Clinical Enzymology and Plasma proteins

Clinical Enzymology

- Introduction:
- **- Enzymes:** are biological catalysts that increase the rate of specific chemical reaction in the living cell

- **- Enzyme properties:**
- 1) Biological catalysis
- 2) Very efficient, can increase reaction rates at the order of x10
- 3) All are proteins, so they are liable to temperature (denaturation)
- 4) Specific to substrates, Partially specific to tissues

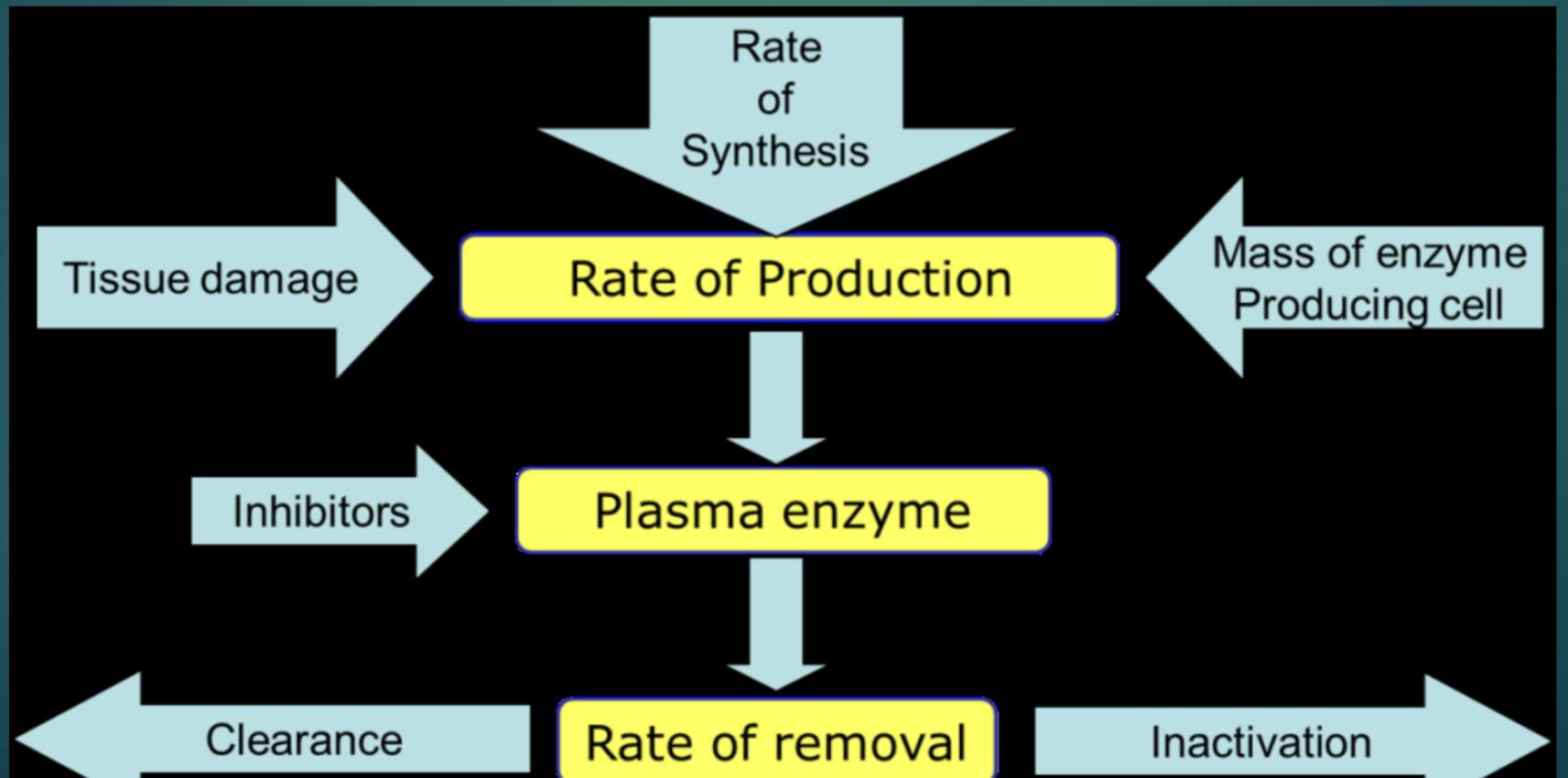
- **Enzyme activity is expressed in International unit (IU)**
- - The amount of enzymes that catalyses the conversion of one micromole (µmol) of substrate to product per minute
- - Katal (Kat): amount of enzyme required to increase the rate of enzyme reaction by 1 mole/s
- - Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted.
- - This entire active complex is referred to as the HOLOENZYME; i.e., protein portion APOENZYME plus non-protein part COFACTOR

- **-** The **COFACTOR** may be:
- 1) Coenzyme: organic substance loosely attached to the protein part
- 2) Prosthetic group: organic substance firmly attached to the protein part
- 3) Metal-ion-activator: K^+, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}

- Plasma enzymes
- **- Enzymes are normally intracellular and LOW concentration in plasma**
- **- Enzyme release (leakage) in the blood indicates cell damage.**
- **- Functional plasma enzymes:** they are enzymes that act on substrate normally present in plasma (coagulation enzymes and LPL)

- **- Non-functional plasma enzymes:** they are enzymes that act on substrate within the cell, these enzymes may transport from cells to plasma due to:
- 1) Cell aging
- 2) Diffusion
- 3) Excretion
- 4) Cell damage

The plasma enzymes level is balanced by **cellular production rate** of the enzymes and the **catabolism of these enzymes**.



Measurement plasma enzymes

- - Sources of non-functional enzymes increased plasma concentration:
- 1) Increase in the rate of enzyme synthesis, e.g. Bilirubin increases the rate of ALP in obstructive liver diseases.
- 2) Obstruction of normal pathways e.g. obstruction of bile ducts increases ALP.
- 3) Increased permeability of cell membrane as in tissue asphyxia
- 4) Cell damage with the release of its content of enzymes into the blood, e.g. myocardial infarction and viral hepatitis.

- Medical importance of non-functional enzymes:

- 1) Diagnosis of diseases; as diseases of different organs causes elevation of different plasma enzymes.
- 2) Prognosis and disease monitoring; can follow up the effect of treatment by measuring plasma enzymes before and after treatment.

▪ Non-specific factors affect plasma enzymes level

- **a) Physiological factors:**
- 1) AST and CK in new-born are more than in adults.
- 2) ALP in children is more than adults.
- 3) ALP in pregnant is high due to the extra secretion from placenta.
- 4) GGT and CK are higher in men than in women
- **b) Drugs:**
- 5) Phenothiazine increases the liver enzymes
- 6) Alcohol and anticonvulsants increase GGT.
- **c) Artefactual elevations:**
- 7) Hemolysis usually increases all enzymes that are present in RBCs (AST, LDH and Aldolase)
- 8) Prostatic massage increases PAP level

Isoenzymes

- - Isoenzymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction (differ in some physical or chemical properties)
- **- Formed of two or more polypeptide chains (Differ in AA sequence)**
- **- Different polypeptide chains are products of different genes**
- - May be separable on the basis of charge (electrophoresis) or the molecular weight (ultracentrifugation)
- **- They are tissue specific.**

a) Creatine Kinase (CK)

- **- Creatine Kinase is a dimer made of 2 monomers occurs in the tissues** - Skeletal muscle contains M subunit.
- - Brain contains B subunit.

- **-** So there are 3 different isoenzymes are formed:

Isoenzyme	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases
CK-2	MB	Myocardium / Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle	Muscular dystrophy After surgery

b) Lactate Dehydrogenase (LDH)

- **-** LDH is a tetrameric protein and made of two types of subunits - Heart contains 2 H subunits
- - Skeletal muscle contains 2 M subunits.

- **- So there are 5 different isoenzymes are formed:**

Isoenzyme	Composition	Present in	Elevated in
LDH1 (H4)	HHHH	Myocardium, RBCs	Myocardial Infarction
LDH2 (H3M1)	HHHM	Myocardium, RBCs, kidney	
LDH3 (H2M2)	HHMM	Brain, Lung, WBCs	
LDH4 (H1M3)	HMMM	Lung, Skeletal muscle	
LDH5 (M4)	MMMM	Skeletal muscle, Liver	Skeletal muscle & liver diseases

Enzymes of clinical interest

Enzyme	Site	Increased Plasma Levels		
		Physiological	Pathological	Artefactual
Aspartate aminotransferase (**AST**).	Liver Muscles Heart RBCs	Newborn	Myocardial Infarction Liver disease especially with liver cell damage	Hemolysis
Alanine aminotransferase (**ALT**)	Liver Muscles Heart		Liver disease especially with liver cell damage	
Alkaline phosphatase (**ALP**).	Bone Liver Intestine Placenta Kidney	Children Pregnancy Heavy meals	Bone diseases, Osteomalacia & rickets Hepatobiliary diseases	

Enzyme	Site	Increased Plasma Levels		
		Physiological	Pathological	Artefactual
PAP	Prostate Liver RBCs		Prostatic cancers	Prost. massage Catheter, Hemolysis Constipation
5-Neucleotidase (5'-NT).	Biliary tract		Hepatobiliary disease	
γ-glutamyltransferase (**GGT**).	Liver Kidney		Liver cirrhosis Alcoholism	

Enzyme	Site	Increased Plasma Levels		
		Physiological	Pathological	Artefactual
CK	Brain Heart Muscles	Male > Female	Myocardial infarction, Muscle diseases	After surgery
Lactate dehydrogenase-LDH	Heart Liver Muscles RBCs		Myocardial infarction Liver disease Hematologic diseases	Hemolysis
Aldolase	Skeletal Muscles Heart		Muscle diseases	Hemolysis
Amylase	Saliva Pancreas Ovaries		Acute pancreatitis	

Plasma proteins

- Introduction
- - Proteins are present in all body fluids, but only plasma proteins are examined most frequently for diagnostic purposes.
- - Over 300 individual proteins have a physiological function in the plasma. The concentrations of many of these are affected by pathological processes.
- - Plasma concentration of total proteins is 6–8 gm% of this 3–5 gm% (60%) is albumin and 2 – 3.5 gm% (35%) are globulins

- **- The concentration of plasma proteins (PP) is determined by 3 main factors:**
- 1) Rate of protein synthesis
- 2) Rate of protein catabolism
- 3) The volume of fluid in which proteins are distributed.

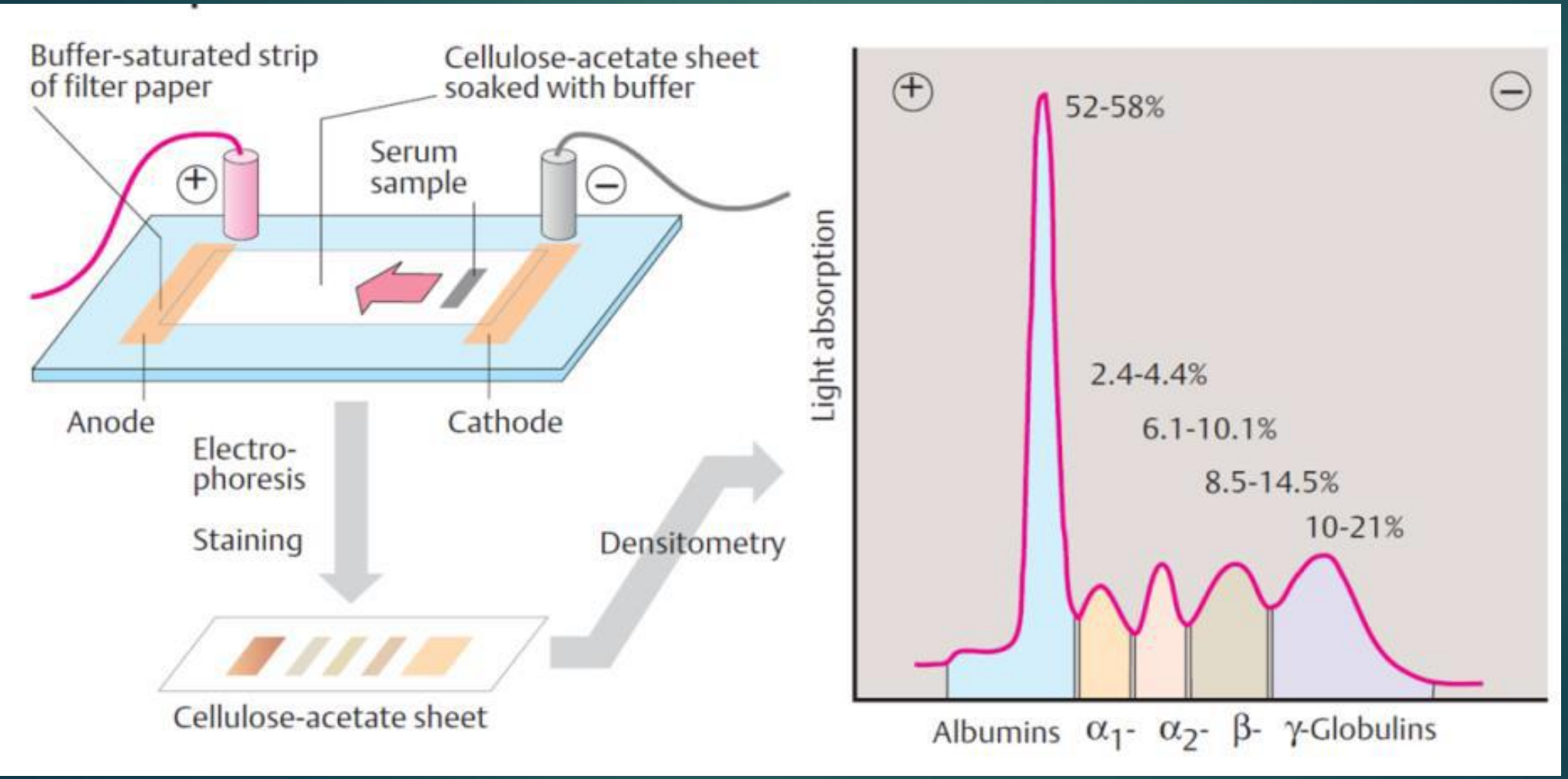
- **a) Synthesis**: All plasma proteins are synthesized in the liver, although some of them are produced in other sites, such as immunoglobulins by lymphocytes.
- **b) Distribution**: Water passes more freely through capillary walls than proteins and therefore the concentration of proteins in the vascular space is affected by fluid distribution. For example
- **-** Posture: an increase in concentration of 10–20% occurs within 30 min of becoming upright after a period of recumbence.
- **-** Prolonged tourniquet application leads to a significant rise in protein concentration. the change in protein concentration is caused by increased diffusion of fluid from the vascular into the interstitial compartment.

- **c) Catabolism**: plasma proteins are degraded throughout the body. The rate of proteins synthesis is equal to the rate of degradation (Proteins turnover)
- **-** Only changes in albumin or immunoglobulins will have a significant effect on the total protein concentration.
- **-** A rapid increase in total plasma protein concentration is always due to a decrease in the volume of distribution (dehydration).
- **-** A rapid decrease is often the result of an increase in plasma volume.

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

- Measurement of plasma proteins
- **a) Quantitative measurement of a specific protein:** by chemical or immunological methods such as ELISA.
- **b) Semi-quantitative measurement by electrophoresis:** Proteins are separated on the basis of their electrical charge.

- Electrophoresis is usually performed on **serum** rather than plasma, because the fibrinogen present in plasma produces a band in the β region that might be mistaken for a para-protein.
- **- Electrophoresis, on cellulose acetate or agarose gel, separates the proteins into 5 bands:**
- Albumin, a1- globulins, a2-globulins, β-globulins and gamma - globulins


Buffer-saturated strip of filter paper
Cellulose-acetate sheet soaked with buffer
Serum sample
Anode
Cathode
Electro-phoresis
Staining
Densitometry
Cellulose-acetate sheet
Light absorption
52-58%
2.4-4.4%
6.1-10.1%
8.5-14.5%
10-21%
Albumins
α1-
α2-
β-
γ-Globulins

Albumins:	Transthyretin Albumin: 45 g · l^{-1}	Transport of thyroxin and triiodothyronin Maintenance of osmotic pressure; transport of fatty acids, bilirubin, bile acids, steroid hormones, pharmaceuticals and inorganic ions.
α_1-Globulins:	Antitrypsin Antichymotrypsin Lipoprotein (HDL) Prothrombin Transcortin Acid glycoprotein Thyroxin-binding globulin	Inhibition of trypsin and other proteases Inhibition of chymotrypsin Transport of lipids Coagulation factor II, thrombin precursor (*3.4.21.5*) Transport of cortisol, corticosterone and progesterone Transport of progesterone Transport of iodothyronins
α_2-Globulins:	Ceruloplasmin Antithrombin III Haptoglobin Cholinesterase (3.1.1.8) Plasminogen Macroglobulin Retinol-binding protein Vitamin D-binding protein	Transport of copper ions Inhibition of blood clotting Binding of hemoglobin Cleavage of choline esters Precursor of plasmin (*3.4.21.7*), breakdown of blood clots Binding of proteases, transport of zinc ions Transport of vitamin A Transport of calciols
β-Globulins:	Lipoprotein (LDL) Transferrin Fibrinogen Sex hormone-binding globulin Transcobalamin C-reactive protein	Transport of lipids Transport of iron ions Coagulation factor I Transport of testosterone and estradiol Transport of vitamin B_{12} Complement activation
γ-Globulins:	IgG IgA IgM IgD IgE	Late antibodies Mucosa-protecting antibodies Early antibodies B-lymphocyte receptors Reagins

▪ Albumin

- - Albumin, the most abundant plasma protein (60% of total plasma proteins).
- - It is synthesized in the liver and has a half-life of 20 days
- - **Functions:**
- 1) Oncotic pressure: Albumin is responsible for ~ 80% of the plasma oncotic pressure (the osmotic pressure due to the presence of proteins) and is an important determinant of the distribution of ECF between the intravascular and extravascular compartments.
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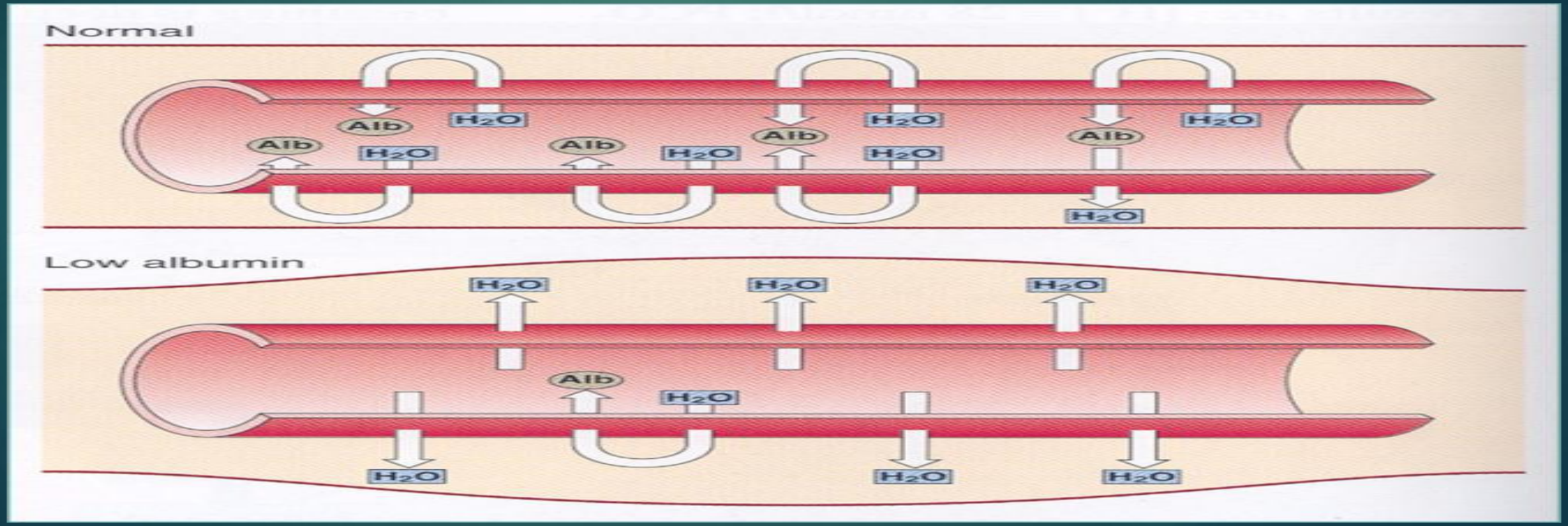
- 2) Buffering effect (remember acid-base balance)
- 3) Transport: Many substances are transported in the blood bound to albumin e.g. Hormones (thyroid & steroid hormones), Calcium Drugs (salicylates & sulfonamides), Free fatty acids, Bilirubin

- Hypoalbuminemia:

- **1) Artefactual:** Diluted samples.
- **2) Physiological:** Pregnancy.
- **3) Decreased amino acids intake:** Reduced essential amino acids in diet & reduced synthesis of non-essential amino acids (ManInutrition/Malabsorption).
- **4) Decreased albumin synthesis:** Chronic liver diseases (half-life of 20 days).
- **5) Increased loss:** From the kidney (Nephrotic syndrome), from GIT (protein-losing enteropathy), and from skin (severe burns).
- **6) Increased catabolism:** surgery, infection, and shock.

- **Hyperalbuminemia:** can be either an artefact, (hemoconcentration) or over-infusion of albumin, or be a result of dehydration.



Globulins:

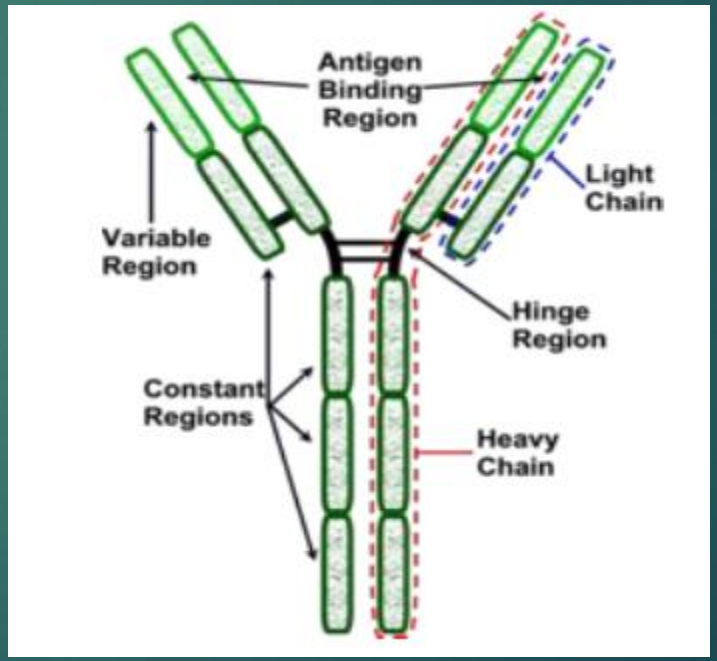
- they are 35% of total plasma proteins, classified into:

- **1) α-1 globulins**, α-fetoprotein (AFP)
- AFP is normally produced by the fetal liver. AFP levels decrease gradually after birth, reaching adult levels by 8 to 12 months. It is used as a tumor marker as it increases in case of hepatocellular carcinoma.
- **2) α-2 globulins**, α-2 macroglobulins act as anticoagulant through binding with several clotting factors thus preventing blood clots.
- **3) β-globulins**, fibrinogen is a soluble protein that forms blood clot.
- **4)** gamma**-globulins**, immunoglobulins (antibodies) including IgA, IgM, IgG, IgE.

Immunoglobulins:

- The immunoglobulins (antibodies [Igs]) are special proteins produced by the body in response to foreign substances including bacteria and viruses; there are five structurally distinct classes of immunoglobulins produced by plasma cells in the bone marrow and other lymphoid tissue that bind to and neutralize foreign substances (antigens). Immunoglobulins are glycoproteins composed of 82%–96% protein and 4%–18% carbohydrate produced by white blood cells, known as B cells that confer humoral immunity. These proteins consist of two identical heavy (H) and two identical light (L) chains linked by two disulfide bonds that can be in the form of monomers with one unit, dimers with two units, or pentamers with five units. There are **five** classes of immunoglobulins (**IgG**, **IgA**, **IgM**, **IgD**, and **IgE**) or isotypes based on the type of heavy chain they possess.

The general structure of an immunoglobulin.
Note the light chain that usually result in paraproteins in malignant conditions



Total Protein and Albumin/Globulin (A/G) Ratio

- The level of total protein in the blood is normally a relatively stable value, reflecting a balance in loss of old protein molecules and production of new protein molecules.
- **Total Protein and Albumin/Globulin (A/G) Ratio** is the calculated ratio of albumin to globulins. The A/G ratio is calculated from measured total protein, measured albumin, and calculated globulin (total protein - albumin).
- Normally, there is a little more albumin than globulins, giving a normal A/G ratio of slightly over 1.
- The A/G ratio may change whenever the proportions of albumin and other proteins shift (increase or decrease) in relationship to each other. Because disease states affect the relative amounts of albumin and globulin, the A/G ratio may provide a clue as to the cause of the change in protein levels.

- **-** The biochemical laboratories routinely measure **total protein** and **albumin** concentrations in serum and report the globulins fraction as: **Globulins = Total protein - Albumin**
- **a) Total proteins: 6 – 8 gm%**
- **b) Albumin: 3 – 5 gm%**
- **c) Globulins: 2 – 3.5 gm%**
- **d) A/G ratio:** Normally, there is more albumin than globulins in plasma, giving a normal A/G ratio > 1.

- **-** A high A/G ratio suggests: underproduction of Igs (leukemias).
- **-** A low A/G ratio may reflect: hypoalbuminemia OR overproduction of globulins (multiple myeloma or autoimmune diseases).

- One of the malignant conditions in which increased plasma proteins is seen is **multiple myeloma.** Multiple myeloma is a malignant disease in which the neoplastic plasma cells proliferate in the bone marrow, & is characterized by the appearance of a monoclonal protein or **paraprotein** in the serum and often in the urine as well. This protein is an intact immunoglobulin molecule, or occasionally, or kappa or lambda light chains only. Paraproteins in multiple myeloma may reach a serum concentration of several grams per deciliter.
- Normally light chains and heavy chains are produced in equal amounts and are always joined to make antibody molecules. This is not always the case in myeloma and sometimes more light chains than heavy chains are produced. Light chains which are not joined to heavy chains are called free light chains.
- Paraprotein is too large to pass through the kidney so it is normally present in blood in myeloma but not in urine. Free light chains can pass through the kidney and may be found in blood, urine or both.

- **Myocardial infarction or Heart attack**
- **- Infarction** is the process by which necrosis (cell or tissues death) results from ischaemia (loss of blood supply)
- **-** Acute myocardial infarction (MI) indicates irreversible myocardial injury resulting in necrosis of a significant portion of myocardium (generally >1 cm).
- **- Pathology of MI:**
- **- Atherosclerosis is an inflammatory process located within the arterial wall.**
- **- These cause narrowing of the coronary arteries leading to reduced coronary perfusion.**
- **-** If an unstable plaque ruptures, the released contents precipitate the formation of a clot (thrombosis) may result in sudden complete occlusion of the affected artery and infarction of the area of myocardium it supplies.

- Diagnosis of MI:
- 1) Myocardial enzymes: when myocardial cells die, they break up and release their contents which are (total CK,CKMB, LDH1, and AST)
- ▪ CKMB is most specific and rises much earlier following MI (1 – 3 hrs post MI). Its diagnostic value can be improved by:
- 1. Using CKMB / Total CK ratio (specificity)
- 2. Measuring the enzyme mass instead of activity (sensitivity)
- 2) Myocardial proteins: **Myoglobin** (95% sensitivity **6 hrs** post MI but not specific for heart) and **Troponins** (100% sensitive **12 hrs** post MI) i.e: MI can be **excluded** with confidence with a –ve troponin results if sample is taken 12 hrs or more after the onset of the chest pain.

Diagnostic markers for MI