Unit : 6 and 7
Plasma Proteins
Clinical Enzymology

❖ *Outlines:*

- Types of plasma proteins
- Measurement of plasma proteins
  1. Albumin
  2. Globulins
  3. A/G ratio
- Plasma enzymes
- Isoenzymes
- Enzymes of clinical interest
- Diagnosis of Myocardial Infarction

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 physiological functions in the plasma. The concentration of many of these is 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. 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**
   1. **Posture: an increase in concentration of 10-20% occurs within 30 min of becoming upright after a period of recumbency.**
   2. **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: PP 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 (over hydration).**

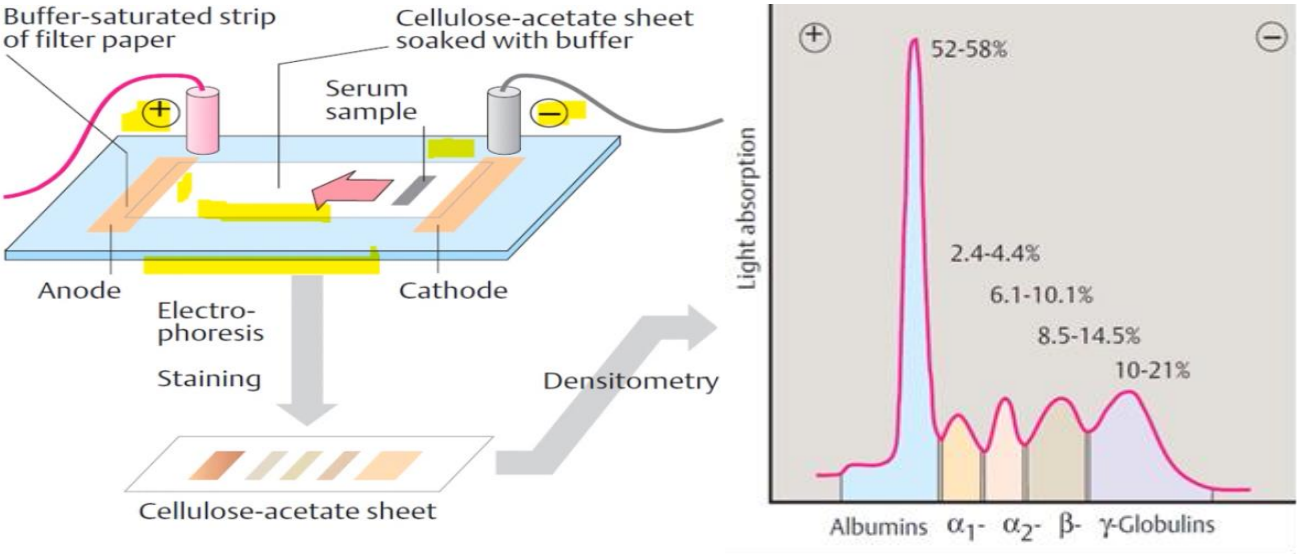
Causes of changes in total plasma protein concentration			
Increase		**Decrease**	
hypergammaglobulinaemia paraproteinaemia	↑ protein synthesis	malnutrition and malabsorption liver disease humoral immunodeficiency	↓ protein synthesis
artefactual	haemoconcentration due to stasis of blood during venepuncture	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 ß region that might be mistaken for a paraprotein.

Electrophoresis, on cellulose acetate or agarose gel, separates the proteins into 5 distinct bands: Albumin, α1- globulins, α 2-globulins, ß -globulins and γ-globulins.



❖ *Albumin*

- **Albumin, the most abundant plasma protein (60% of total plasma proteins).**
- **It is synthesized in the liver & has a half-life of 20 days**
- **Functions:**

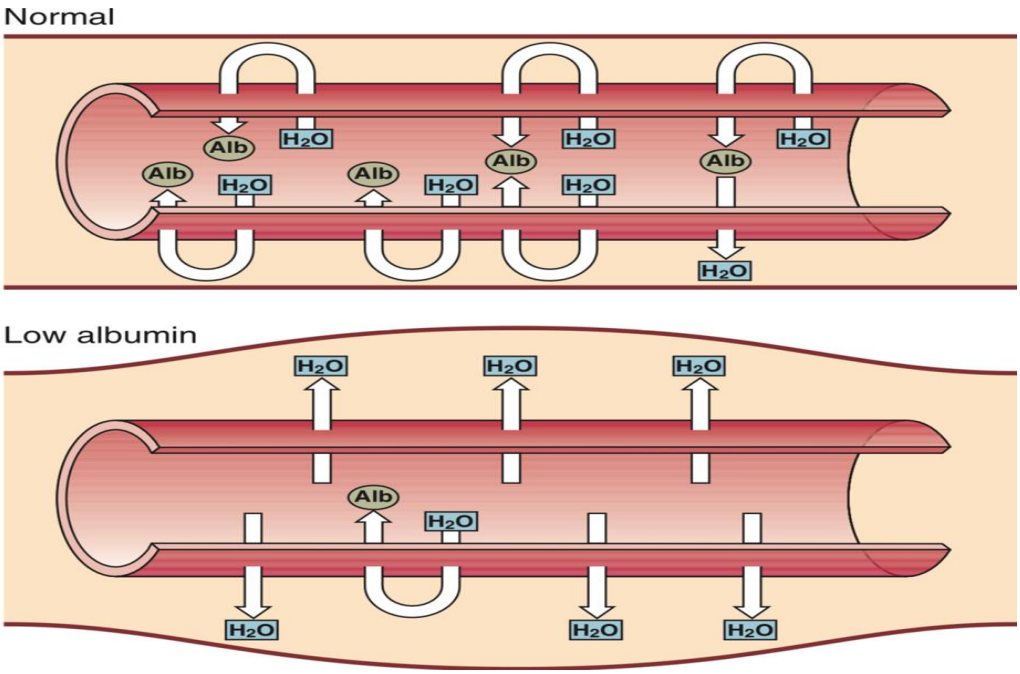
1. **Oncotic pressure: Albumin is responsible for 80% of the plasma oncotic pressure (the osmotic pressure due to the presence of proteins) and is an important determinant of the distribution of ECF between the intravascular and extravascular compartments.**
2. **Buffering effect (remember acid-base balance)**
3. **Transport: Many substances are transported in the blood bound to albumin e.g- Hormone (thyroid & steroid hormones) , Calcium, Drugs (salicylates & sulfonamides) , Free fatty acids, Bilirubin.**

➢ Hypoalbuminemia:

1. **Artefactual: Diluted samples**
2. **Physiological: Pregnancy; recumbence**
3. **Decreased amino acids intake: Reduced essential amino acids in diet & reduced synthesis of non-essential amino acids (Malnutrition or 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**

➢ **Hypoalbuminemia: can be either an artifact; (haemoconcentration) or over infusion of albumin, or be a result of dehydration.**

Oedema in Hypoalbuminemia



❖ Globulins

- Globulins are 35% of total plasma proteins, classified into

1. α-1globulins, 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

❖ α-2 globulins, α-2 macroglobulins

- Acts as anticoagulant through binding with several clotting factors thus preventing blood clots.

2. ß-globulins, as fibrinogen
   - Fibrinogen is a soluble protein that forms blood clot,
3. γ-globulins, immunoglobulins (antibodies) including IgA, IgM, IgG, IgE.

❖ Measurement of plasma proteins

- The biochemical laboratories routinely measure total protein and albumin concentrations in serum and report the globulins fraction as:

Globulins = Total protein Albumin

A. Total protein 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)

Enzymes

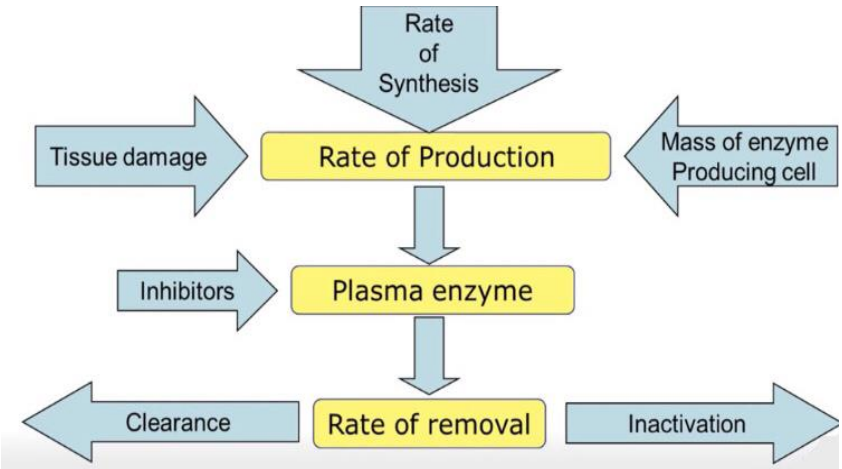
- Enzymes: are biological catalysts that increase the rate of specific chemical reaction in the living cell
- Enzyme properties:
  1. Biological catalysis
  2. Very efficient; can increase reaction rates at the order of $x10^7$
  3. All are proteins, so are liable to temperature (denaturation)
  4. Specific 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 cofactor 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 (NAD, FAD,…).**
  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 Enzyme*

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

❖ **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 plasma 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.**

❖ *Nonspecific factors affect non-functioning plasma enzymes level*

❖ Physiological factors:

1. **AST and CK in newborns 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.**

❖ Drugs

1. **Phenothiazine increases the liver enzymes.**
2. **Alcohol and anticonvulsants increase GGT.**

❖ Artefactual elevations:

1. **Haemolysis usually increases all enzymes that are present in RBC (AST; LDH ,Aldolase and Glucose -6- phosphate Dehydrogenase) .**
2. **Prostatic massage increases Prostatic Acid phosphatase (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**

e.g: Creatine Kinase (CK) ; Lactate Dehydrogenase (LDH)

Creatine Kinase (CK)

- **Creatine Kinase is a dimer made of 2 monomers occurs in the tissues**
  1. **Skeletal muscle contains M subunit.**
  2. **Brain contains B subunit.**
- **So there are 3 different isoenzymes are formed.**

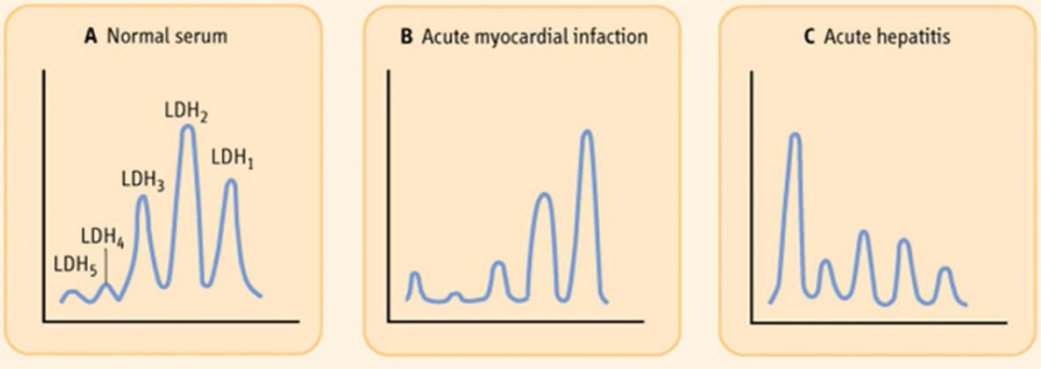
Isoenzyme	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases
CK-2	MB	Myocardium / Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle	Muscular dystrophy After surgery

Lactate Dehydrogenase (LDH)

- **LDH is a tetrameric protein and made of two types of subunits.**
  1. **Heart contains *2 H* subunits**
  2. **Skeletal muscle contains *2 M* subunits.**
- **So there are 5 different isoenzymes are formed**

Isoenzyme	Composition	Present in	Elevated in
LDH1 (H_4)	HHHH	Myocardium, RBCs	Myocardial Infarction
LDH2 (H_3M_1)	HHHM	Myocardium, RBCs, kidney	
LDH3 (H_2M_2)	HHMM	Brain, Lung, WBCs	
LDH4 (H_1M_3)	HMMM	Lung, Skeletal muscle	
LDH5 (M_4)	MMMM	Skeletal muscle, Liver	Skeletal muscle & liver diseases

Densitometric patterns of LDH isozymes in normal and patient serum



Common abbreviation for Enzyme of Clinical Interest

1	Aspartate Transaminase Glutamate Oxaloacteate Transferase	AST GOT
2	Alanine Transaminase Glutamate Pyruvate Transferase	ALT GPT
3	Alkaline Phosphatase	ALP
4	Prostatic Acid Phosphatase	PAP
5	γ-Glutamyl Transferase	GGT
6	Creatine Kinase Creatine Phosphokinase	CK CPK
7	Lactate Dehydrogenase	LDH
8	Aldolase	
9	Amylase	
10	5' Nucleosidase	

Enzymes of clinical insert

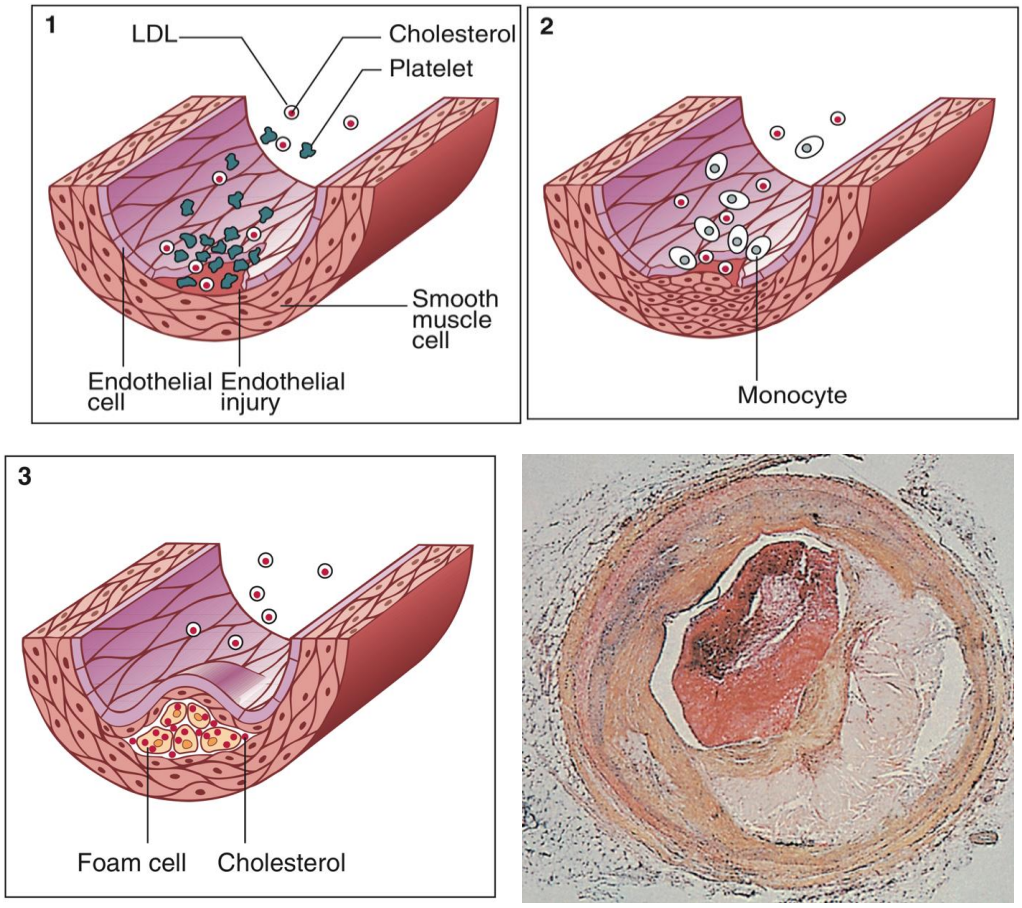
Enzyme	Site	Increased Plasma Levels		
		Physiological	Pathological	Artefactual
AST	Liver Muscles Heart RBCs	Newborn	Myocardial Infarction Liver disease especially with liver cell damage	Hemolysis
ALT	Liver		Liver disease especially with liver cell damage	
ALP	Bone Liver Intestine Placenta Kidney	Children Pregnancy Heavy meals	Bone diseases, Osteomalacia & rickets Hepatobiliary diseases	
PAP	Prostate Liver RBCs		Prostatic cancers	Prost. massage Catheter, Hemolysis Constipation
5' Nuc.	Biliary tract		Hepatobiliary disease	

Enzyme	Site	Increased Plasma Levels		
		Physiological	Pathological	Artefactual
GGT	Liver Kidney		Liver cirrhosis Alcoholism	
CK	Brain Heart Muscles	Male > Female	Myocardial infarction, Muscle diseases	After surgery
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	
G6PD	RBCs	Decreased in **Favism (In-born Errors of Metabolism)**		

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 in the form of athermanous plaques. 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**
- **Symptoms: severe chest pain radiating down the left arm (angina pectoris).**

Fig. Development of atheroma in coronary arteries with histopathological section (bottom right)



Diagnosis of Myocardial infarction MI

- **Diagnostic markers: After MI, a number of intracellular proteins are released from the damaged cells.**

1) **Myocardial Enzymes: when myocardial cells die, thy break up and release their contents which are (total CK, isoenzyme CK_{MB} , LDH_1 and AST)**
   - **CK_{MB} is most specific and rises much earlier following MI (1 -3 h post Ml). Its diagnostic value can be improved by:**
     1. **Using CK_{MB} / Total CK ratio (↑ specificity)**
     2. **Measuring the enzyme mass instead of activity(↑ sensitivity 2)**
2) **Myocardial proteins: Myoglobin (95% sensitivity 6 h post MI but not specific for heart) and Troponins (100% sensitive 12 h post MI) i.e: MI can be excluded with confidence with a-ve troponin results if sample is taken 12 h or more after the onset of the chest pain.**

Myocardial enzymes	Myocardial proteins
1. Total CK	1. Myoglobin
2. CK-MB	2. Troponins (T and I)
3. AST	
4. LDH1	

Marker	Onset	Peak	Duration
CK-MB	3-6 hr	18-24 hr	36-72 hr
Troponins	4-10 hr	18-24 hr	8-14 days
LDH	6-12 hr	24-48 hr	6-8 days
AST	24-36 hr	4-5 d	10-12 days
Myoglobin	1-4 hr	6-7 hr	24 hr

Marker	Size of infarction detectable
ECG	5 – 10 gm
CK	0.2 gm
CK_{MB}	0.02 gm
Troponin	0.0003 gm