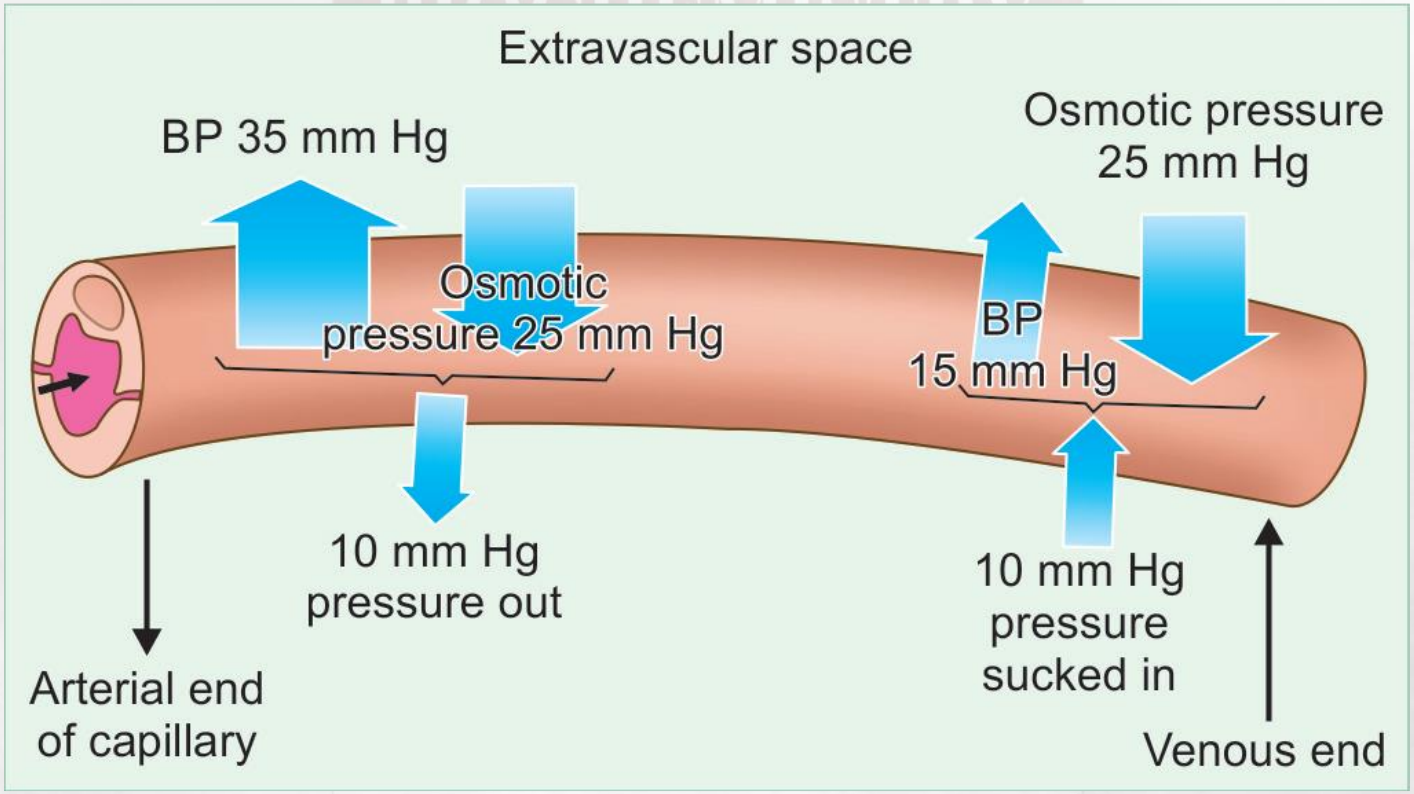
2. Transport of hydrophobic substances
3. Buffering (histidine residues)
4. Nutritional



Total Osmotic Pressure : 5000 mm Hg

Effective Osmotic pressure: 25 mmHg

80% by albumin; 20% globulins



Edema when Albumin < 2 g / dl

1) Albumin is reduced

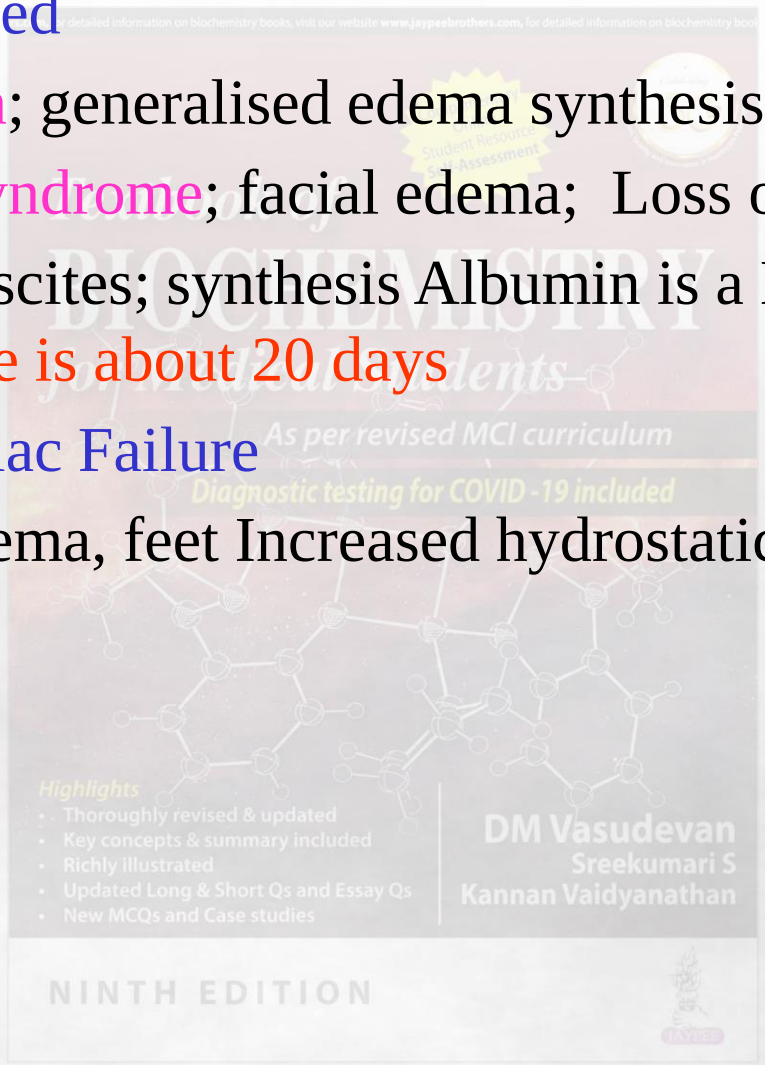
A) Malnutrition; generalised edema synthesis is reduced

B) Nephrotic syndrome; facial edema; Loss of albumin

C) Cirrhosis; Ascites; synthesis Albumin is a Liver Function Test Half life is about 20 days

2) Congestive Cardiac Failure

Dependent edema, feet Increased hydrostatic P in vein



Functions of Albumin

1. Effective Osmotic Pressure

2.Transport of hydrophobic substances

Specific carriage

Bilirubin

Non-esterified fatty acids (NEFA)

Non-specific carriage

Steroids, Thyroxin, Calcium, Copper Aspirin, Sulpha drugs

Dicoumarol, Phenytoin

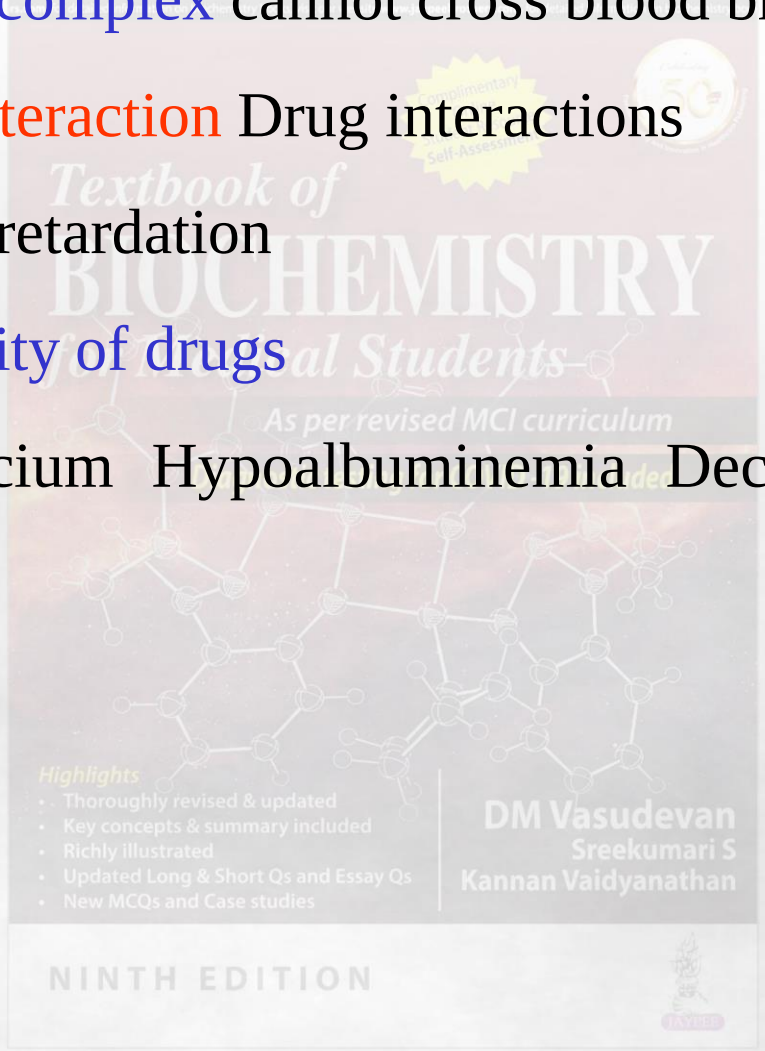
Albumin-fatty acid complex cannot cross blood brain barrier

Bilirubin- aspirin interaction Drug interactions

Kernicterus mental retardation

Biological availability of drugs

Protein bound calcium Hypoalbuminemia Decreased calcium in blood



Normal Albumin level in blood:

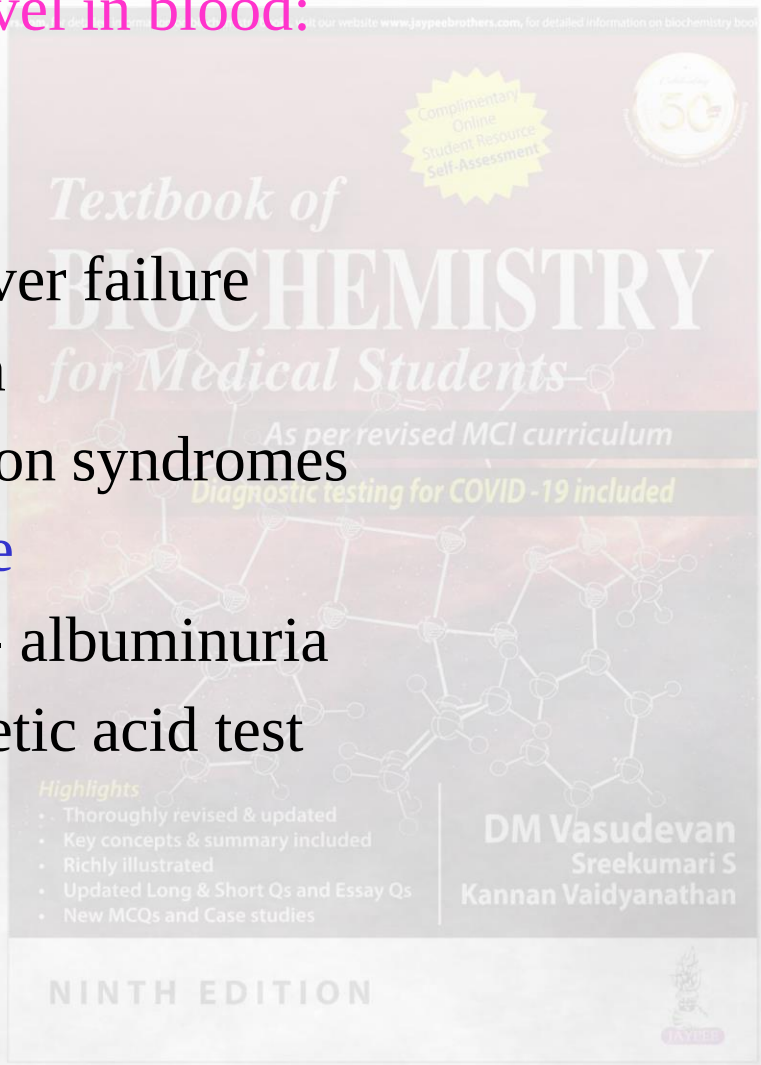
3.5 - 5 g /dl

Hypoalbuminemia

- Cirrhosis, liver failure
- Malnutrition
- Malabsorption syndromes

Nephrotic syndrome

- Proteinuria - albuminuria
- Heat and acetic acid test



Proteinuria

Glomerular proteinuria

Micro albuminuria

Minimal albuminuria

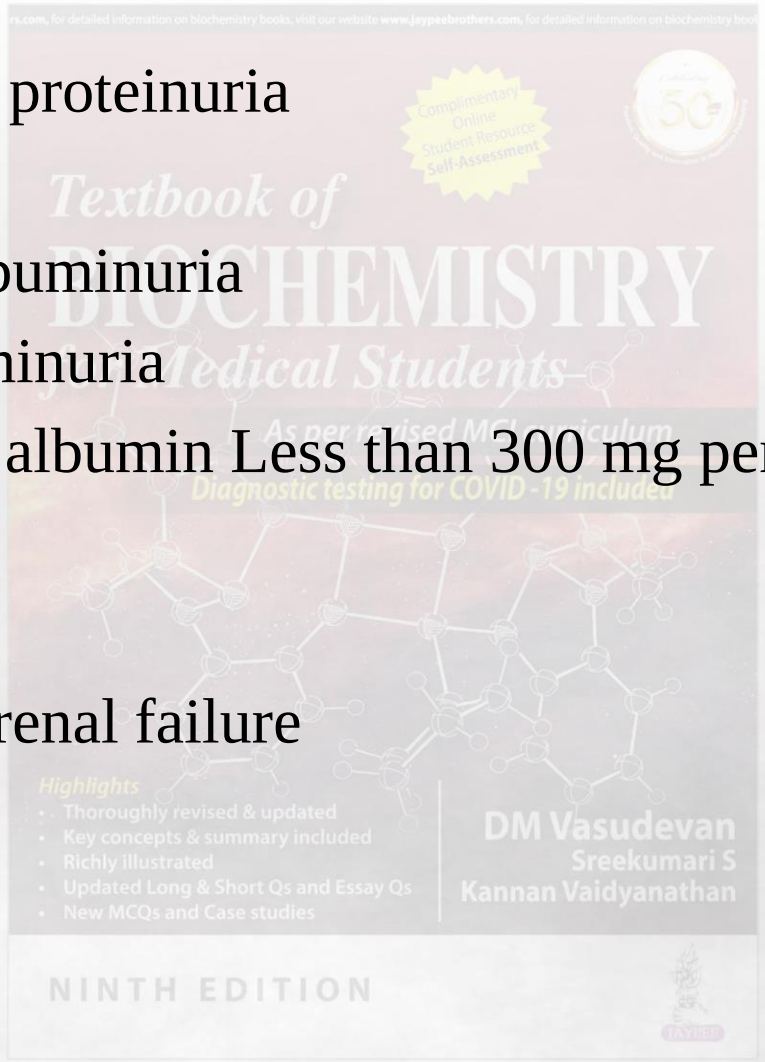
Pauci albuminuria

Small quantities of albumin Less than 300 mg per day

Diabetes mellitus

Hypertension

Indicator of future renal failure



Hypoproteinemia

Cirrhosis

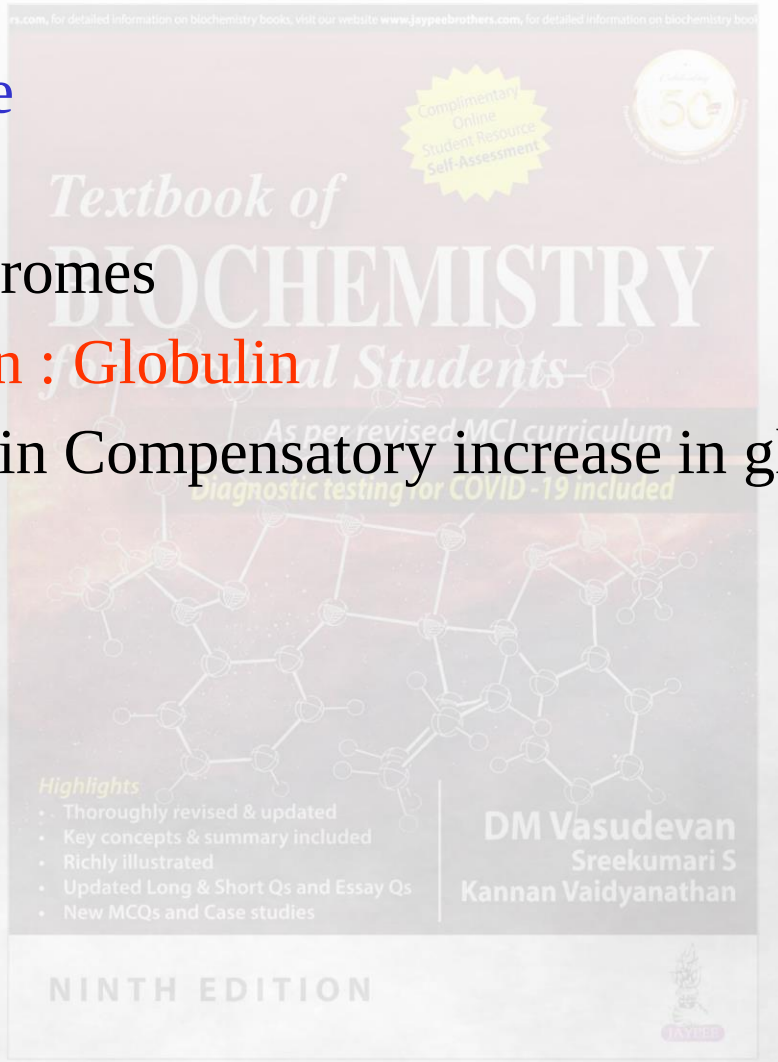
Nephrotic syndrome

Malnutrition

Malabsorption syndromes

Reversal of Albumin : Globulin

Reduction of albumin Compensatory increase in globulin fractions



Hyper Globulinemias

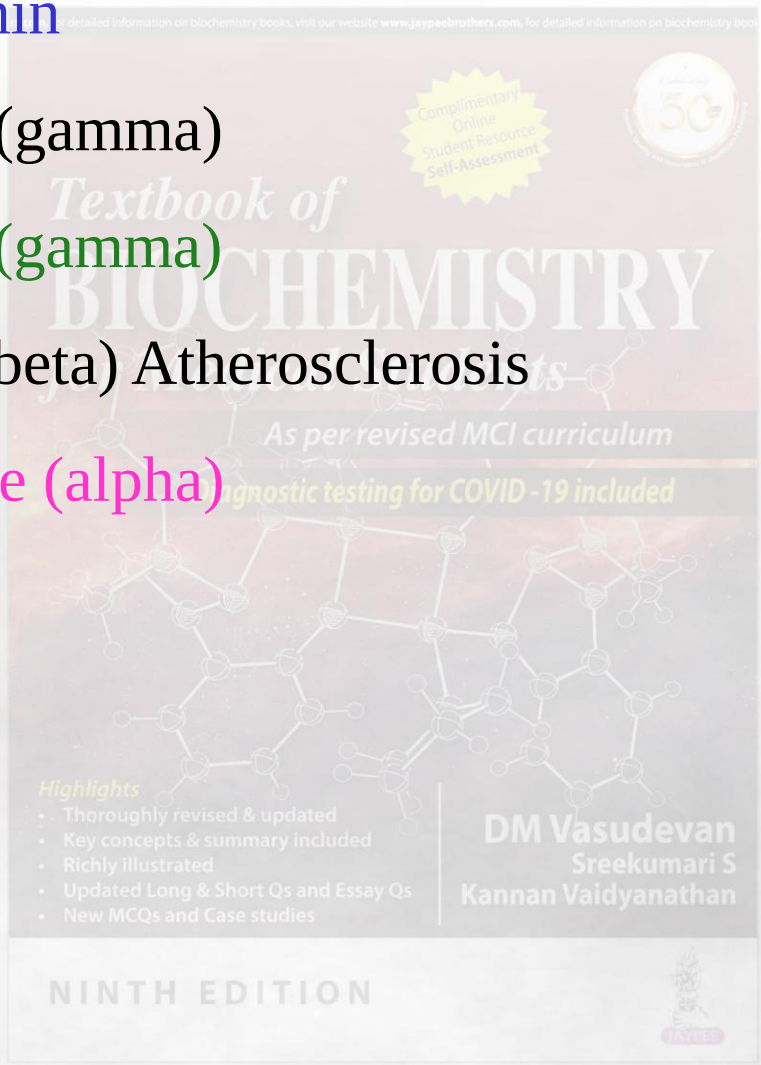
Reduction of albumin

Chronic infections (gamma)

Multiple myeloma (gamma)

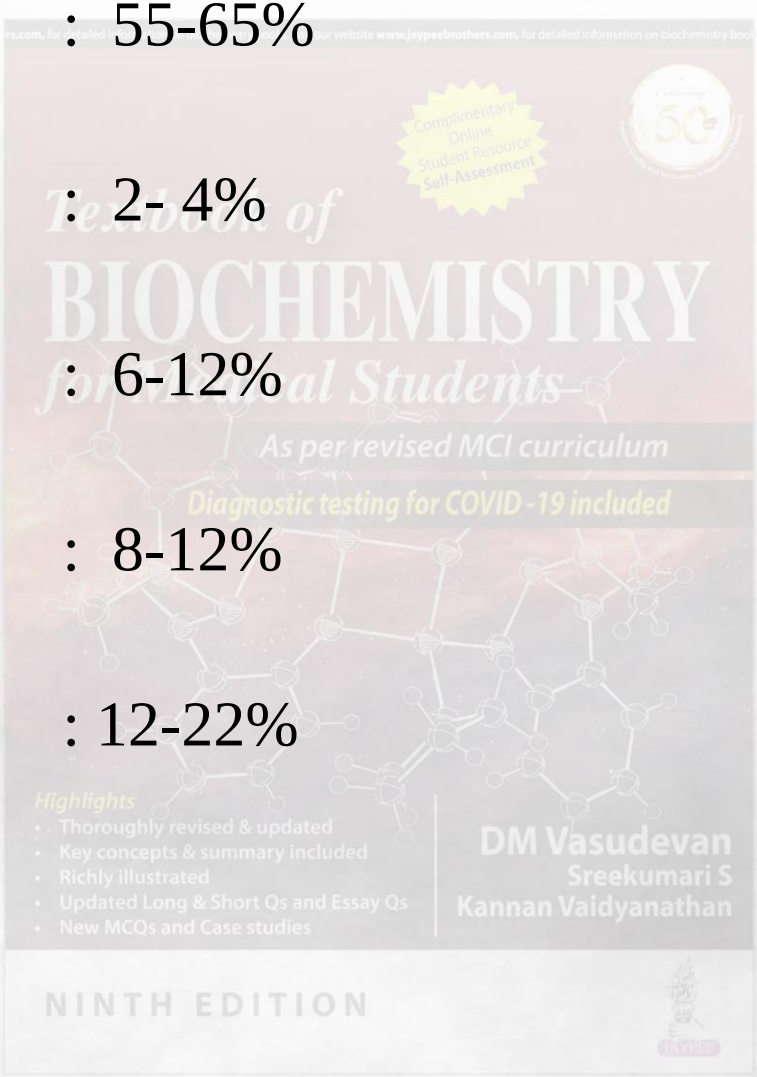
Lipoproteinemias (beta) Atherosclerosis

Nephrotic syndrome (alpha)



Normal Values

Albumin	: 55-65%
Alpha-1-globulin	: 2- 4%
Alpha-2-globulin	: 6-12%
Beta-globulin	: 8-12%
Gamma-globulin	: 12-22%



Globulins

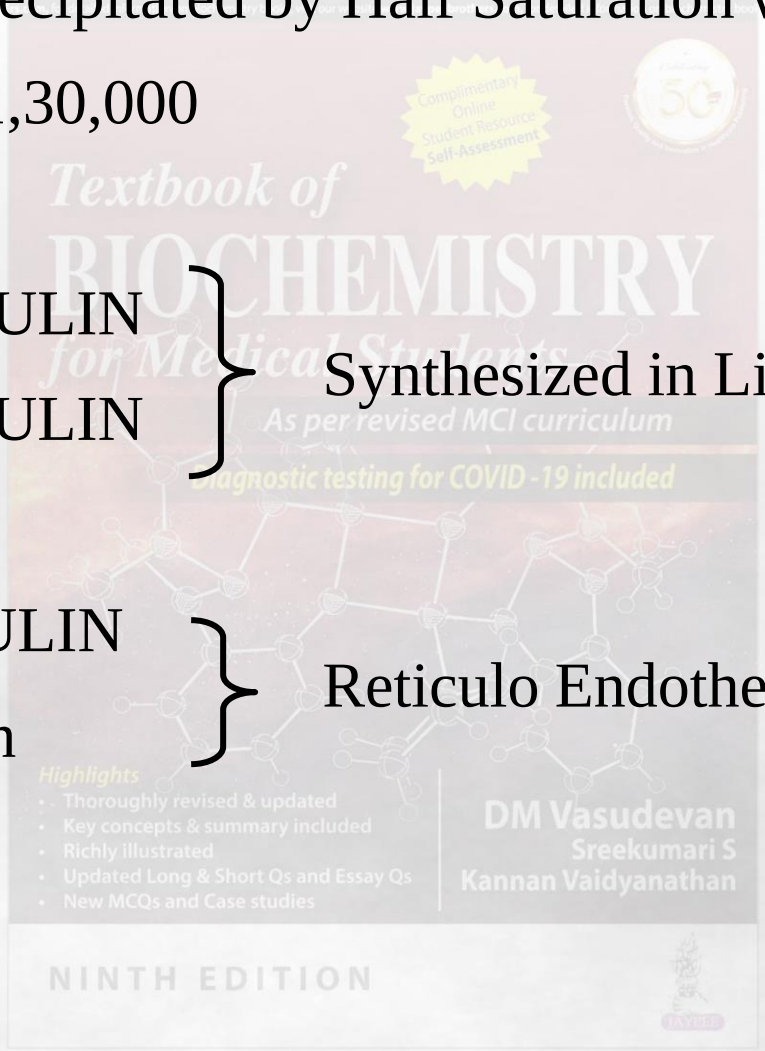
- Globulins are Precipitated by Half Saturation with Salts
- M.Wt 90,000 - 1,30,000

- α_1 GLOBULIN
- α_2 GLOBULIN

} Synthesized in Liver

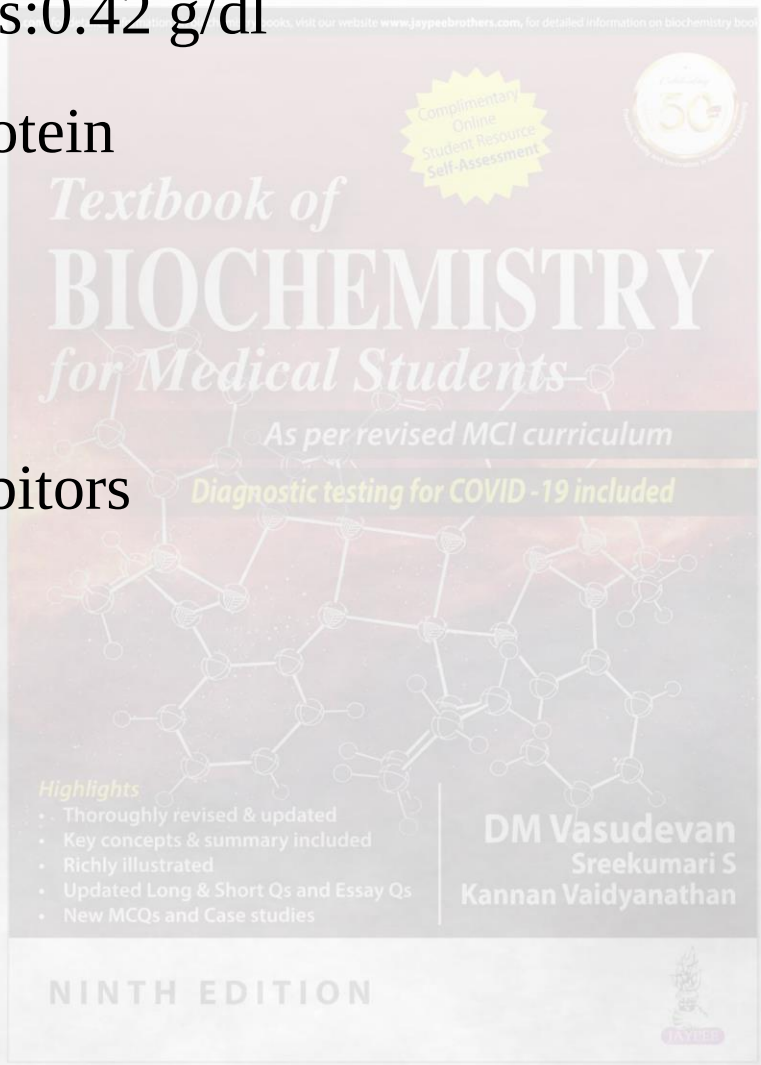
- β GLOBULIN
- γ Globulin

} Reticulo Endothelial System



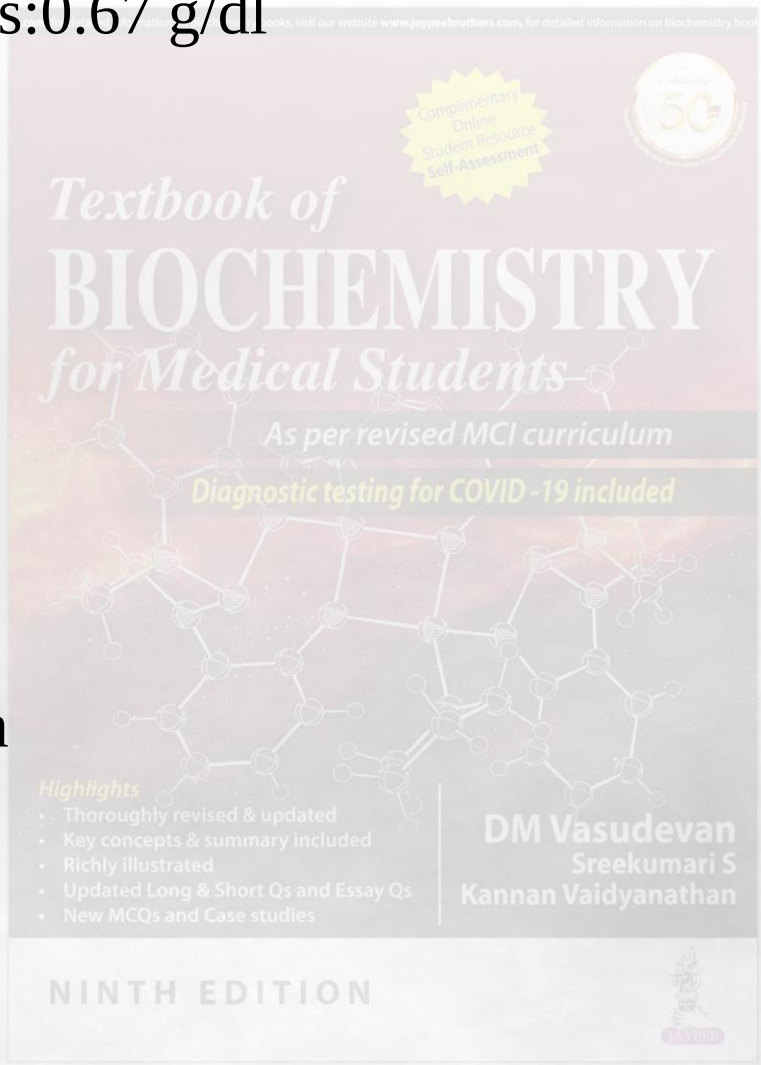
α_1 Globulin

- Complex Proteins:0.42 g/dl
- α_1 Acid Glycoprotein
- α_1 Lipoprotein
- α_1 fetoprotein
- α_1 Globulin Inhibitors
- α_1 Antitrypsin



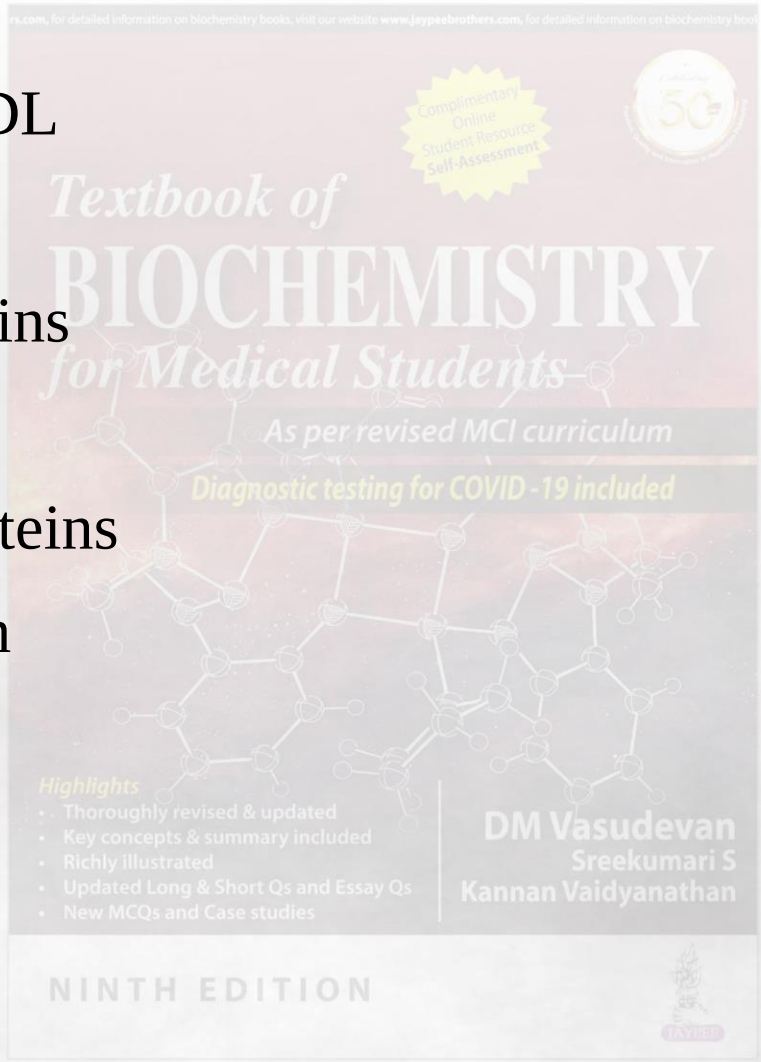
α_2 Globulin

- Complex Proteins:0.67 g/dl
- α_2 Glycoprotein
- Caeruloplasmin
- Haptoglobin
- Plasminogen
- Prothrombin
- α_2 Macroglobulin



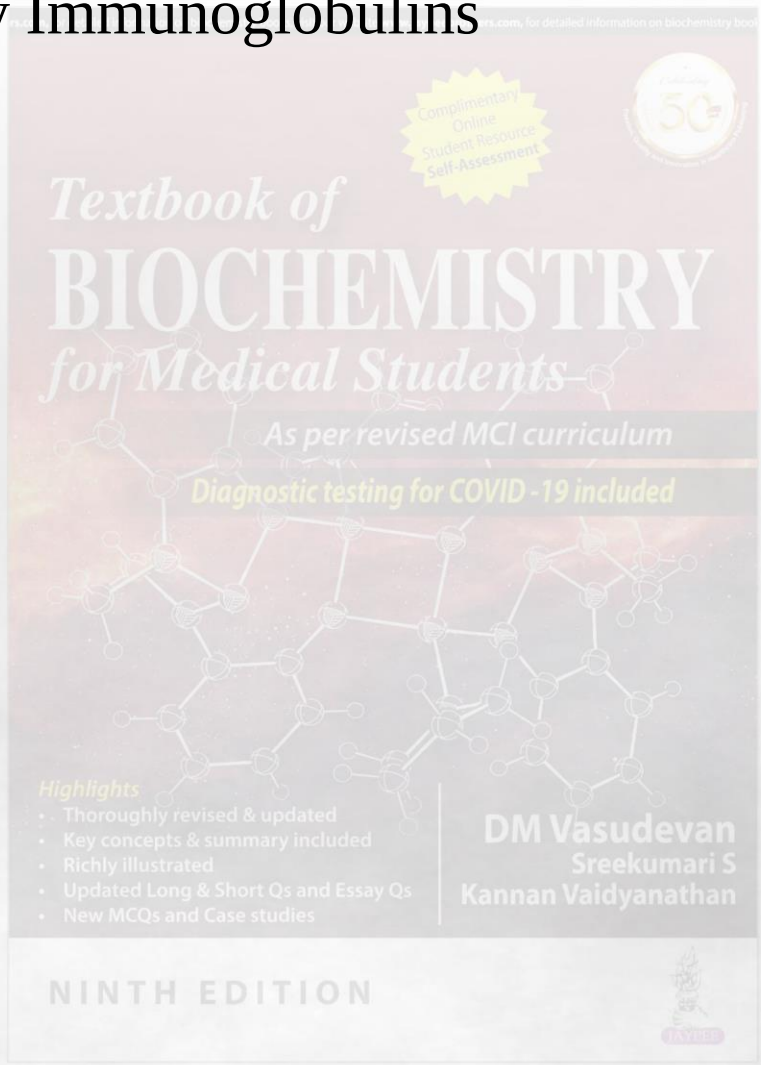
β Globulin

- 0.91 d/dl
- β Lipoprotein-LDL
- Transferrin
- C Reactive Proteins
- Hemopexin
- Complement Proteins
- β Micro Globulin



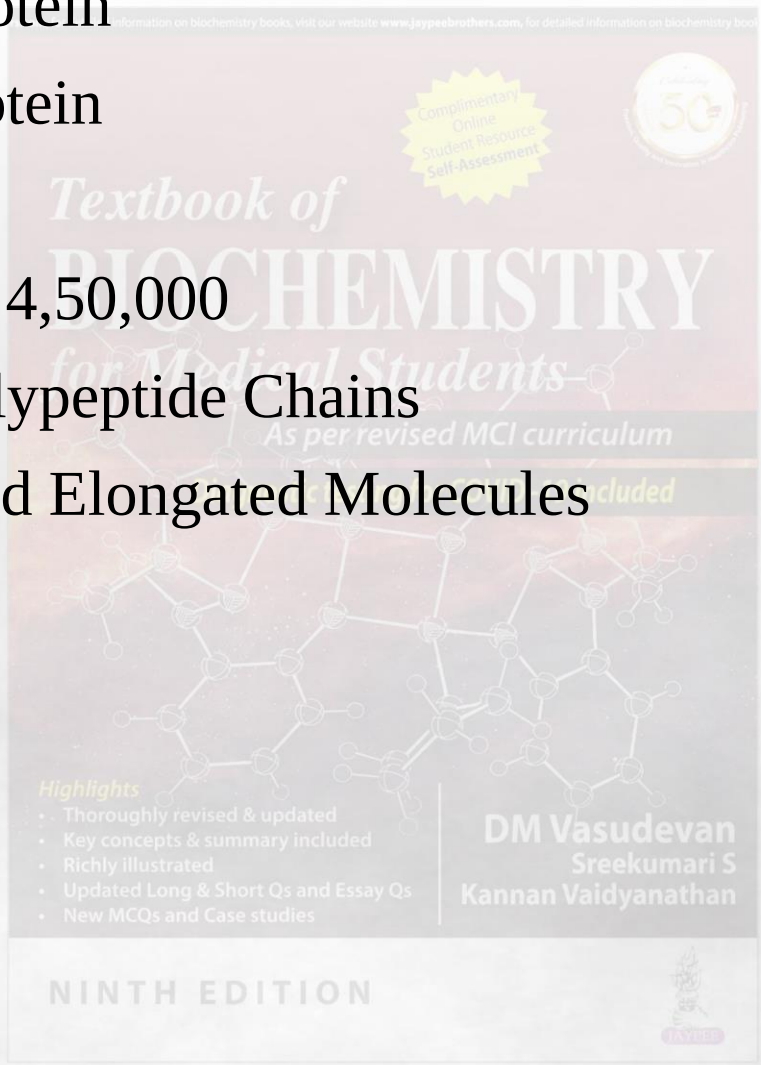
γ Globulin

- These are Mainly Immunoglobulins



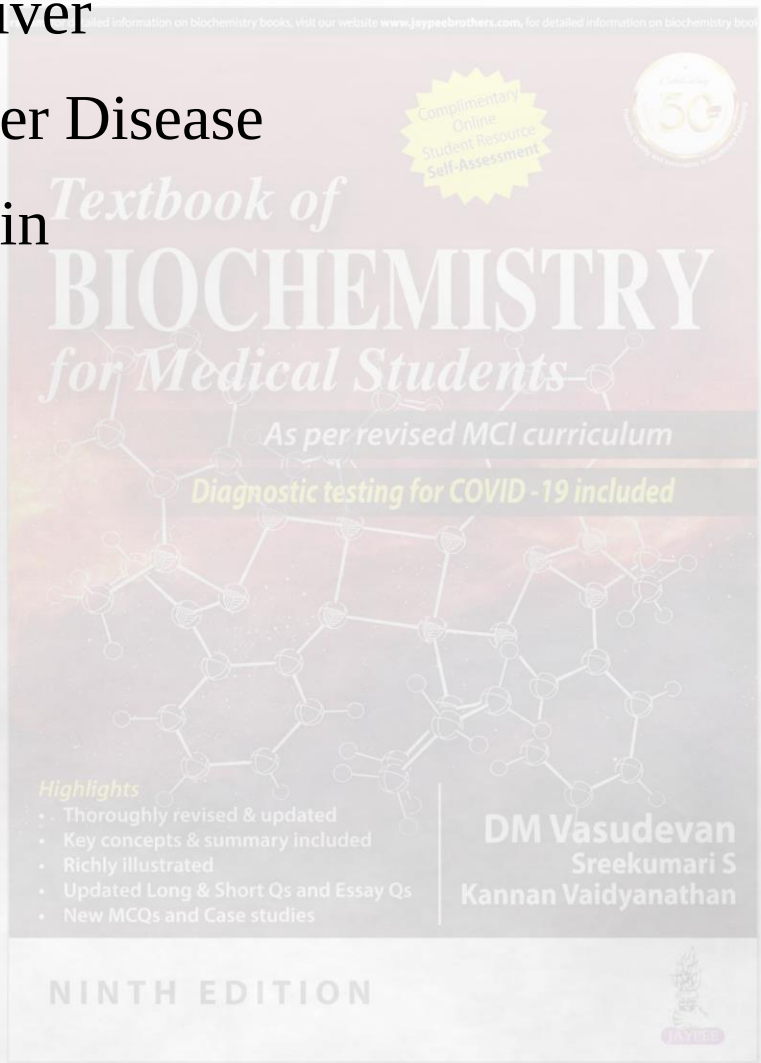
Fibrinogen

- Soluble Glycoprotein
- 4-6% Plasma Protein
- 200 - 400 mg/dl
- M Wt 3,50,000 - 4,50,000
- Made up of 6 Polypeptide Chains
- Asymmetrical and Elongated Molecules



Fibrinogen

- Synthesized in Liver
- Decreased in Liver Disease
- Precursor of Fibrin



Transport Proteins

for carrying lipid substances

1. ALBUMIN

   Bilirubin, fatty acids, Calcium, drugs

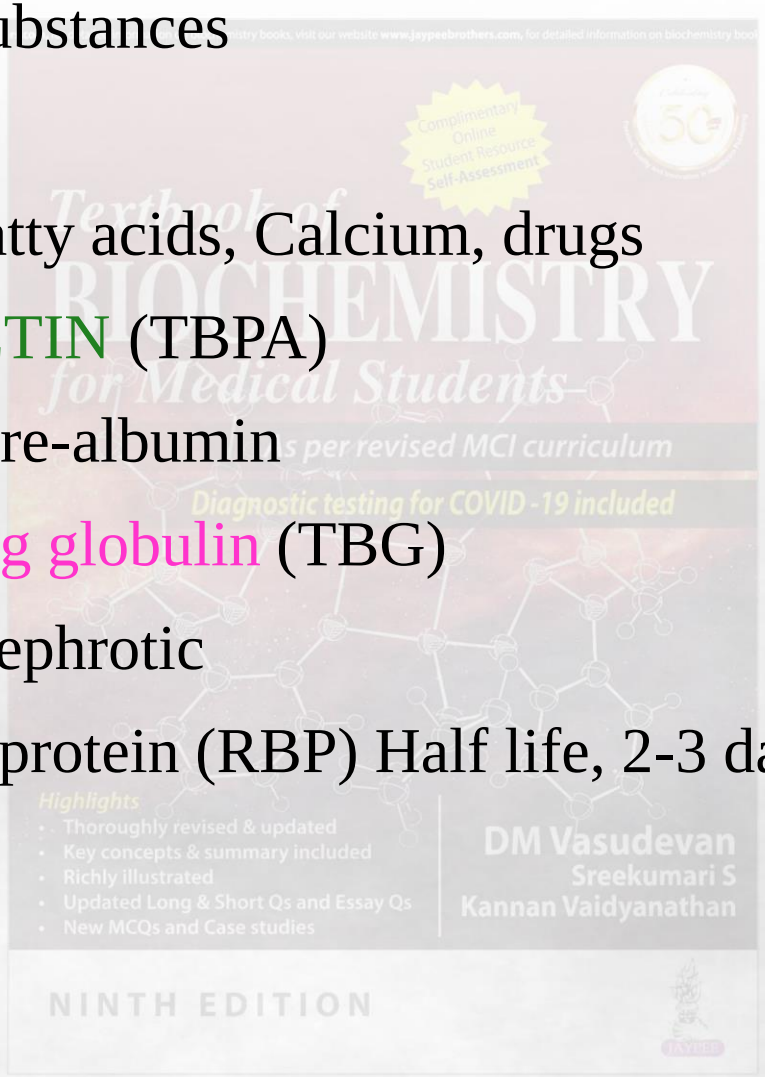
2. TRANSTHYRETIN (TBPA)

Thyroxin binding pre-albumin

3. Thyroxin binding globulin (TBG)

↑ Pregnancy; ↓ Nephrotic

4. Retinol binding protein (RBP) Half life, 2-3 days



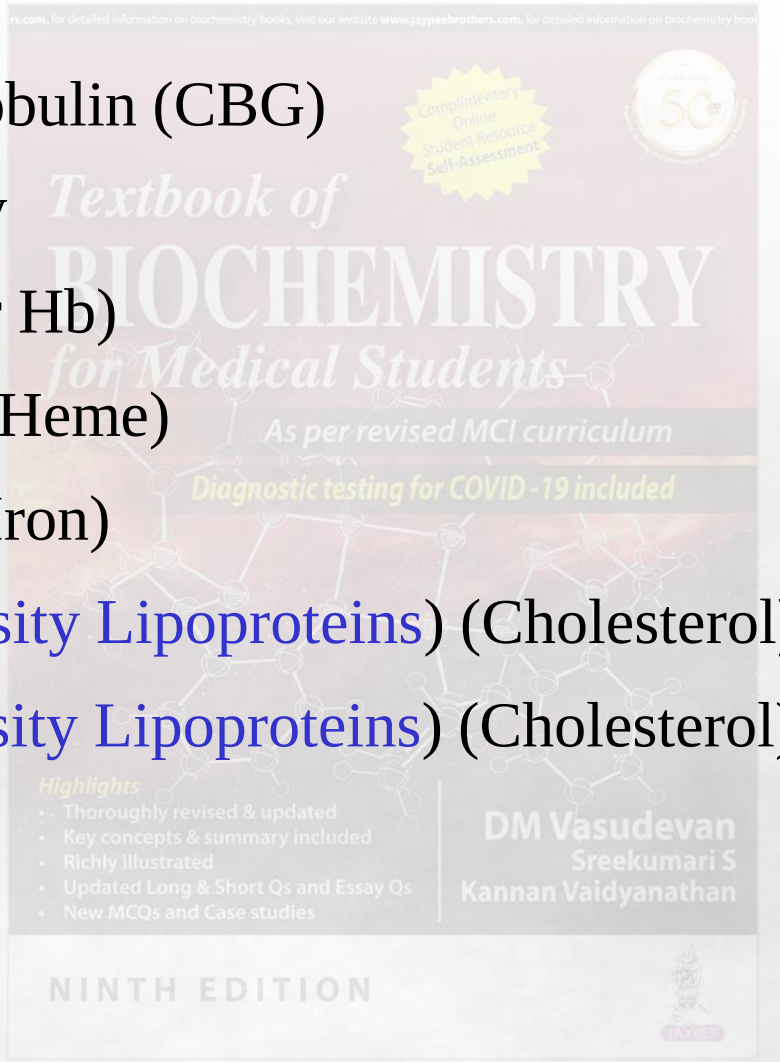
Transport Proteins

5. Transcortin

Cortisol binding globulin (CBG)

↑ Pregnancy

6. Haptoglobin (for Hb)
7. Hemopexin (for Heme)
8. Transferrin (for Iron)
9. HDL (High Density Lipoproteins) (Cholesterol)
10. LDL (Low Density Lipoproteins) (Cholesterol)



Carrier Proteins or Transport Proteins of Plasma

Name	Plasma level	Compound bound or transported	Biological and clinical significance
Albumin	3.5–5 g/dl	Fatty acids, bilirubin, calcium, thyroxine, heavy metals, drugs	Bilirubin competes with aspirin for binding sites on albumin
Pre-albumin (Trans- thyretin)	25–30 mg/dl	Steroid hormones, Thyroxine, Retinol	Rich in tryptophan. Half-life is 1day. It is a negative acute phase protein. Transports T3 and T4 losely.
Retinol binding protein (RBP)	3–6 mg/dl	Retinol (Vitamin A)	Synthesised by liver. RBP has a short half-life. Level indicates vitamin A status. Useful to assess the protein turn over.

Carrier Proteins or Transport Proteins of Plasma

Name	Plasma level	Compound bound or transported	Biological and clinical significance
Thyroxine binding globulin (TBG)	1–2 mg/dl	Thyroxine	Assessment of the binding sites on TBG is important in studying thyroid function. It is synthesised in liver
Trans-cortin; Cortisol Binding globulin	3–3.5 mg/dl	Cortisol and Cortico-sterone	Synthesised by liver. Increased in pregnancy. Free unbound fraction of hormone is biologically active.
Hapto-globin (Hp)	40-175mg/dl	Hemoglobin	Synthesised in liver. Low level indicates hemolysis. Half-life of Hp is 5 days; but that of Hb-Hp is only 90 minutes. It is an acute phase protein

Carrier Proteins or Transport Proteins of Plasma

Name	Plasma level	Compound bound or transported	Biological and clinical significance
Transferrin	200–300 mg/dl	Iron 33% saturated	Conserves iron by preventing iron loss through urine
Hemopexin	50–100 mg/dl	Free heme	Helps in preventing loss of heme (and so iron also) from body.
HDL (High density lipo-protein)		Cholesterol Phospho-lipids	The lipoprotein contains apoprotein-A. Serves to transport cholesterol from tissues to liver for elimination through bile. It is anti-atherogenic.
LDL (Low density lipo-protein)		Cholesterol; Phospho-lipids; TG	Contains apoprotein-B. Trans-ports cholesterol to tissues. It increases risk for MI.

Acute Phase Proteins

50-1000 times

Inflammatory / neoplastic diseases

Induction by Interleukins released by macro / lymphocytes

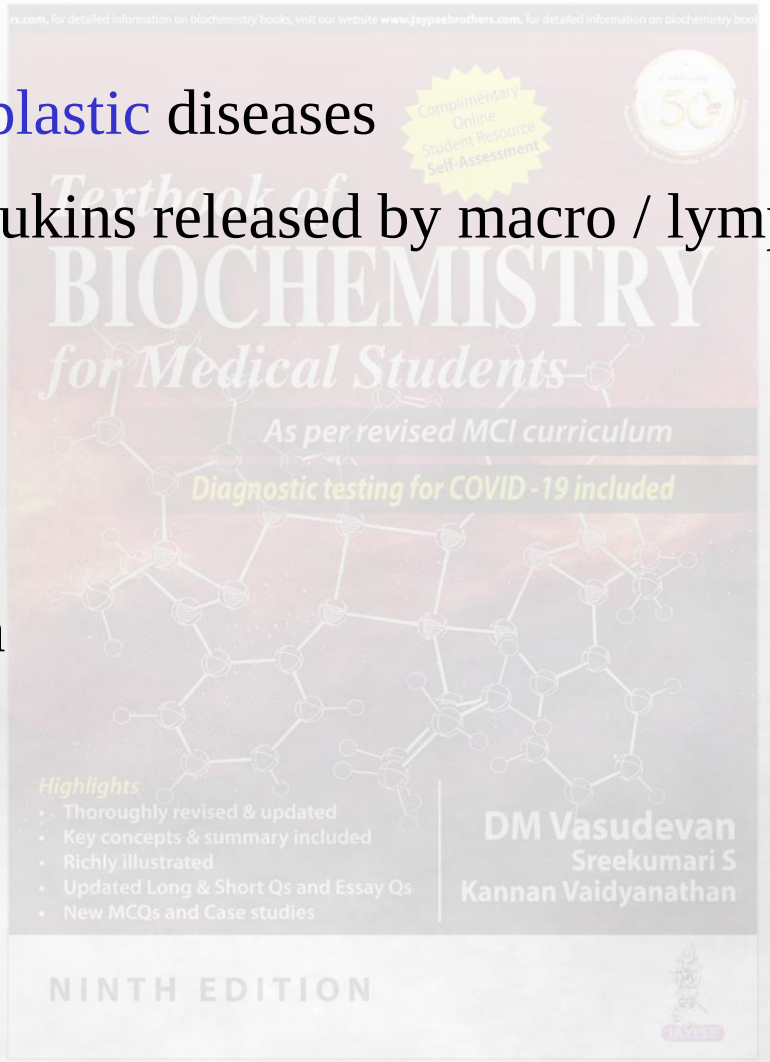
C-reactive protein

Ceruloplasmin

Haptoglobin

Alpha-1 anti trypsin

Fibrinogen



C-reactive Protein (CRP)

C-polysaccharide of capsule of pneumococci Beta globulin; Synthesised in liver

CRP+Bacteria -(similar to antibodies) Complement activation Bacteria killed CRP level parallels with

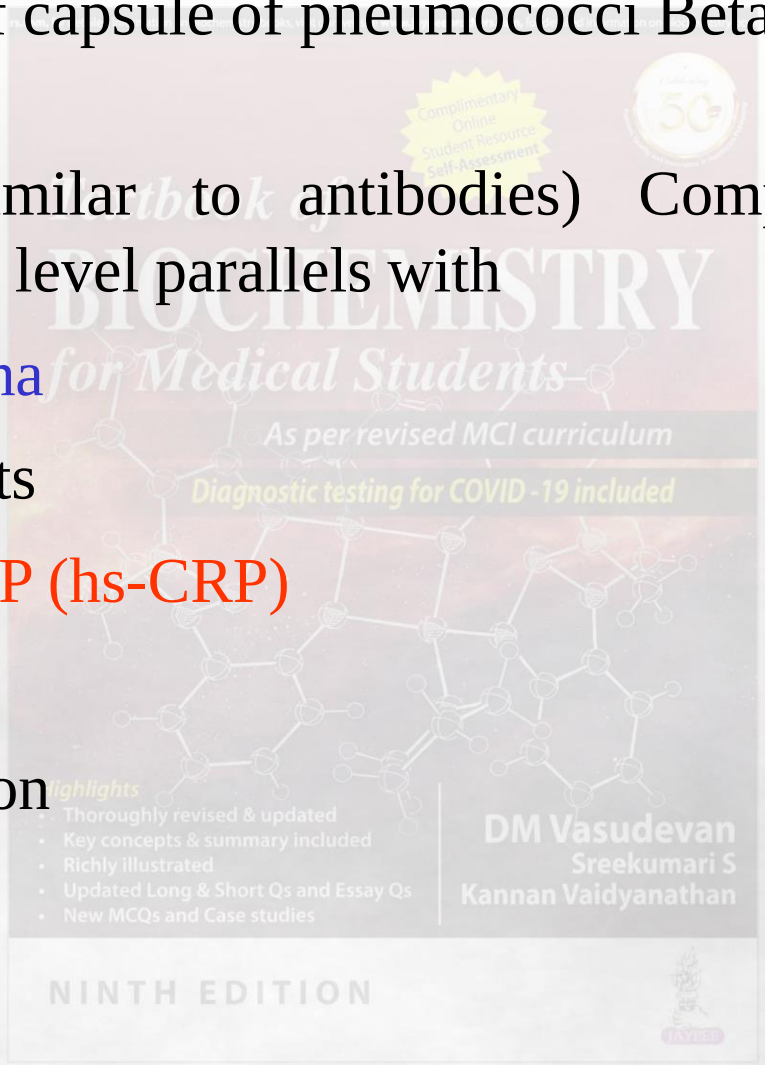
inflammation, trauma

Follow up of patients

High sensitivity CRP (hs-CRP)

Predictive value for

Myocardial infarction



Ceruloplasmin

Latin, blue

Alpha-2 globulin

Mol wt. 160,000

Synthesised in liver

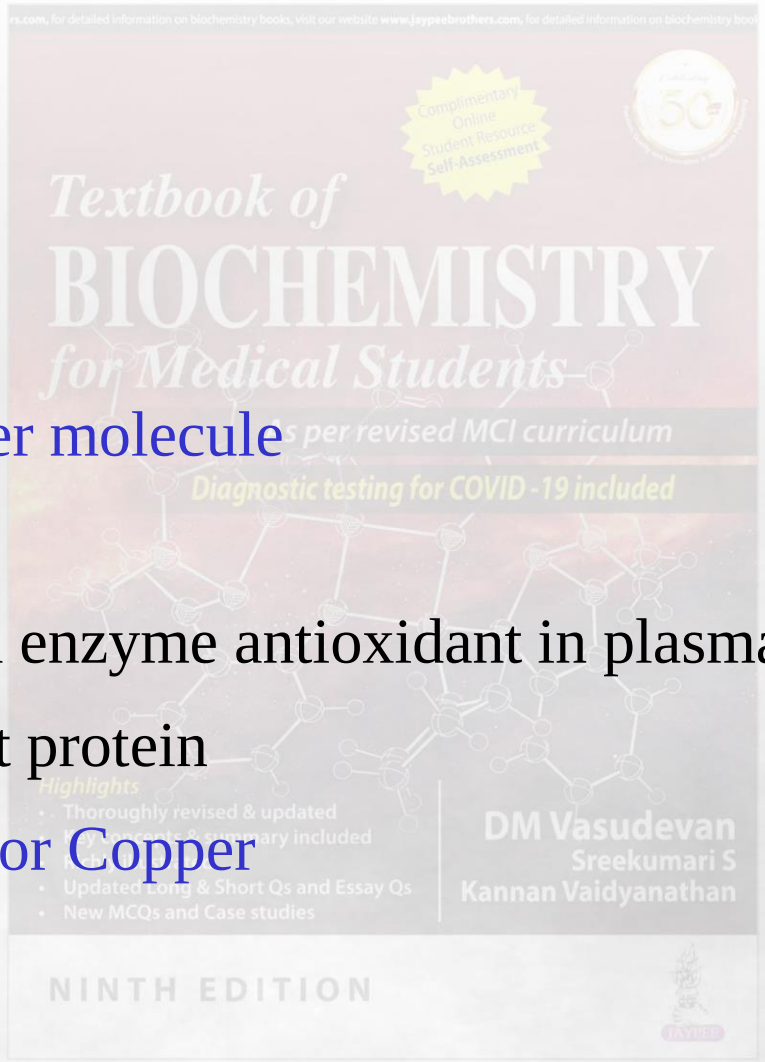
6-8 copper atoms per molecule

FERROXIDASE

Ceruloplasmin is an enzyme antioxidant in plasma

Cp is not a transport protein

Albumin is carrier for Copper



Ceruloplasmin

Normal blood level: 25-50 mg /dl

Reduced in Wilson's disease

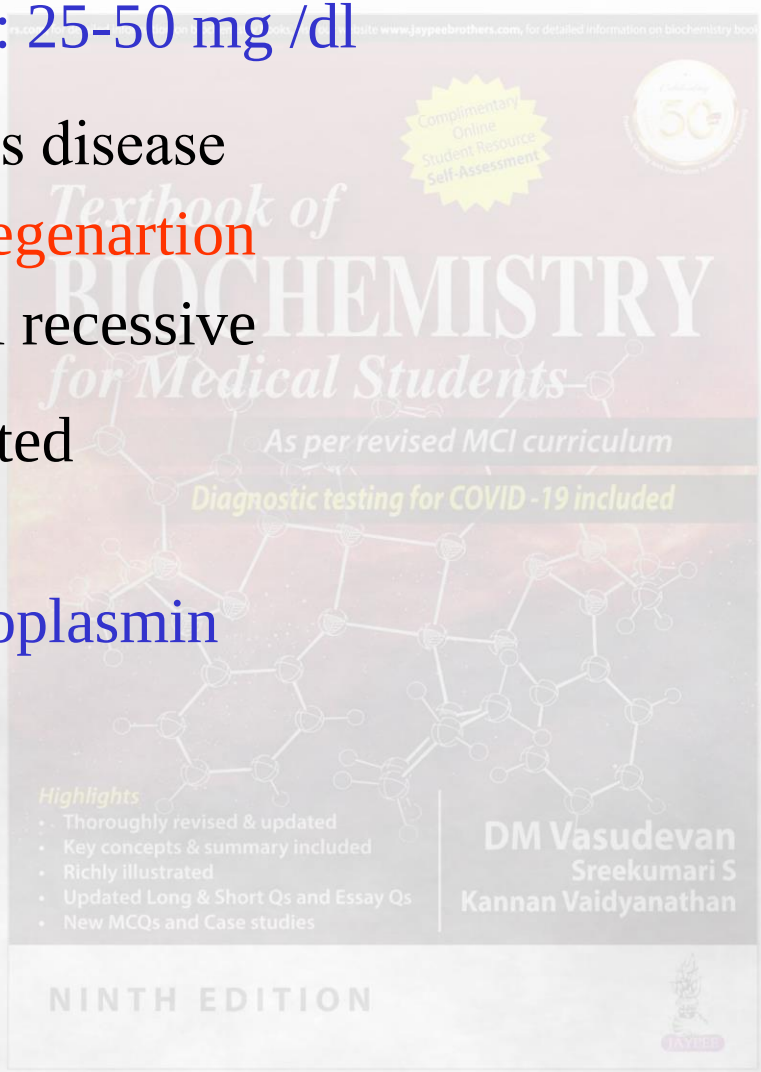
Hepato lenticular degenartion

Inherited autosomal recessive

Copper is not excreted

Copper toxicity

↓ copper into ceruloplasmin



Copper Deposited in Organs

Hepatic degeneration Cirrhosis

Basal ganglia;

Lenticular degeneration

Neurological symptoms

Kidney - Renal failure

Bone marrow: Hemolytic anemia

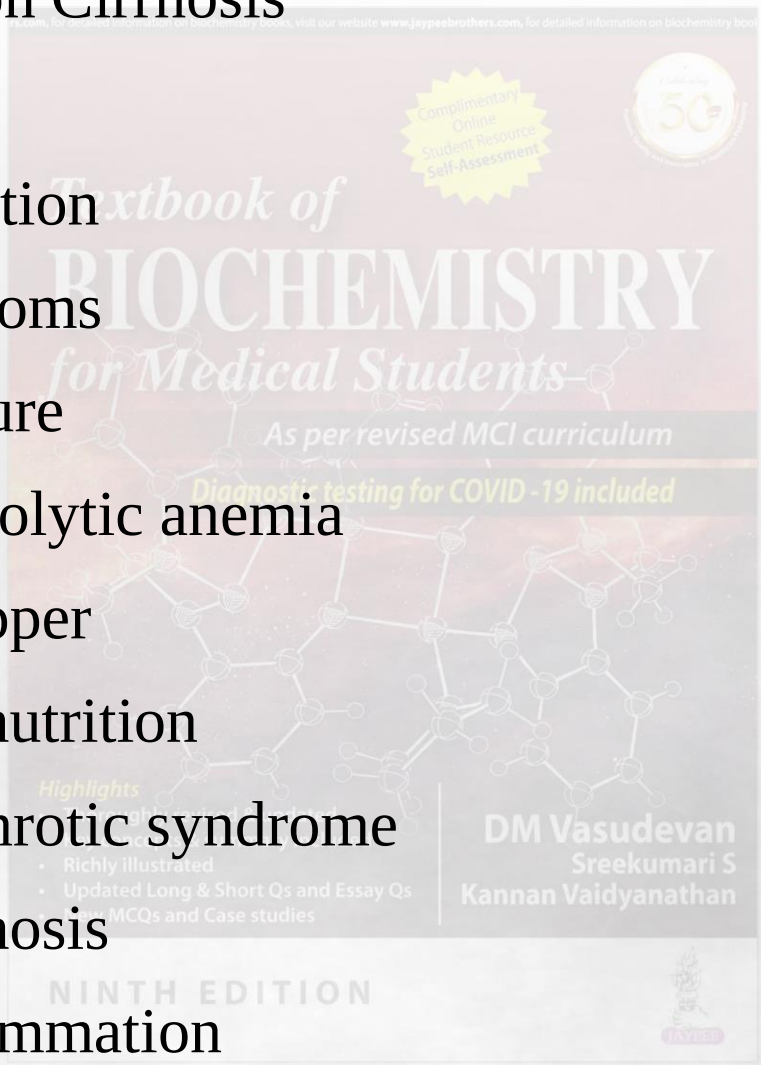
Treatment: Low copper

Low Cp: Malnutrition

Nephrotic syndrome

Cirrhosis

Increase Cp: Inflammation



Alpha-1 Antitrypsin (AAT)

Alpha anti proteinase

Protease inhibitor (Pi)

inhibits plasmin, thrombin, cathepsin, trypsin, chymotrypsin, elastase

Serine Protease Inhibitors

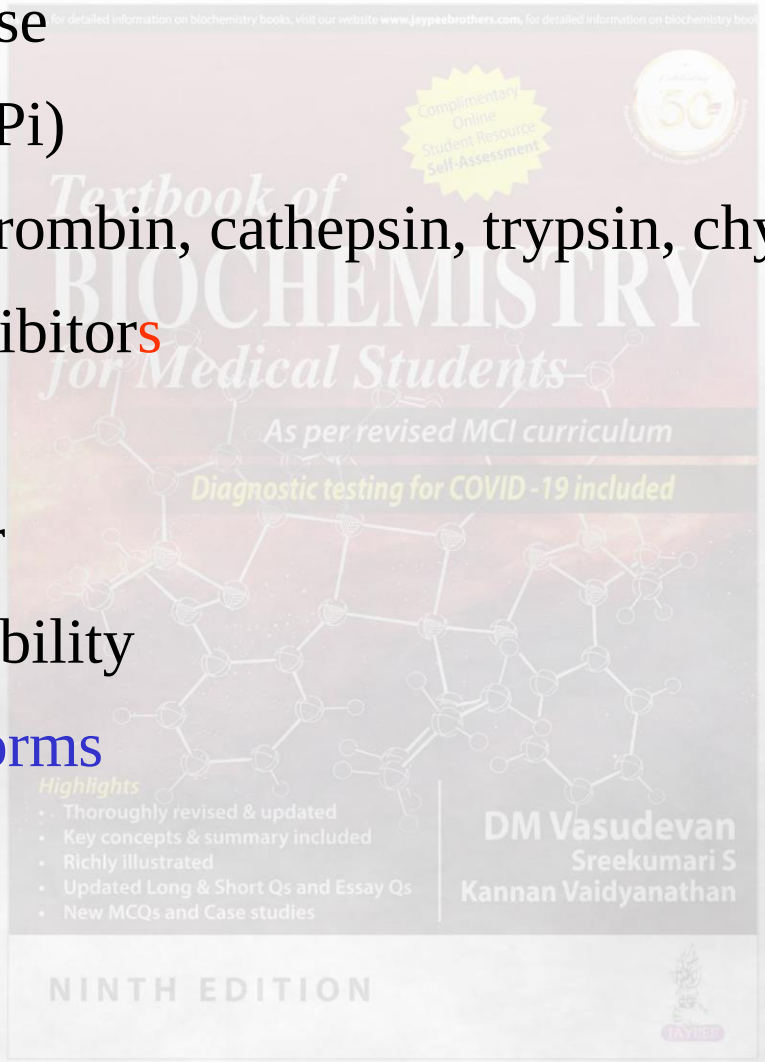
SERPINs

Synthesised in liver

Bulk of alpha-1 mobility

Multiple allelelic forms

75 variant forms



Polymorphism

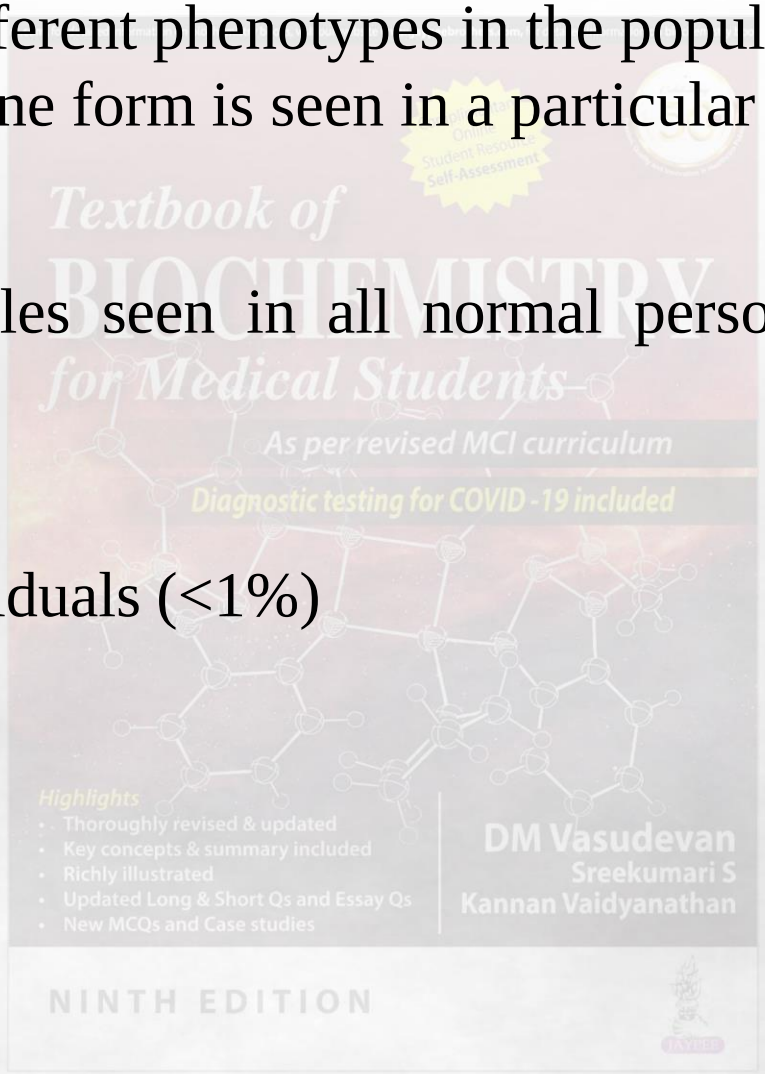
Protein exists in different phenotypes in the population (> 1%) ; but only one form is seen in a particular person

ISOFORMS

Variants in molecules seen in all normal persons, eg, Classes of immunoglobulins

MUTATION

Only in a few individuals (<1%)



AAT Deficiency

Alpha-1 band absent

1 in 1000; most common inborn error

Emphysema

Lung infection → macrophage → Elastase → Tissue destruction → Normally this is opposed by AAT In the deficient person →

continued action of elastase destruction of lung → Emphysema

About 5% cases of emphysema are due to AAT deficiency

Smoking will inactivate AAT So, in smokers, emphysema is common

Alpha-2 Macroglobulin (AMG)

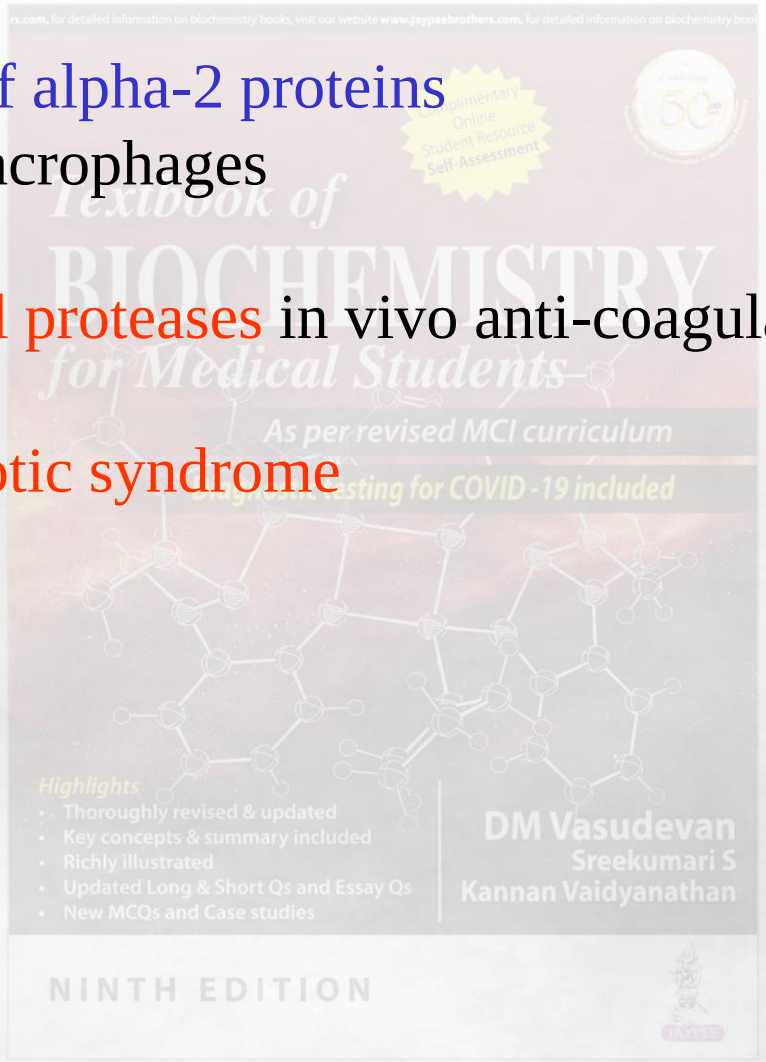
Mol.wt. 725,000

Major component of alpha-2 proteins

Hepatocytes and macrophages

AMG inactivates all proteases in vivo anti-coagulant

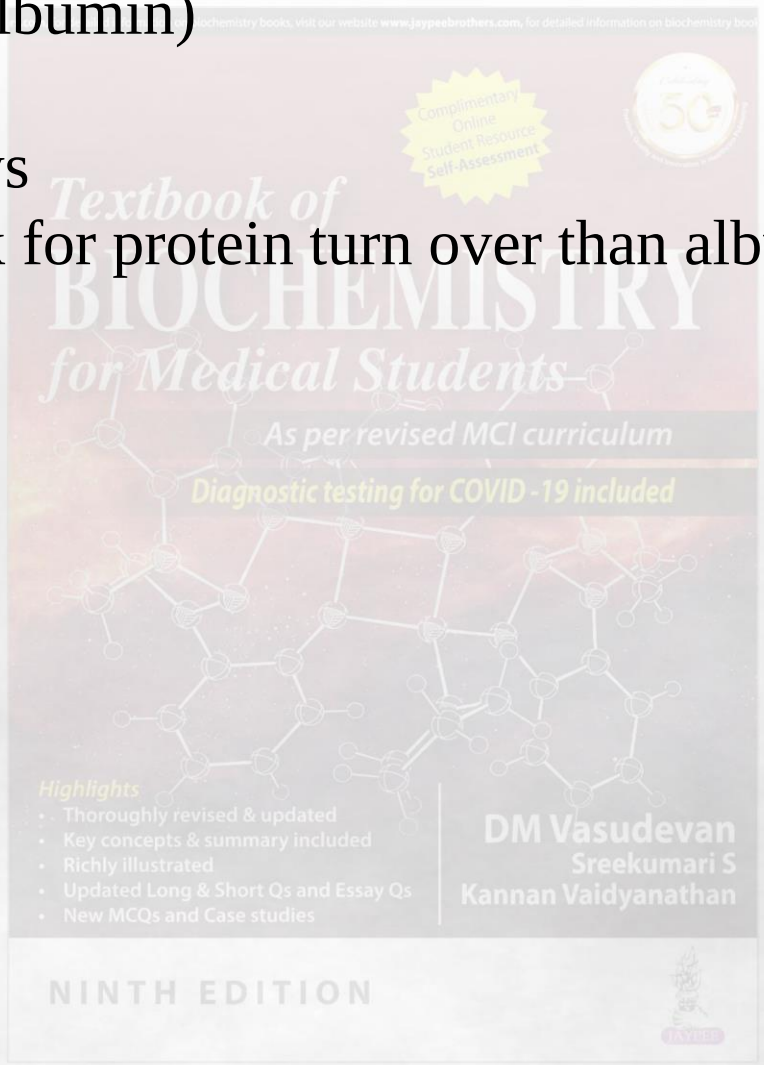
Increased in Nephrotic syndrome



Negative Acute Phase Proteins

Transthyretin (Pre-albumin)

Half life of 7-10 days
Hence a better index for protein turn over than albumin



C-Reactive Protein (CRP)

C-polysaccharide of capsule of pneumococci

Beta globulin; Synthesised in liver

CRP + Bacteria = Complement

activation = Bacteria killed

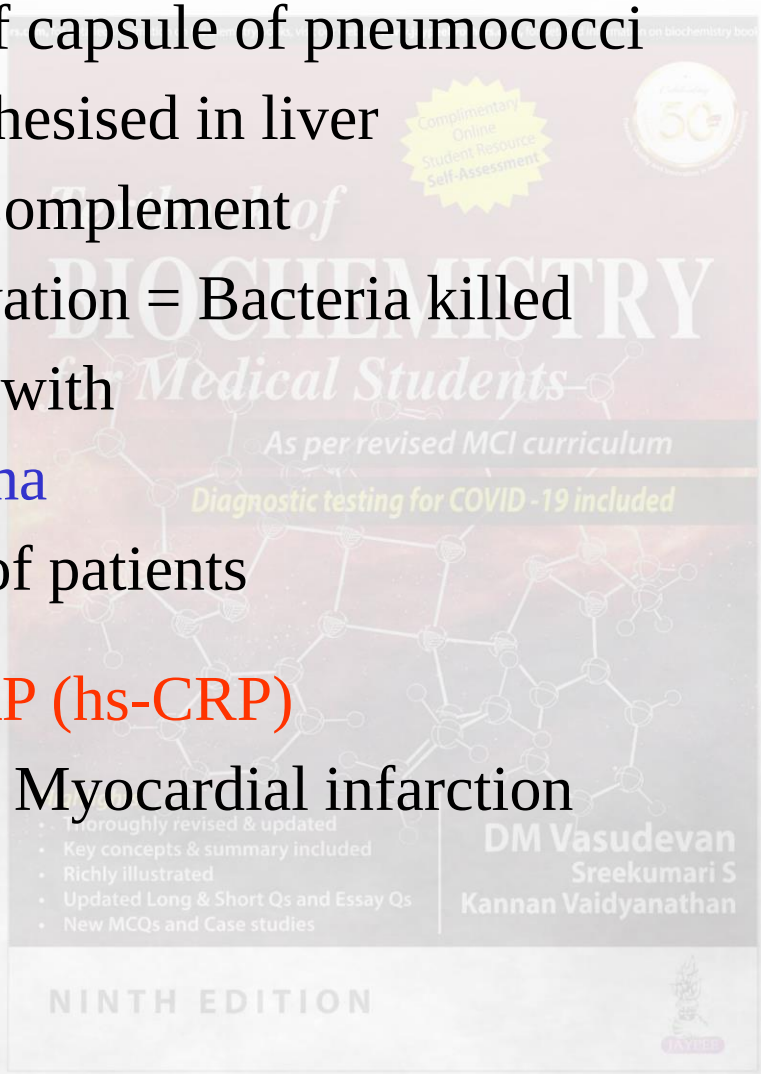
CRP level parallels with

inflammation, trauma

Follow up of patients

High sensitivity CRP (hs-CRP)

Predictive value for Myocardial infarction



Ceruloplasmin

Latin, blue

Alpha-2 globulin

Mol wt. 160,000

Synthesised in liver

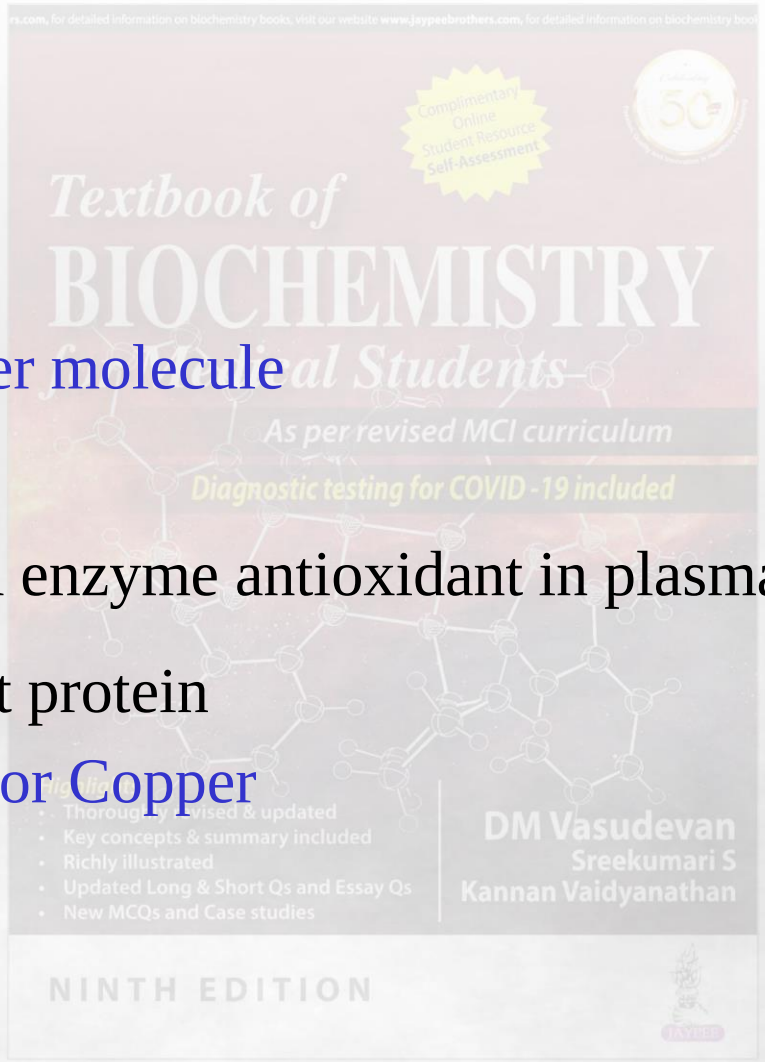
6-8 copper atoms per molecule

FERROXIDASE

Ceruloplasmin is an enzyme antioxidant in plasma

Cp is not a transport protein

Albumin is carrier for Copper



Ceruloplasmin

Normal blood level: 25-50 mg /dl

Reduced in Wilson's

Hepato lenticular degenartion

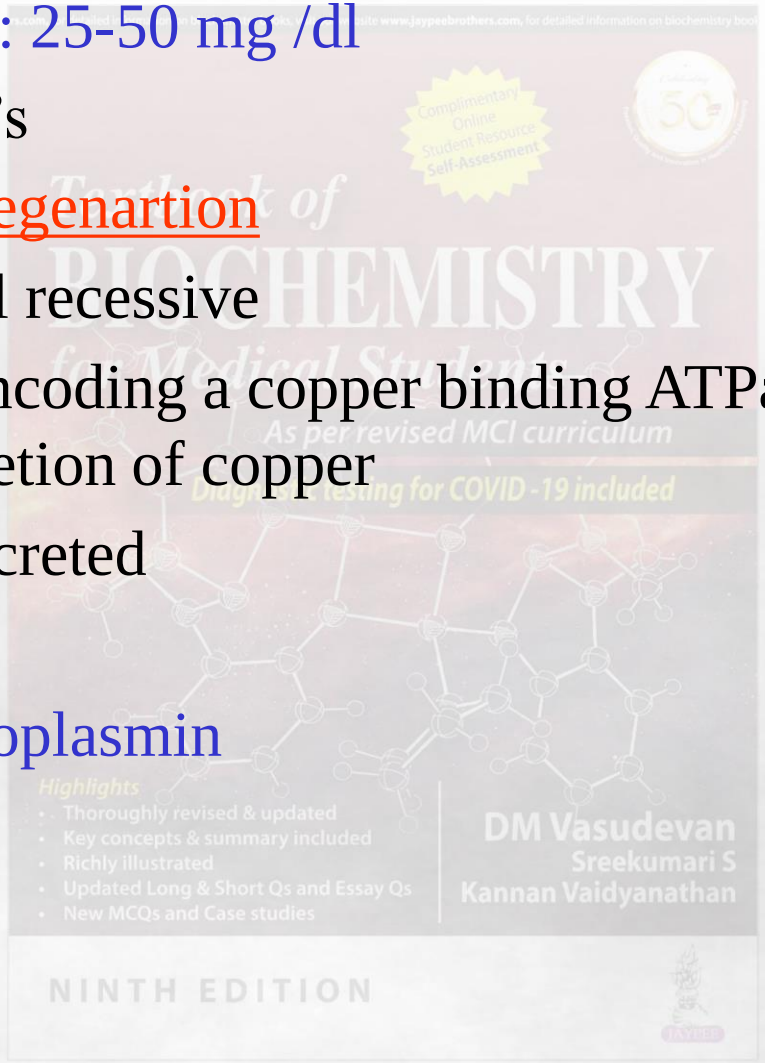
Inherited autosomal recessive

Mutation in gene encoding a copper binding ATPase in cells which is required for excretion of copper

So copper is not excreted

Copper toxicity

↓ copper into ceruloplasmin



Copper Deposited in Organs

Hepatic degeneration Cirrhosis

Basal ganglia;

Lenticular degeneration

Neurological symptoms

Kidney Renal failure

Bone marrow: Hemolytic anemia

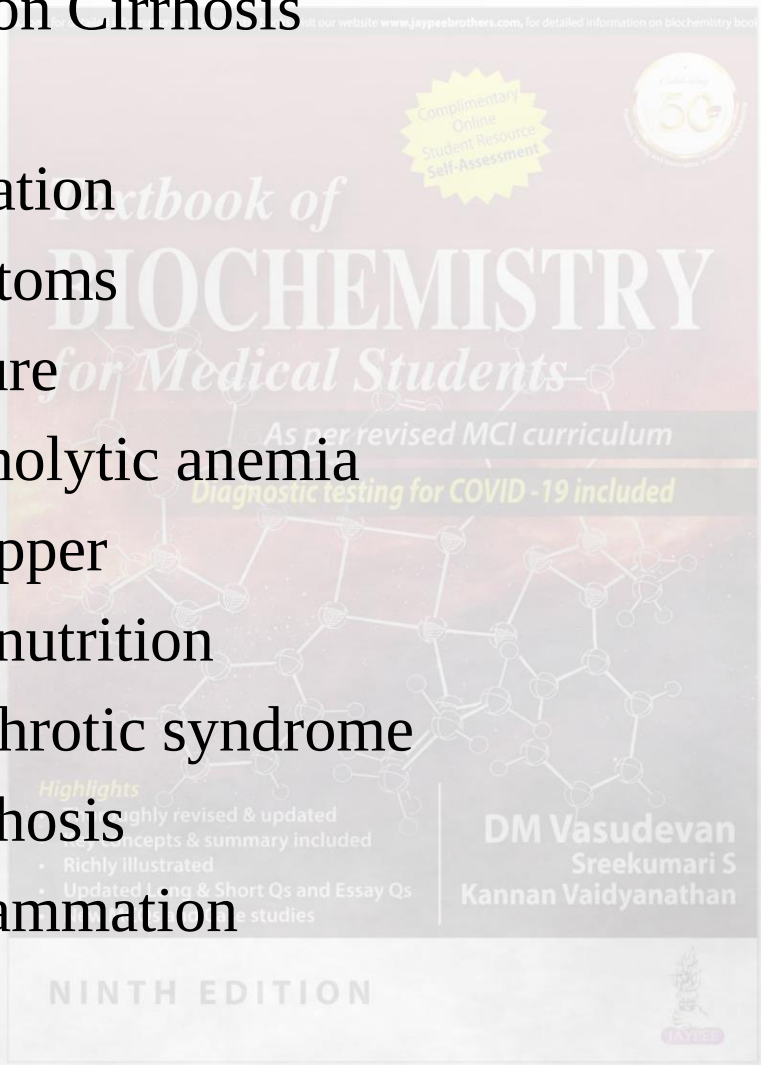
Treatment: Low copper

Low Cp: Malnutrition

Nephrotic syndrome

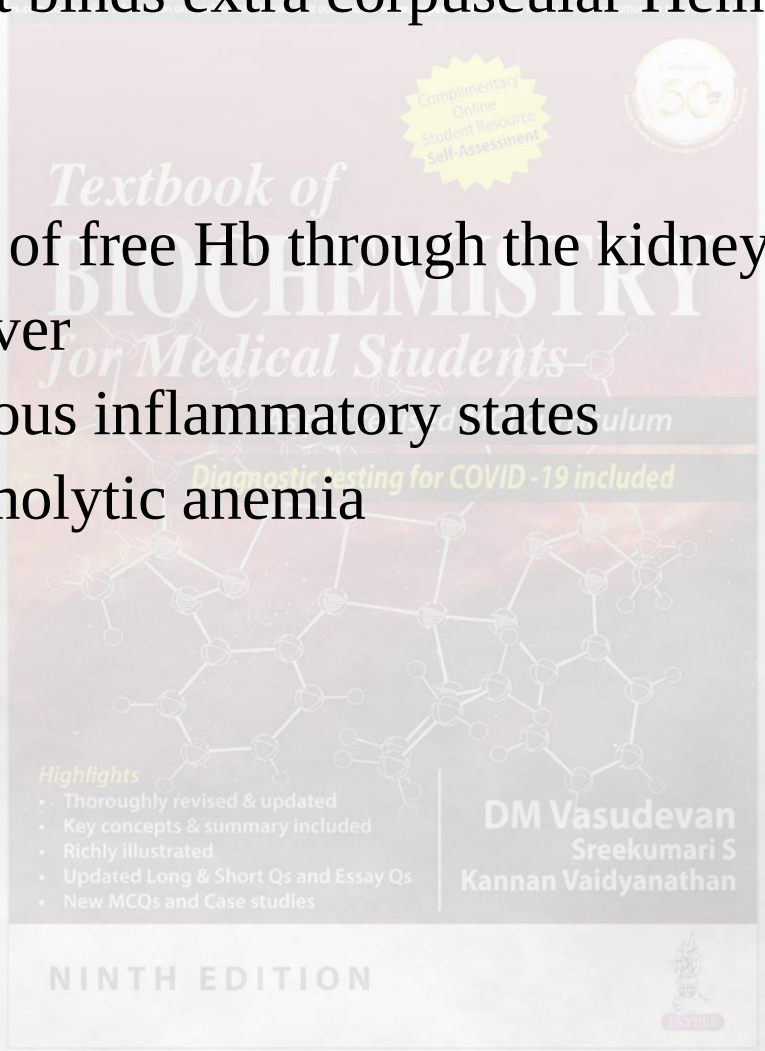
Cirrhosis

Increase Cp: Inflammation



Haptoglobin

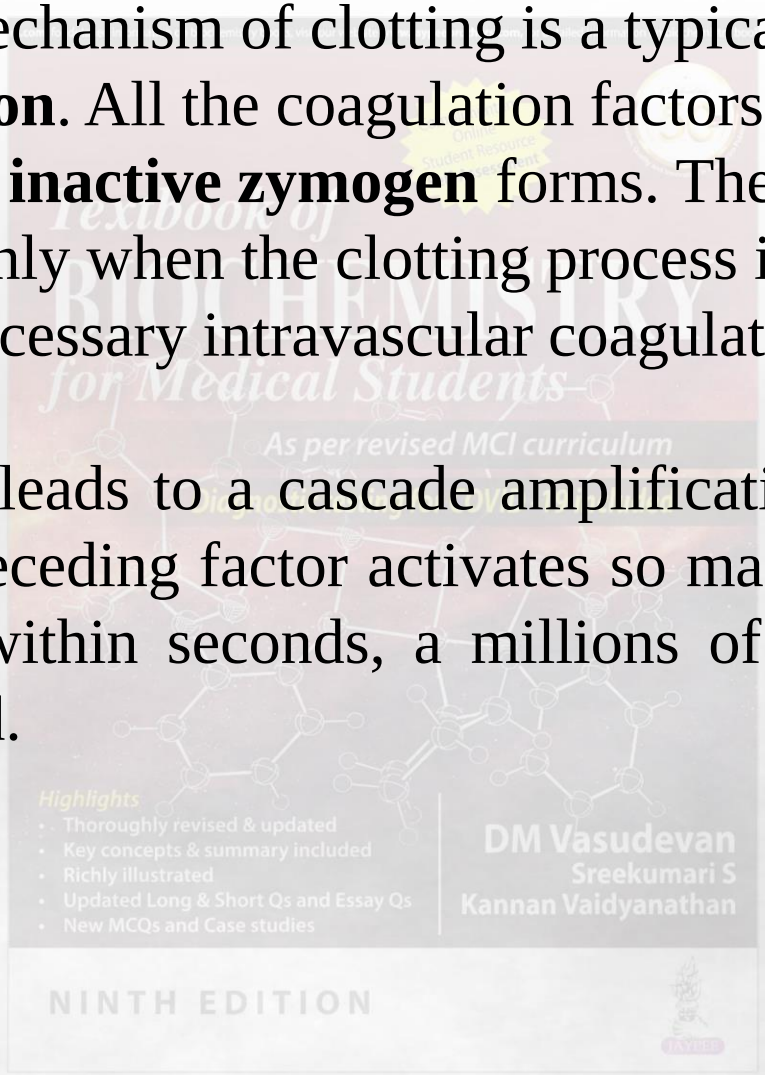
- Glycoprotein that binds extra corpuscular Hemoglobin
- 40 -180 mg/dl
- M wt: 90,000
- Prevents the loss of free Hb through the kidney
- Synthesized in liver
- Increased in various inflammatory states
- Decreased in hemolytic anemia



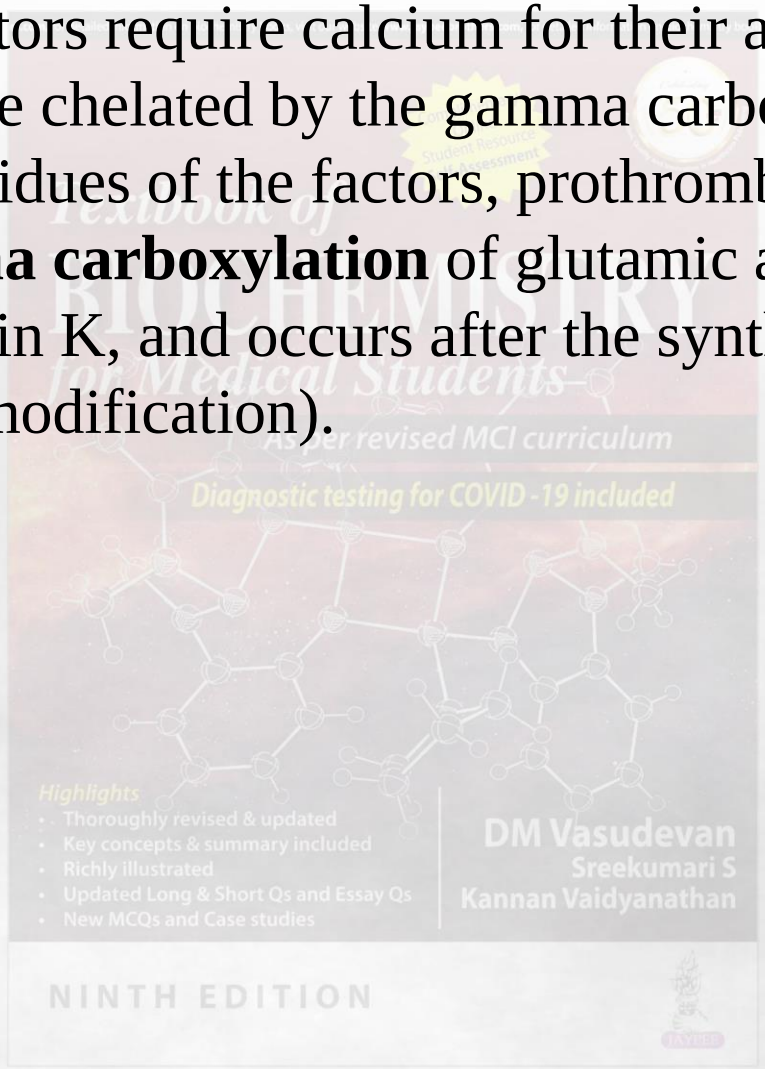
Clotting Factors

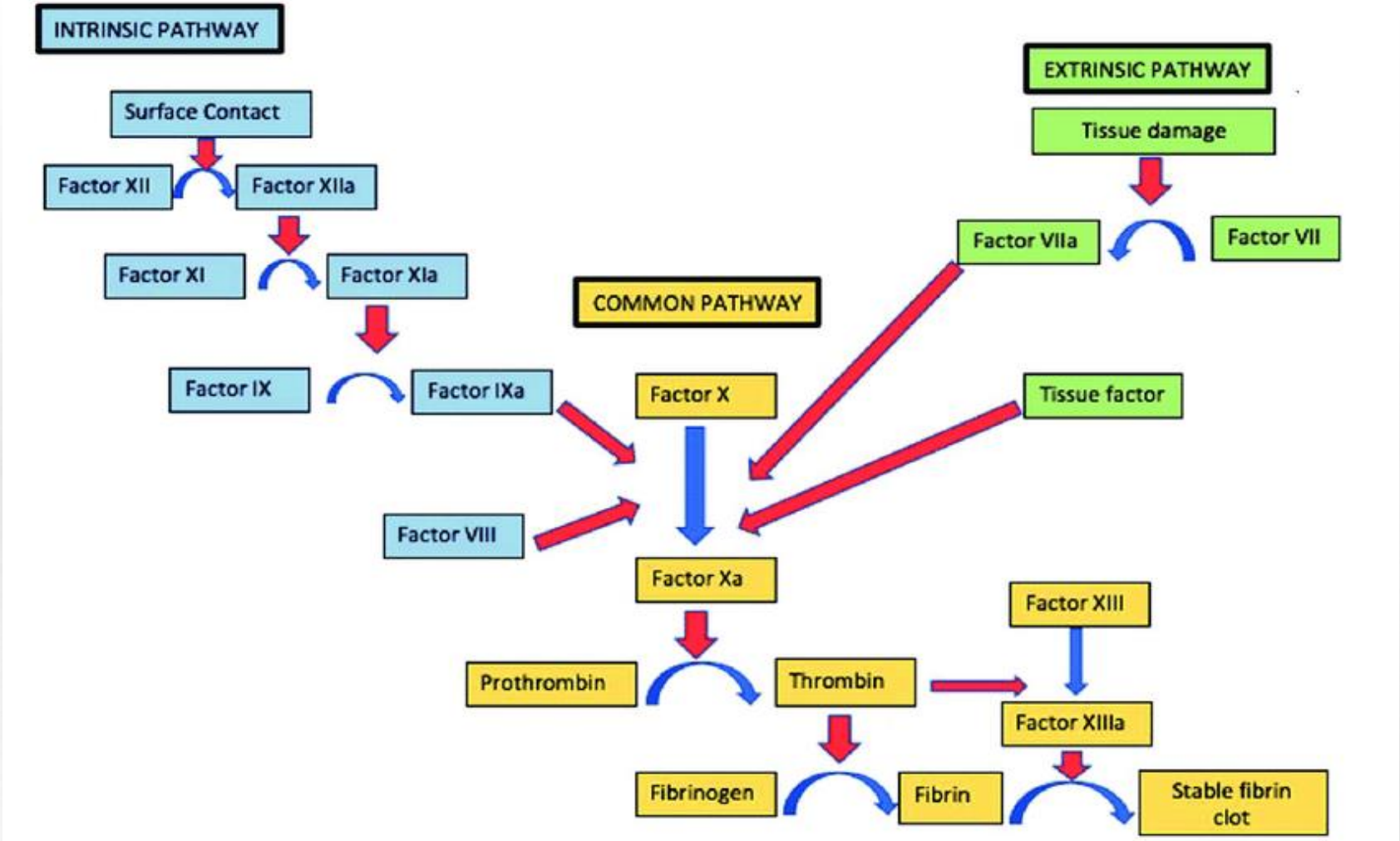
The biochemical mechanism of clotting is a typical example of **cascade activation**. All the coagulation factors are present in the circulation as **inactive zymogen** forms. They are converted to their active forms only when the clotting process is initiated. This would prevent unnecessary intravascular coagulation.

Activation process leads to a cascade amplification effect, in which one molecule of preceding factor activates so many molecules of the next factor. Thus within seconds, a millions of molecules of final factors are activated.



Several of these factors require calcium for their activation. The calcium ions are chelated by the gamma carboxyl group of glutamic acid residues of the factors, prothrombin, VII, IX, X, XI and XII. The **gamma carboxylation** of glutamic acid residue is dependent on vitamin K, and occurs after the synthesis of the protein (post-translational modification).





Cascade Pathway of Coagulation

Factors Involved in Coagulation Process

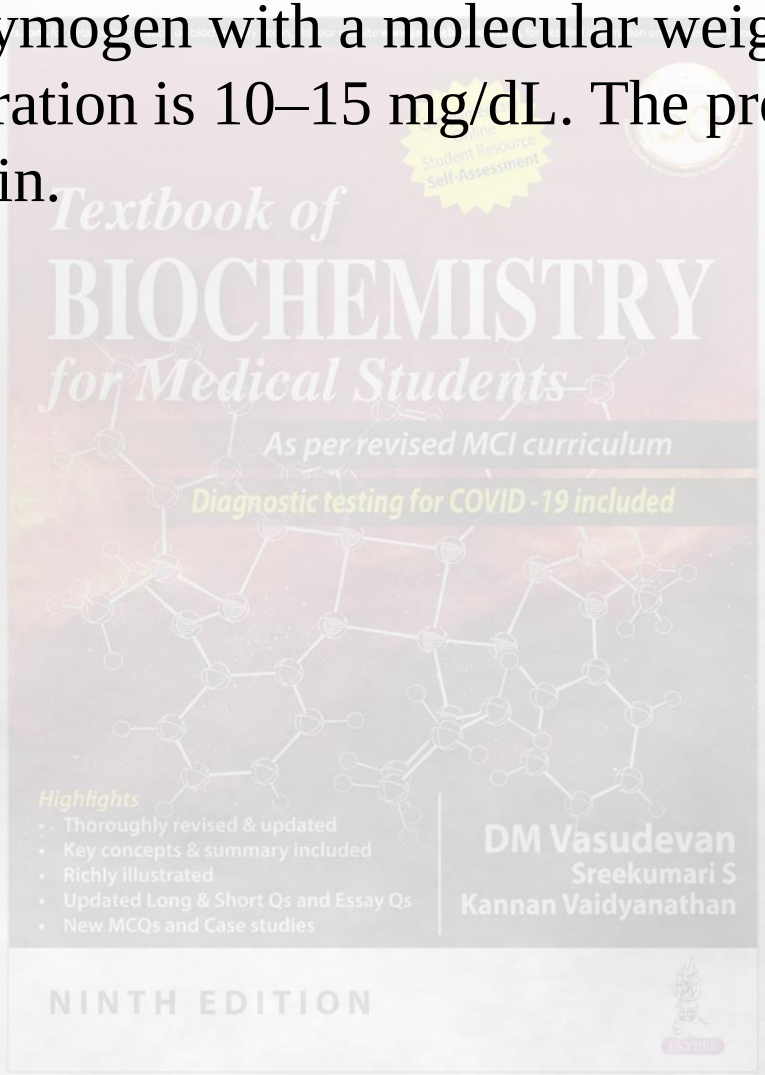
No.	Name	Activated by	Function
I	Fibrinogen	Thrombin	Forms the clot (fibrin)
II	Prothrombin	Factor Xa	Activation of fibrinogen and factors XIII, VIII and V
IV	Calcium	—	Activation of factor II, VII, IX, X, XI and XII
V	Labile factor	Thrombin	Binding of prothrombin to platelet
VII	Proconvertin; (SPCA)	Thrombin	Activation of factor X
VIII	Antihemophilic globulin (AHG)	Thrombin	Activation of factor X

Factors Involved in Coagulation Process

No.	Name	Activated by	Function
IX	Plasma thromboplastin-component (PTC); Christmas factor	Factor XIa	Activation of factor X
X	Stuart Prower factor	Factor IXa	Activation of prothrombin
XI	Plasma thromboplastin anticedent (PTA)	Factor XIIa	Activation of factor IX
XII	Hageman factor	Kallikrein	Activation of factor XI
XIII	Fibrin stabilising factor (Liki Lorand factor)	Thrombin	Stabilization of fibrin clot by forming cross-links

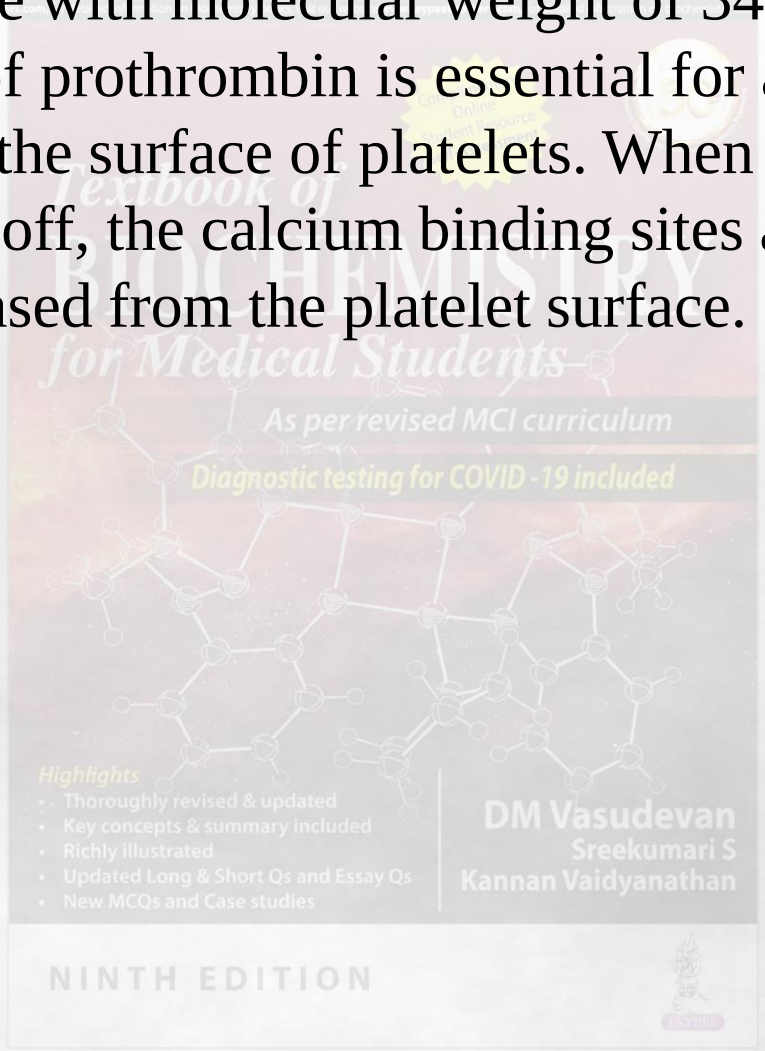
Prothrombin

It is a single chain zymogen with a molecular weight of 69,000D. The plasma concentration is 10–15 mg/dL. The prothrombin is converted to thrombin.



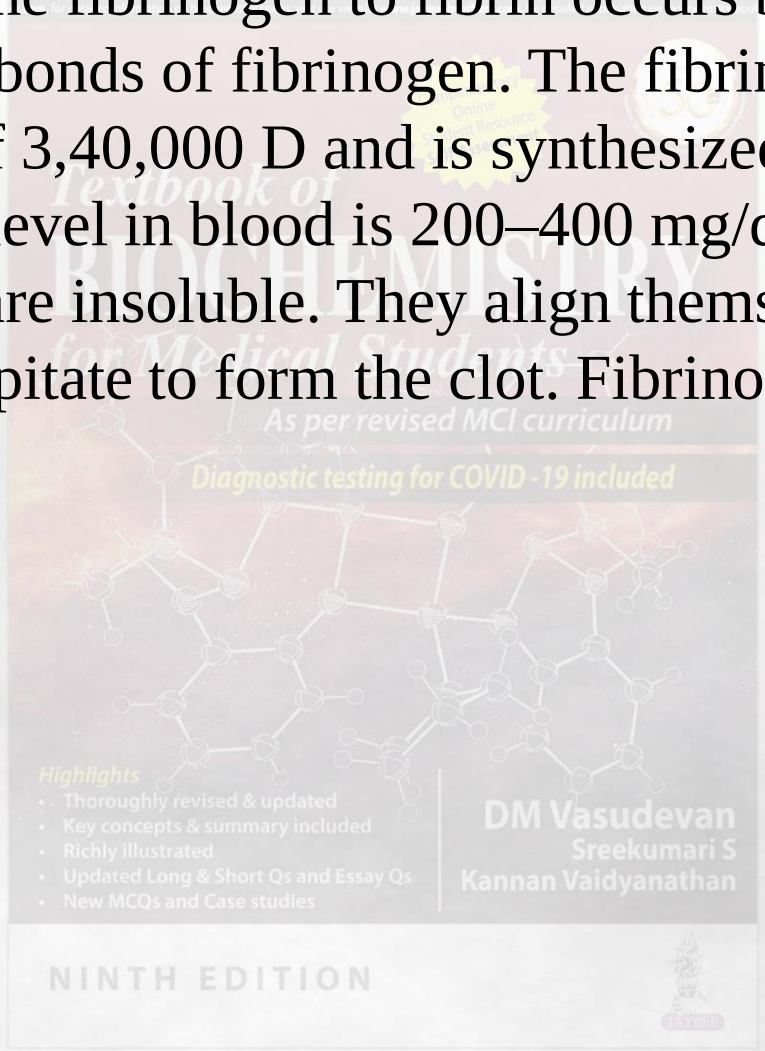
Thrombin

It is a serine protease with molecular weight of 34,000 D. The Ca++ binding of prothrombin is essential for anchoring the prothrombin on the surface of platelets. When the terminal fragment is cleaved off, the calcium binding sites are removed and so, thrombin is released from the platelet surface.



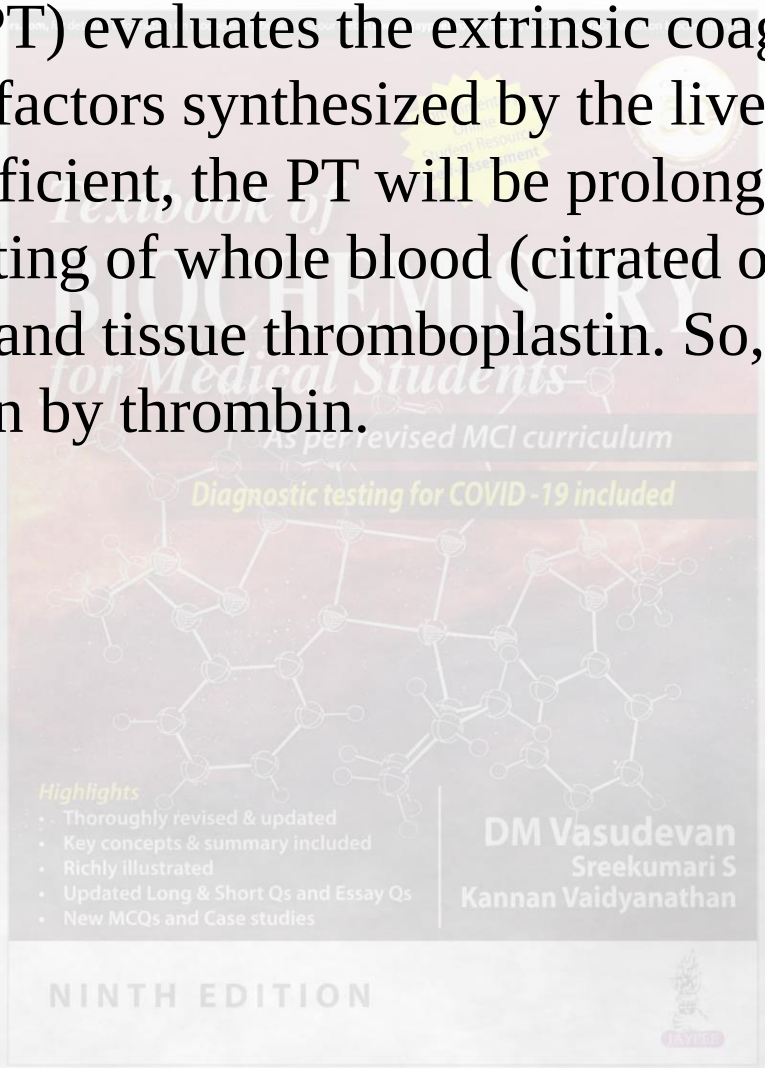
Fibrinogen

The conversion of the fibrinogen to fibrin occurs by cleaving of Arg-Gly peptide bonds of fibrinogen. The fibrinogen has a molecular weight of 3,40,000 D and is synthesized by the liver. Normal fibrinogen level in blood is 200–400 mg/dL. The fibrin monomers formed are insoluble. They align themselves lengthwise, aggregate and precipitate to form the clot. Fibrinogen is an **acute phase protein**.



Prothrombin Time

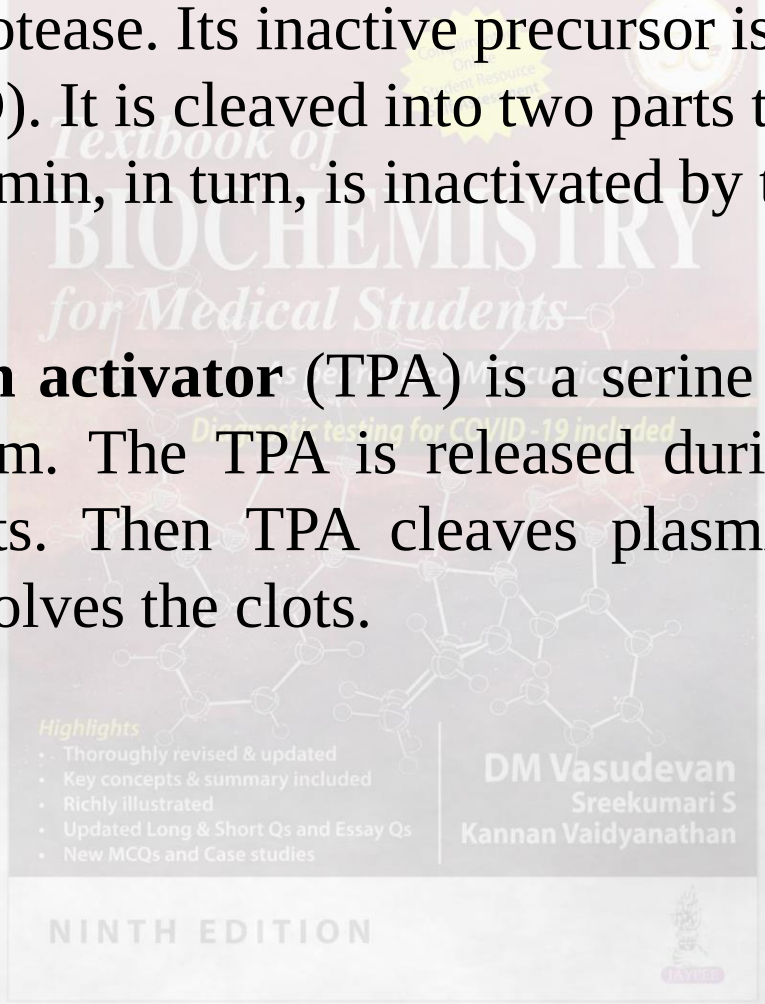
Prothrombin time (PT) evaluates the extrinsic coagulation pathway, so that if any of the factors synthesized by the liver (factors I, II, V, VII, IX and X) is deficient, the PT will be prolonged. PT is the time required for the clotting of whole blood (citrated or oxalated) after addition of calcium and tissue thromboplastin. So, fibrinogen is polymerized to fibrin by thrombin.



Fibrinolysis

Unwanted fibrin clots are continuously dissolved in vivo by plasmin, a serine protease. Its inactive precursor is plasminogen (90 kD). It is cleaved into two parts to produce the active plasmin. Plasmin, in turn, is inactivated by the alpha-2 antiplasmin.

Tissue **plasminogen activator** (TPA) is a serine protease present in vascular endothelium. The TPA is released during injury and then binds to fibrin clots. Then TPA cleaves plasminogen to generate plasmin, which dissolves the clots.



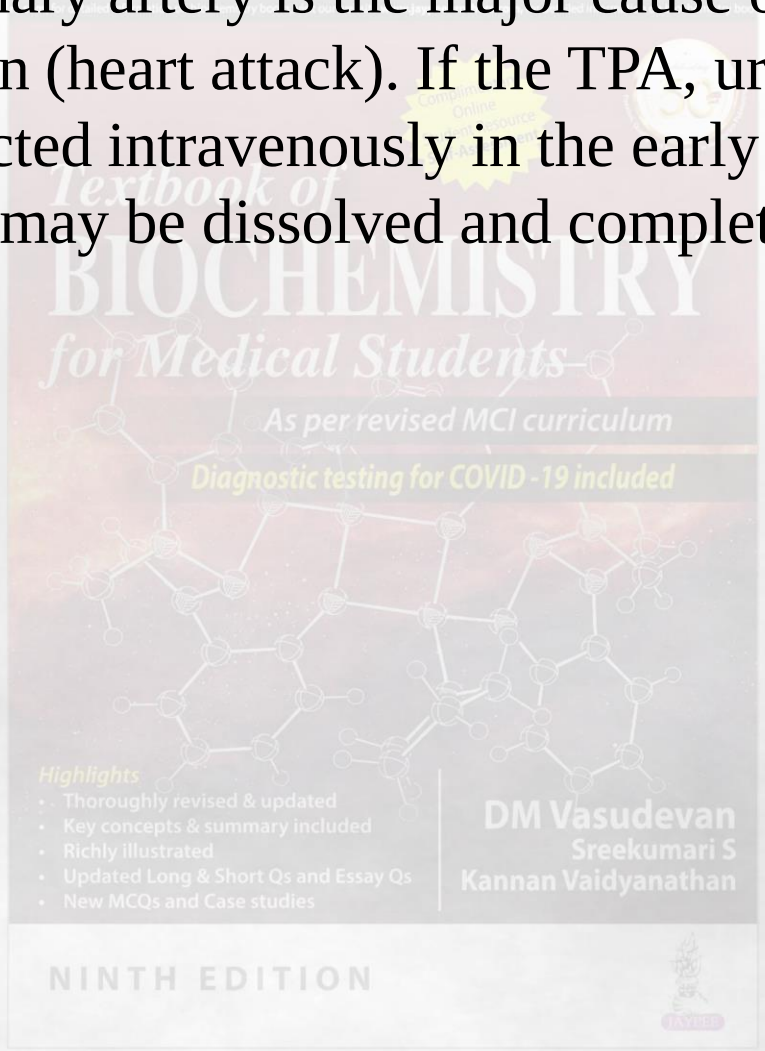
D-Dimer

It is the protein fragment that forms in blood soon after the degradation of a blood clot and later gets cleared by the kidney. In Covid pandemic complications, the formation of higher level of blood clots in blood will lead to severe fatal pneumonia, pulmonary embolism or vein thrombosis. The venous thromboembolism can be identified by the D-dimer test.

D-dimer is one of the natural biomarkers for the extent of blood clotting. So, people who have a tendency for blood clotting disorders should mandatorily do a D-dimer test to know the extent of clot formation. The D-dimer level of above 0.5 milligrams per mL of blood or higher values means abnormal blood clotting and needs immediate treatment.

Clinical Significance of Clotting Factors

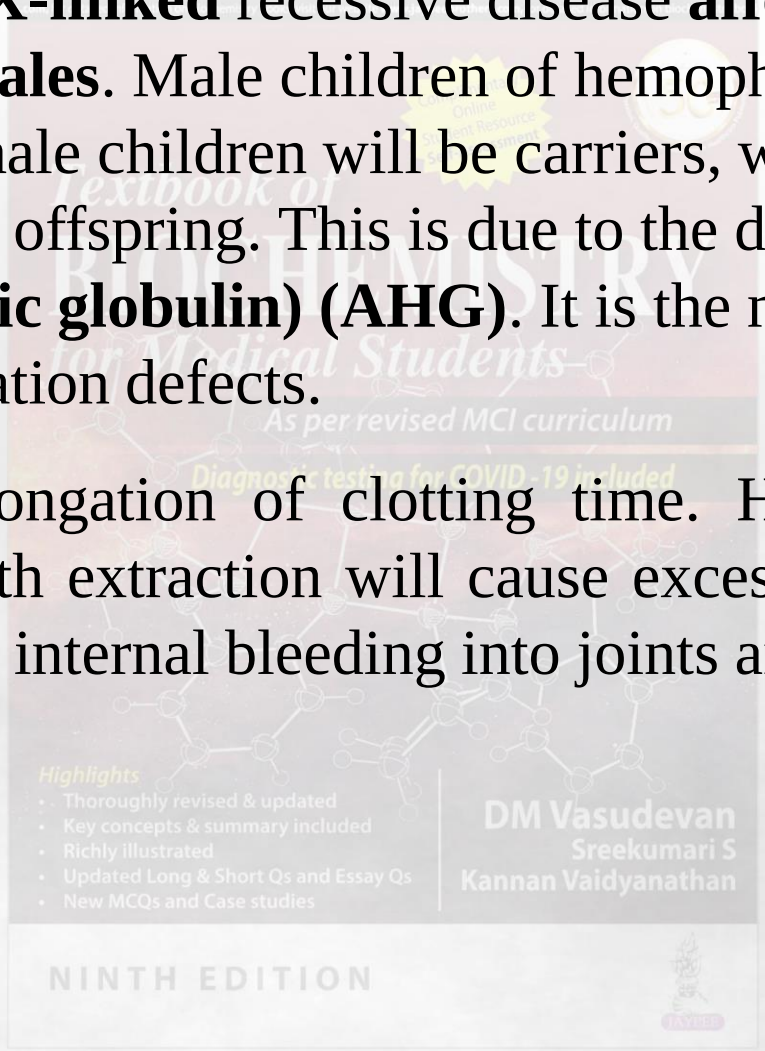
Thrombosis in coronary artery is the major cause of myocardial infarction (heart attack). If the TPA, urokinase or streptokinase is injected intravenously in the early phase of thrombosis, the clot may be dissolved and complete recovery of patient is possible.



Hemophilia A (Classical Hemophilia)

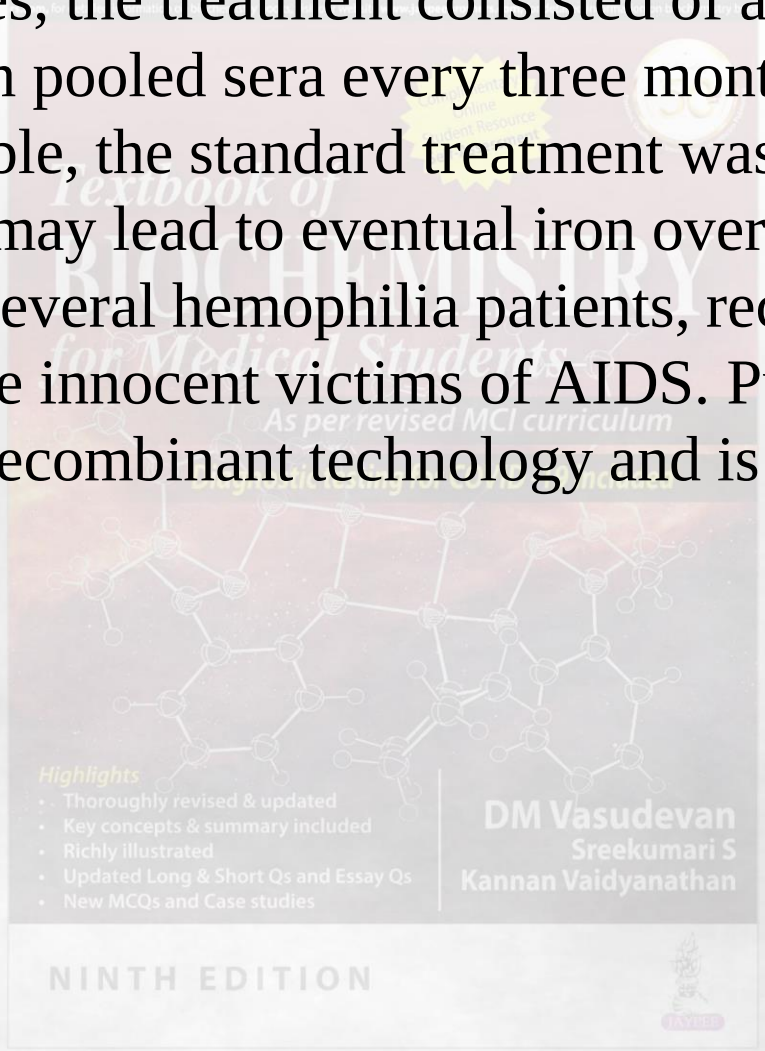
This is an inherited **X-linked** recessive disease **affecting males and transmitted by females**. Male children of hemophilia patients are not affected; but female children will be carriers, who transmit the disease to their male offspring. This is due to the deficiency of **factor VIII (antihemophilic globulin) (AHG)**. It is the most common of the inherited coagulation defects.

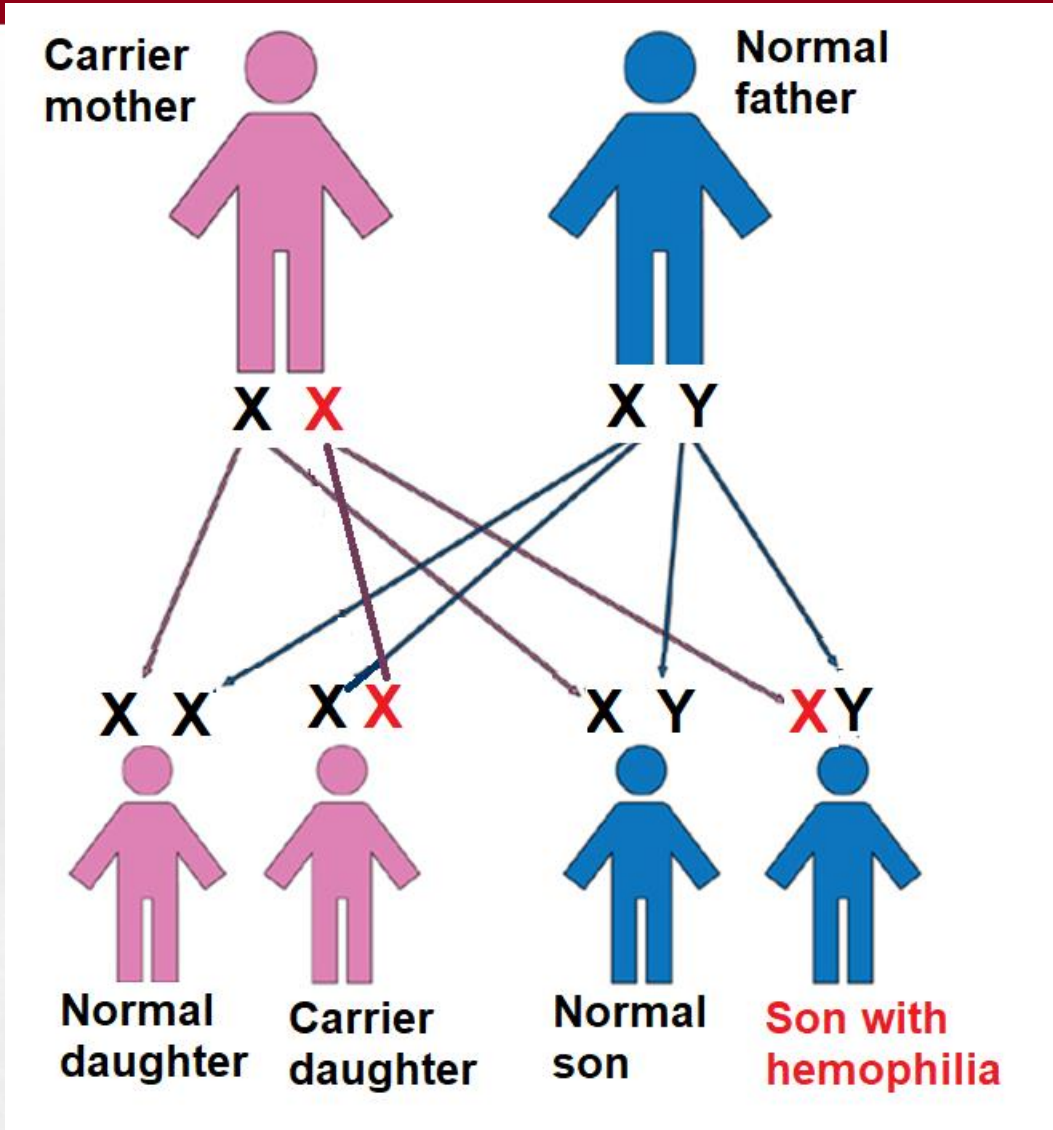
There will be prolongation of clotting time. Hence, even trivial wounds such as tooth extraction will cause excessive loss of blood. Patients are prone to internal bleeding into joints and intestinal tract.



Hemophilia A (Classical Hemophilia)

Until the recent times, the treatment consisted of administration of AHG, prepared from pooled sera every three months. Since this was not generally available, the standard treatment was to transfuse blood periodically, which may lead to eventual iron overload, called hemochromatosis. Several hemophilia patients, receiving repeated trans-fusions became innocent victims of AIDS. Pure AHG is now being produced by recombinant technology and is the treatment of choice.



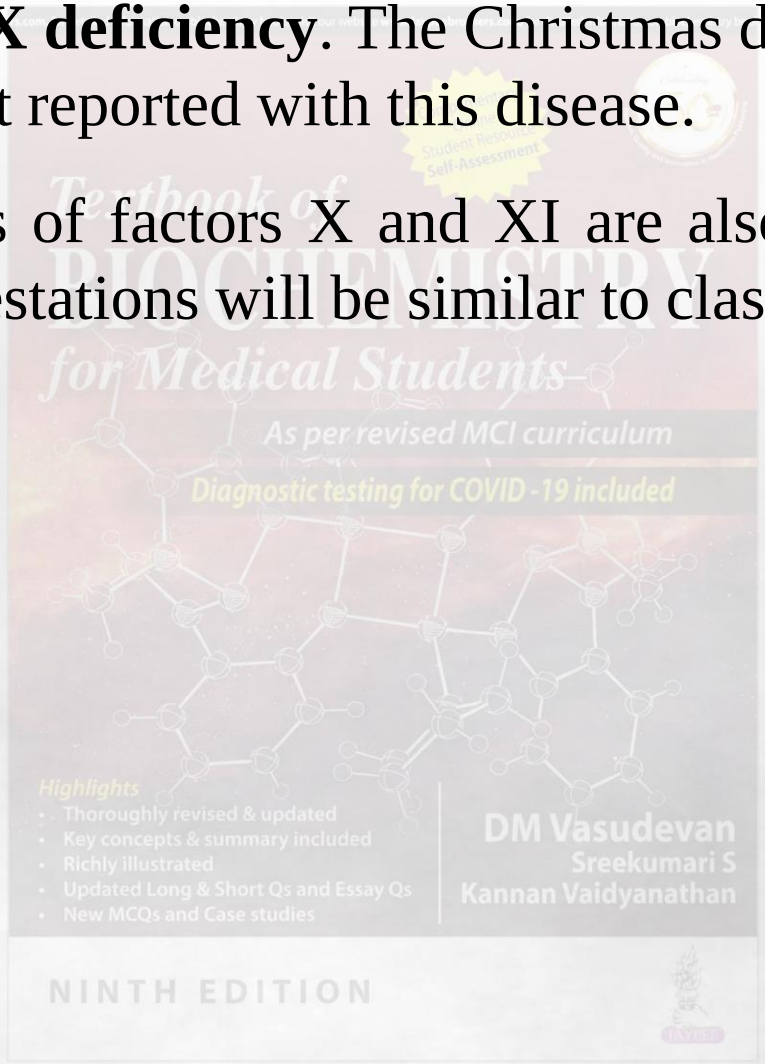


Inheritance Pattern of Hemophilia.

Hemophilia B or Christmas Disease

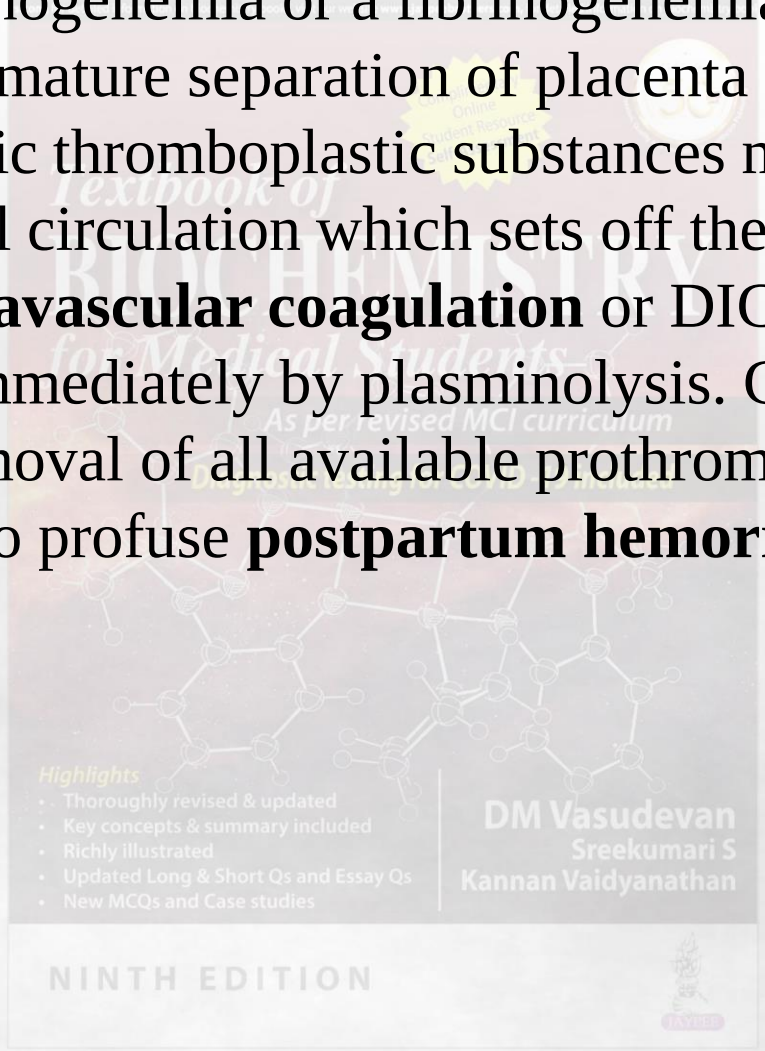
It is due to **factor IX deficiency**. The Christmas disease is named after the first patient reported with this disease.

Similar deficiencies of factors X and XI are also reported. In these diseases, the manifestations will be similar to classical hemophilia.



Other Disorders

Acquired hypofibrinogenemia or a fibrinogenemia may occur as a complication of premature separation of placenta or **abruption placenta**. Proteolytic thromboplastic substances may enter from placenta to maternal circulation which sets off the clotting cascade (**disseminated intravascular coagulation** or DIC). But the clots are usually degraded immediately by plasminolysis. Continuation of this process leads to removal of all available prothrombin and fibrinogen molecules leading to profuse **postpartum hemorrhage**.



Anticoagulants

They are two types: 1. Acting *in vitro* to prevent coagulation of collected blood, and 2. Acting *in vivo* to prevent and regulate coagulation. The first group of anticoagulant removes calcium which is essential for several steps on clotting. Oxalates, citrate and EDTA belong to this group.

Heparin and antithrombin III are the major *in vivo* anticoagulants. Heparin is also used as an anticoagulant for *in vitro* system, e.g. in dialysis and for the treatment of thrombo-embolic diseases. It is also used in the treatment of intravascular thrombosis. Low molecular weight heparin is used to prevent DVT. Antagonists to vitamin K are used as anticoagulants, especially for therapeutic purposes, e.g. Dicoumarol and Warfarin.