IMPORTANT BIOCHEMISTRY LECTURES

BY

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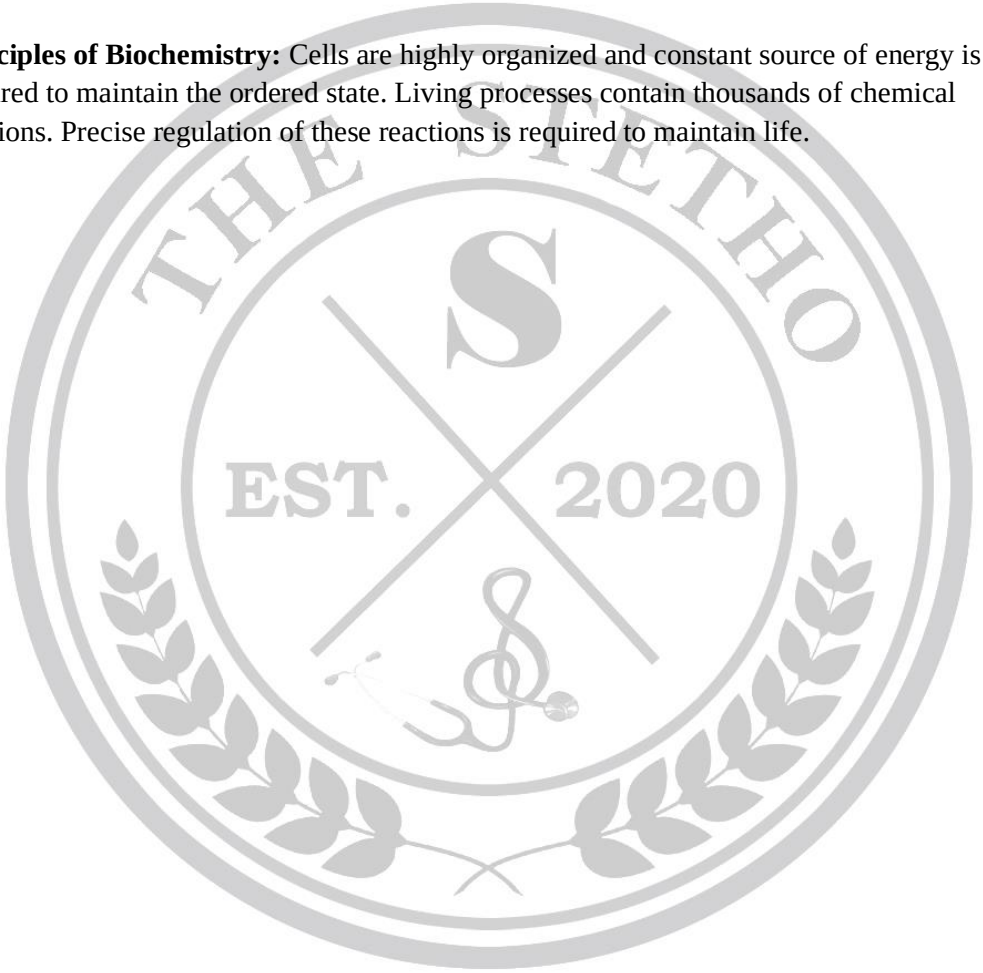
CONTENTS

Introduction to Biochemistry

Biochemistry is chemistry of life. Biochemists use physical and chemical principles to explain biology at the molecular level. Biochemistry is the study of chemical processes in living organisms. It deals with the structure and function of cellular components such as proteins, carbohydrates, lipids, nucleic acids and other bio-molecules.

Principles of Biochemistry: Cells are highly organized and constant source of energy is required to maintain the ordered state. Living processes contain thousands of chemical reactions. Precise regulation of these reactions is required to maintain life.



Proteins

Proteins are nitrogenous compound made up of large no of amino acids joined together by peptide bond.

Amino acids: These are the building blocks of proteins and amino acid with side chain R-bounded to form alpha carbon.

Peptide linkage: These are acid amide linkage between carbonyl group of one amino acid and alpha amino group of another amino acid. One molecule of H2o is eliminated in peptide linkage.

Importance:

- Proteins are structural components of cytoskeleton.
- Immunoglobin are proteins which are 1st line of defence against bacterial or viral infections.
- Several hormones are protein in nature.
- Proteins by means of exerting osmotic pressure helps to maintain electrolytes and H2o molecules.

Essential amino acids: Amino acid which are not produced in the body.

Non- essential amino acid: Amino acid which are synthesized in the body.

Albumin: It is H2O soluble protein found in plants and animals. E-g legumin in plants and eggs in animals. They are coagulated by heat. They can be precipitated by full saturation with aluminium sulphate.

Globulin: It is protein which is insoluble in H2O and soluble in salt solution. Globulin is also coagulated by heat. Globulin are more easily precipitated than albumin. It molecular wt ranges from 90,000 to 130,000.

Test for Proteins:

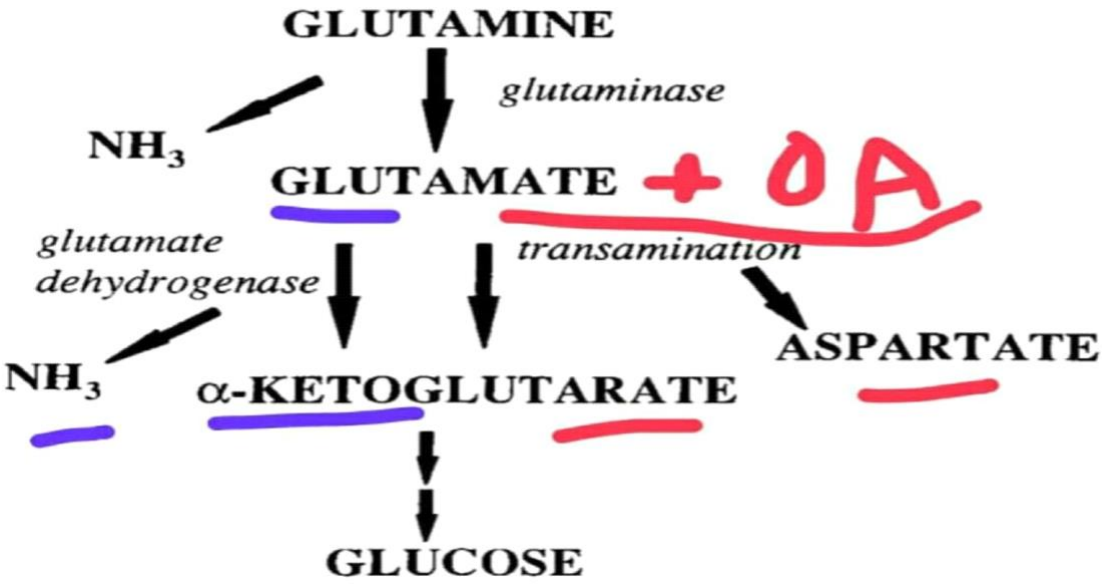
- **Biuret test**
- **Ninhydrin test**
- **Xanthoproteic test**

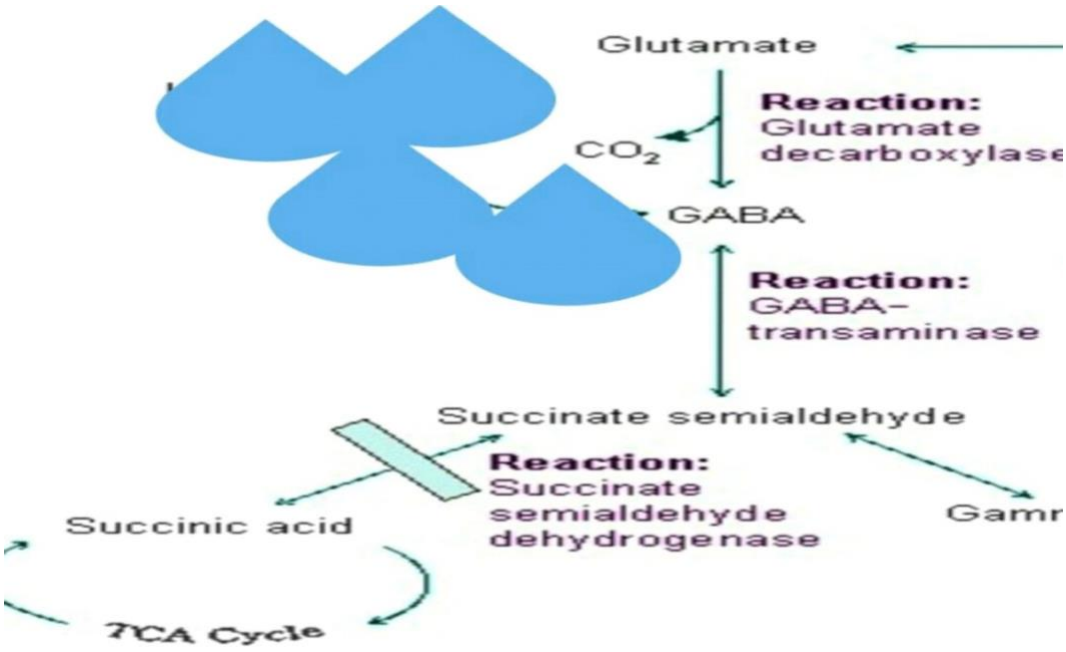
Amino acids Metabolism

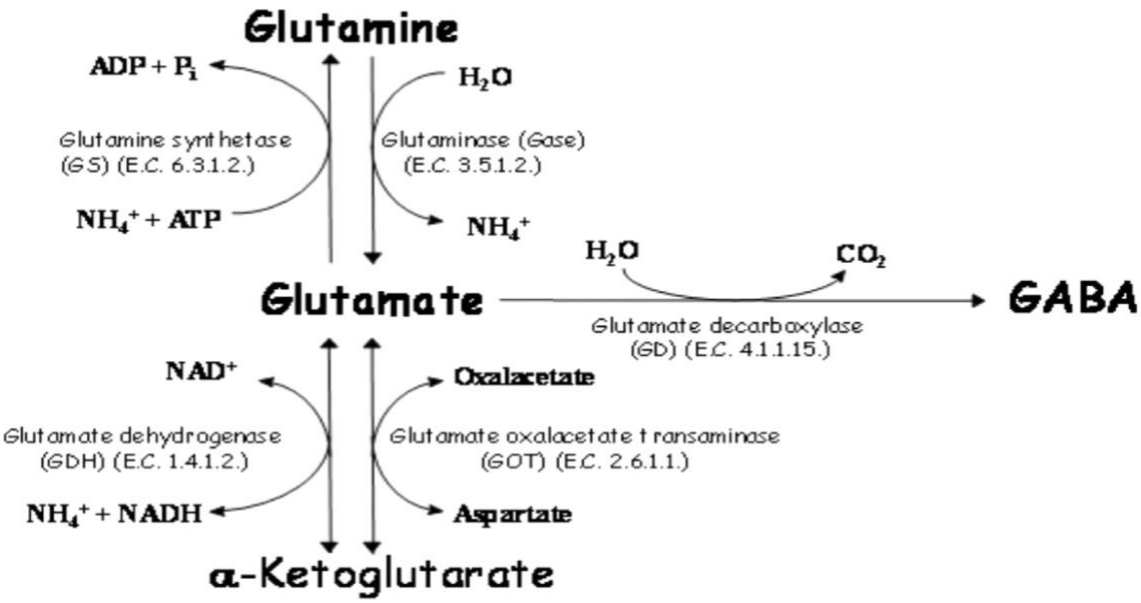
Metabolism of following amino acids will be discussed:

- **Glutamate**
- **Glutamine**
- **Proline**
- **Lysine**
- **Threonine**

GLUTAMATE AND GLUTAMINE:



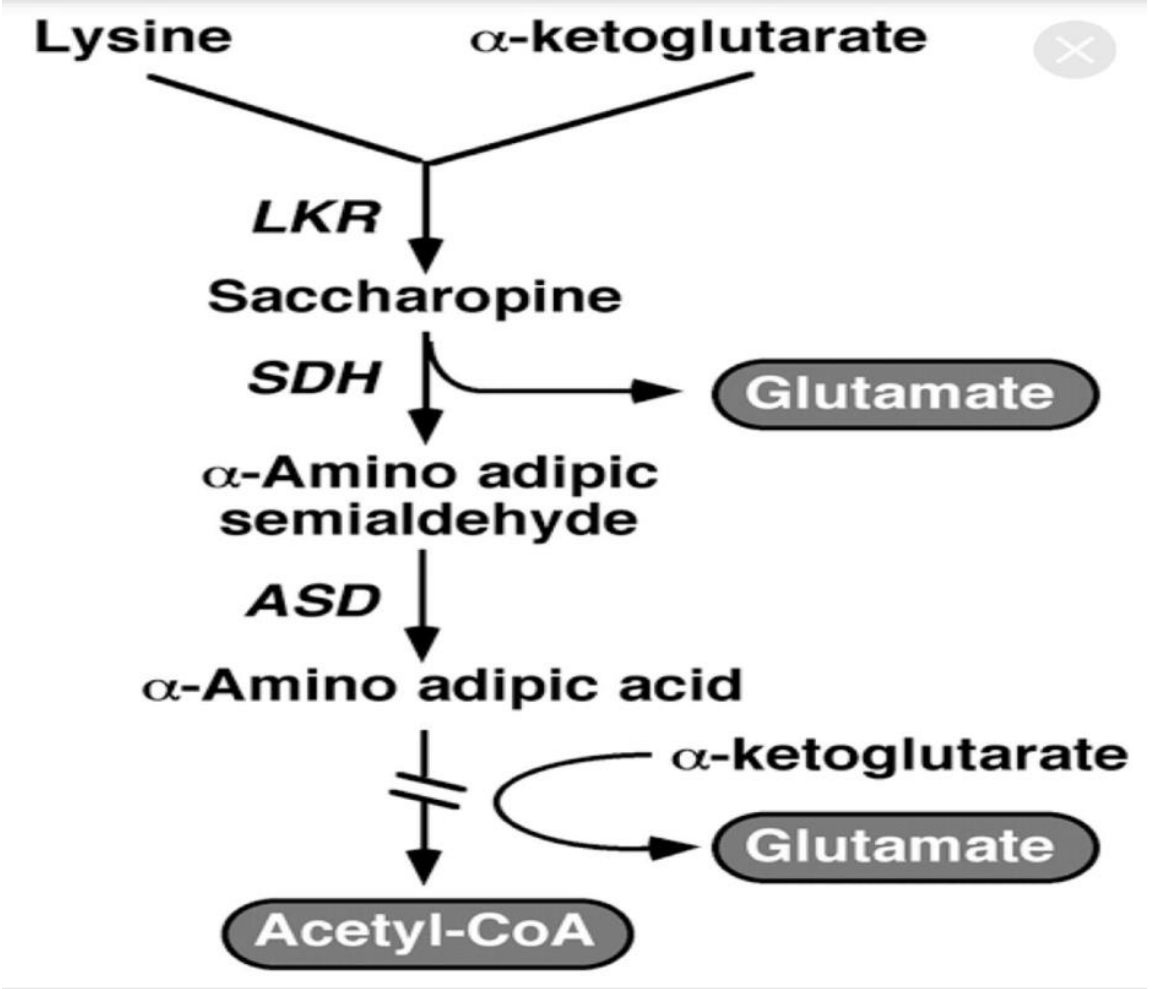

Glutamate
Reaction:
Glutamate
decarboxylase
CO_2
GABA
Reaction:
GABA-
transaminase
Succinate semialdehyde
Reaction:
Succinate
semialdehyde
dehydrogenase
Succinic acid
TCA Cycle


Glutamine
ADP + P_i
H_2O
Glutamine synthetase
(GS) (E.C. 6.3.1.2.)
Glutaminase (Gase)
(E.C. 3.5.1.2.)
NH_4^+ + ATP
NH_4^+
H_2O
CO_2
Glutamate
GABA
Glutamate decarboxylase
(GD) (E.C. 4.1.1.15.)
NAD^+
Oxalacetate
Glutamate dehydrogenase
(GDH) (E.C. 1.4.1.2.)
Glutamate oxalacetate transaminase
(GOT) (E.C. 2.6.1.1.)
NH_4^+ + NADH
Aspartate
α-Ketoglutarate

LYSINE:

Metabolism of Lysine

- Lysine is a nutritionally essential amino acid.
- Lysine is ketogenic amino acid.
- Its deficient in cereals
- It contains two amino groups, neither of which can undergo direct transamination.



**LKR: Lysine-ketoglutarate reductase/saccharopine dehydrogenase

SDH: Saccharoprine Dehydrogenase

ASD: Adipic semi aldehyde Dehydrogenase

**(Amino transferase) alpha ketoadipate

Then get glutaryl coa

Then acetyl coa

Catabolism of Lysine

- Lysine is degraded by a complex pathway in which *saccharopine, α-ketoadipate* and *crotonyl-CoA* are synthesized as an intermediates.

Importance of Lysine

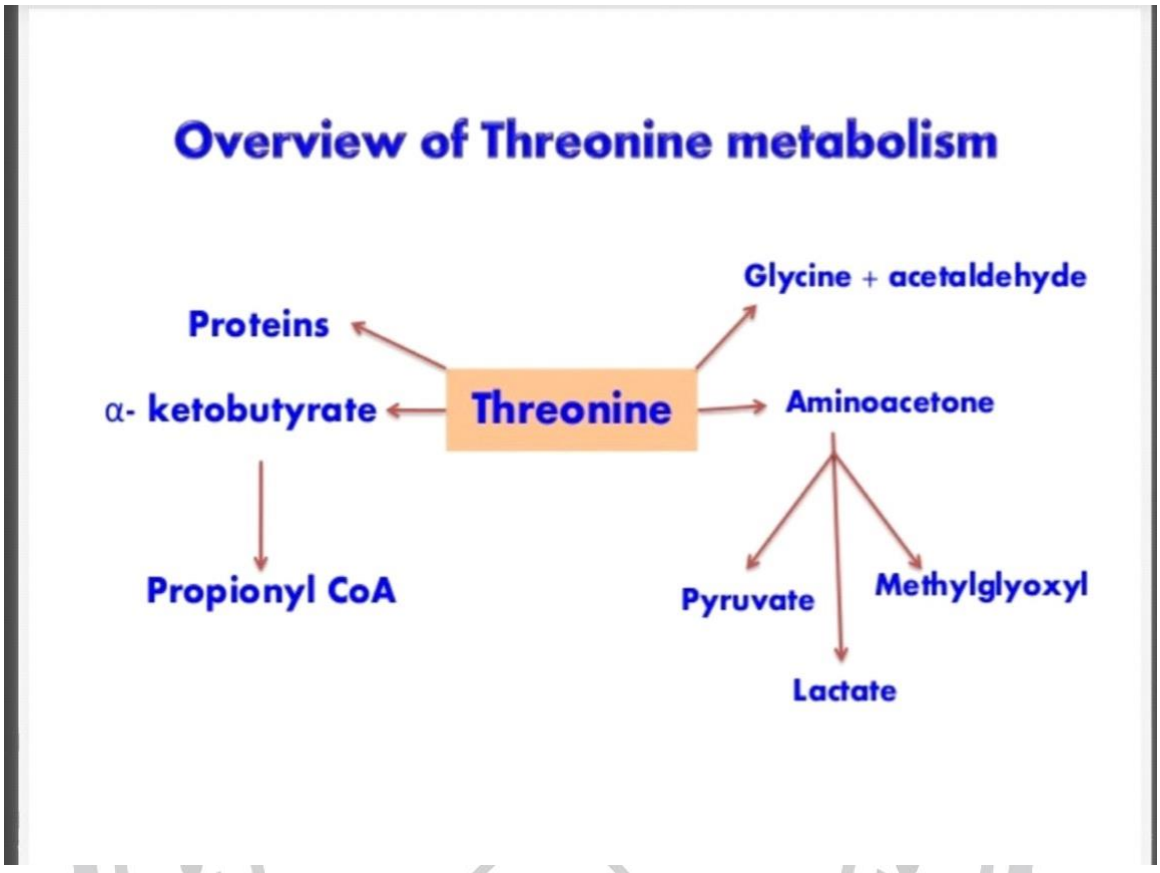
- 1. Hydroxyl lysine is important for the covalent crosslinking of collagen
- 2. Lysine along with methionine forms carnitine
- 3. Decarboxylation of lysine forms cadaverine
- 4. Histone proteins are lysine reach

THREONINE:

Threonine

- Threonine is essential amino acid.
- It is glycogenic & does not participate in transamination reactions.
- Threonine is also a carrier of phosphate group in the protein structure.
- Threonine undergoes deamination by threonine dehydratase to α-ketobutyrate which is converted to propionyl CoA.

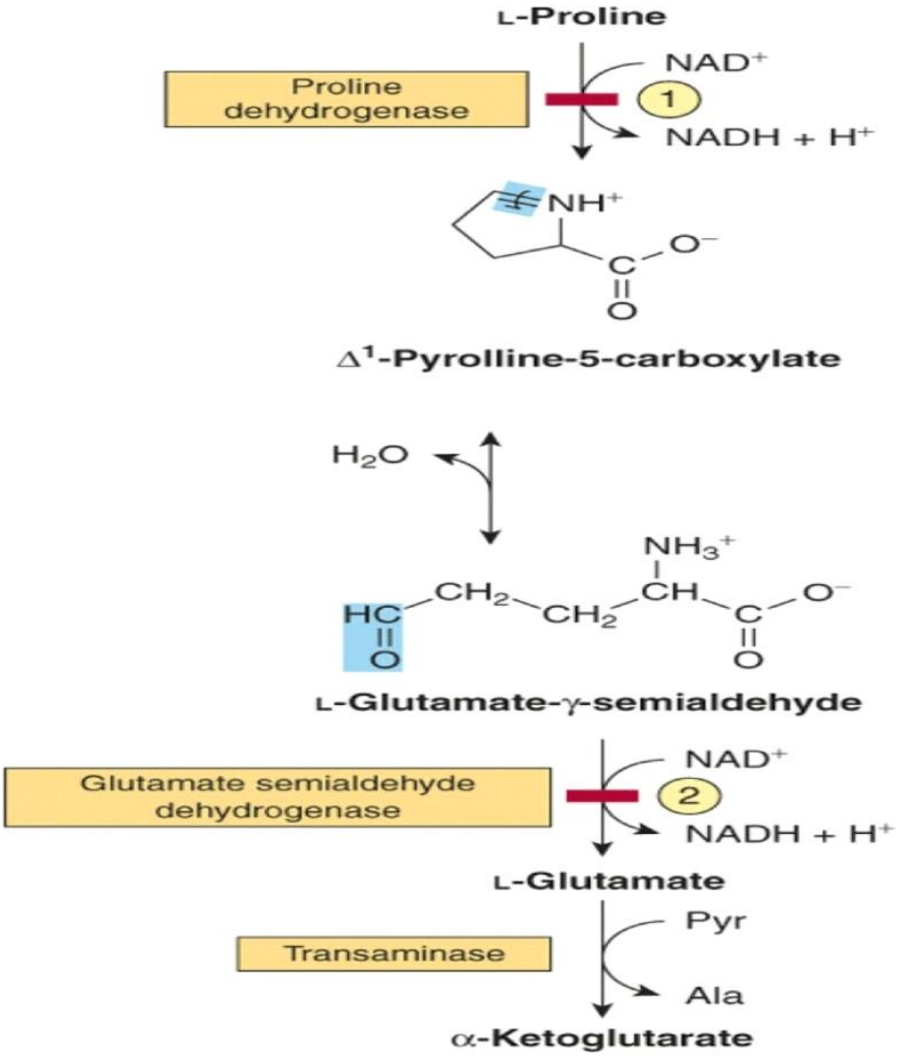
- Threonine can be cleaved to glycine & acetaldehyde by serine hydroxymethyl transferase.
- Dehydrogenation followed by decarboxylation of threonine results in aminoacetone which may be converted to pyruvate or lactate.



Glycine formed directly:1) threonine aldolase

***alpha ketobutyrate*then convert to propionyl coa,Then succinyl coa,That enters tca

PROLINE:


L-Proline
Proline dehydrogenase
NAD+
1
NADH + H+
NH+
O−
C
O
Δ1-Pyrolline-5-carboxylate
H2O
NH3+
CH2
CH2
CH
C
O−
HC
O
L-Glutamate-γ-semialdehyde
Glutamate semialdehyde dehydrogenase
NAD+
2
NADH + H+
L-Glutamate
Transaminase
Pyr
Ala
α-Ketoglutarate


Essential,E
Non essential NE
GlucoG/ketogenic K
Fates
Glutamate/Glutamine
NE
G
1]Alpha ketoglutarate
2]GABA
3]Glutamine
Lysine
E
K
1]Acetyl CoA(via adipic acid)
Threonine
E
G
1]glucose/TCA cycle
2]Glycine
3]Proteins
Proline
NE
G
1]Glutamate, alpha ketoglutarate

4) LIPIDS

Definition of simple lipids: “Simple lipids are the esters of fatty acids with various alcohols”

Types:

1. Natural Fats or TAG
2. Waxes

FATS & OILS:

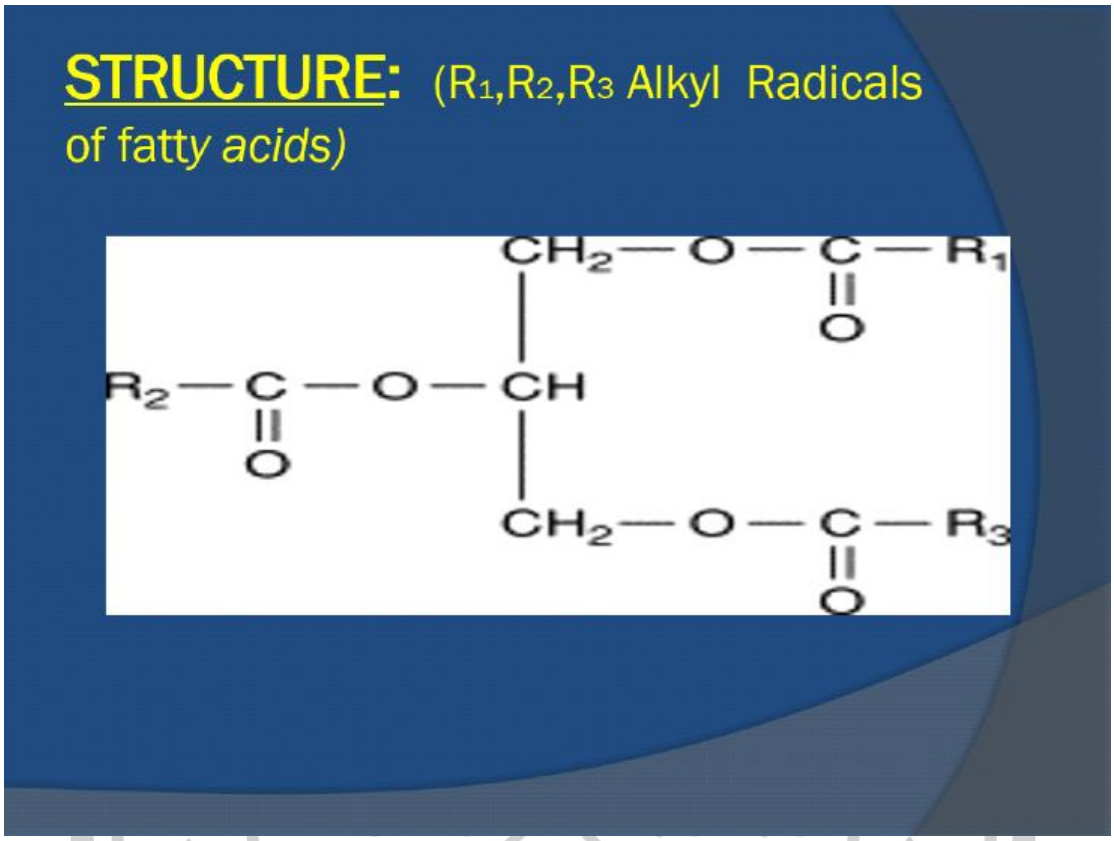
a) These are esters of fatty acids with alcohols.

b) These are natural fats because non-ionizable groups.

1. Esterification of glycerol with 1FA makes monoglycerids.
2. Esterification of glycerol with 2FA makes diglycerids.
3. Esterification of glycerol with 2FA makes triglycerids.

c) The difference is on the basis of their physical states at room temperature. A lipid is a fat if it is solid at 25 C and oil if it is a liquid at the same temperature.

SOURCE: Nuts, Seed, Fats deposits of animals.



STORAGE:

Storage form of fats in adipocytes as source of energy. (Triglycerides)

PHYSICAL PROPERTIES:

1. Neutral fats are colorless, odorless and tasteless substances. The color and taste of some of the naturally occurring fats is due to extraneous substances.

2. Solubility: They are insoluble in water but soluble in organic fat solvents. (e.g. chloroform)

3. Specific gravity: The specific gravity of all fats is less than 1.0, consequently all fats float in water.

4. Emulsification: Emulsions of fat may be made by shaking vigorously in water and by emulsifying agents such as gums, soaps and proteins which produce more stable emulsions.

5.

5. Melting point and consistency: The hardness or consistency of fats is related to their MP glycerides of lower FA melt at lower temperature than those of the higher fatty acids, and the unsaturated fatty acids glycerides at still lower temperature.

CHEMICAL PROPERTIES:

1. On Hydrolysis: Yield fatty acids + glycerol.

2. Saponification: Boiling of triacylglycerols with alkalis e.g. NaOH, KoH from Soaps.

3. Hydrogenation: It is the addition of hydrogen to unsaturated double bond present in oils which are then converted to saturated fatty acids.

4. Halogenation: Unsaturated fatty acids in free or combined form react with halogens (F2, I2, Br2, Cl2) Addition takes place at double bond.

5. Oxidation: Fats very rich in unsaturated fatty acids such as linseed oil undergo spontaneous oxidation at the double bond forming aldehydes, ketones and resins which form transparent coating on the surfaces to which the oil is applied. These are called drying oils and are used in the manufacture of paints and varnishes.

6. Rancidity: The term Rancid is applied to any fat or oil that develops a disagreeable odor, taste or abnormal color after exposure to atmospheric oxygen, light, heat, moisture and bacterial or fungal contamination. Hydrolysis and oxidation reactions are responsible for causing rancidity. Saturated fatty acids resist the rancidity then unsaturated.

WAXES:

Waxes are the esters of fatty acid with alcohols other than glycerol usually monohydroxy alcohol.

Waxes are distributed widely in plants and animals.

Not a food source.

There is no enzyme in humans to break them.

Types OF WAXES:

• True waxes are esters of higher fatty acids with acetyl alcohol (C16H33OH) or other higher straight chain alcohols. (Bee's Wax)

• Cholesterol esters are esters of fatty acid with cholesterol.

• Vit A and Vit D esters are palmitic or stearic acids esters of Vit A (Retinol) or Vit D respectively.

5)LIPIDS METABOLISM:

Lipid Transport in Blood

Lipids are not water soluble so they are packed in proteins for transport

****Four classes of lipoproteins**

◦◦ Chylomicrons

◦◦ VLDL

◦◦ LDL

◦◦ HDL

◦ **Lipid Digestion/Absorption ◦ Five different phases:**

◦ hydrolysis of triglycerides (TG) to free fatty acids (FFA) and monoacylglycerols

◦ solubilization of FFA and monoacylglycerols by detergents (bile acids) and transportation from the intestinal lumen toward the cell surface

◦ uptake of FFA and monoacylglycerols into the cell and resynthesis to triglyceride

◦ packaging of TG's into chylomicrons

◦ exocytosis of chylomicrons into lymph

◦ **Enzymes Involved in Digestion of Lipids**

} lingual lipase: provides a stable interface with aqueous environment of stomach

◦} pancreatic lipase: major enzyme affecting triglyceride hydrolysis

◦} colipase: protein anchoring lipase to the lipid

◦} lipid esterase: secreted by pancreas, acts on cholesterol esters, activated by bile

◦} phospholipases: cleave phospholipids, activated by trypsin

◦ **Repacking in the Liver:**

◦ } Lipid is repackaged in the liver to VLDL or very low density lipoprotein

◦ } Lipoproteins are classified by density

◦ } Lipoproteins transport lipid to the rest of the body

◦ **Lipid Transport**

◦ } Resynthesis of TAG and Cholesteryl ester

◦ } Secretion from Enterocytes

◦ } Use of Dietary Lipid

◦ Free fatty acids transported as complex with albumin in blood

◦ Glycerol converted to glycerol-3-phosphate, deliver to glycolysis pathway

◦**Oxidation of Fatty Acid**

◦β-Oxidation

◦α-Oxidation

◦ω-Oxidation

◦ **β-Oxidation** ◦ LCFA enter in cell and form CoA derivatives in cytosol

◦ Enzyme: Fatty acyl CoA synthetase

◦ Carnitine derivatives ◦ LCFA Translocation

◦ Transfer acyl group from CoA to carnitine by palmitoyltransferase-I

◦ Acyl carnitine is transported into mitoch-matrix by carnitine-acylcarnitine translocase

◦ Carnitine-palmitoyltransferase-II transfer acyl gp to CoA and regenerate free carnitine

◦**α- and Beta-oxidation of fatty acids are specialized pathways**

◦**α -oxidation** i.e., removal of one carbon at a time from the carboxyl end of the molecule has been detected in brain tissue. It does not generate CoA intermediates and does not generate high-energy phosphates.

◦**Beta-oxidation** is a minor pathway and is brought about by cytochrome P450 in the endoplasmic reticulum. CH3 group is converted to a -CH2OH group that subsequently is oxidized to -COOH, thus forming a dicarboxylic acid. They subsequently undergo
□-oxidation and are excreted in the urine

◦**Unsaturated Fatty Acids**

◦} Unsaturated fatty acids must be saturated before beta-oxidation

◦◦ Isomerase converts cis to trans and moves double bond to the 2 position

◦◦ In polyunsaturated: need reductase ◦– Add H's to second double bond

◦ **Odd Chain Fatty Acids**

◦} Minor species, odd chains made by microbes, degradation of AA's

◦} B-oxidation occurs to end: ◦◦ Left with 3 carbon + CoA

◦} Vitamin B12 cobalamin co-enzyme ◦◦ Catalyzes conversion of propionyl CoA (3 C) to succinyl-CoA (4 C)

◦ – TCA cycle intermediate

◦**KETOGENESIS** ◦ It occurs when there is a high rate of fatty acid oxidation in the liver.

◦Acetoacetic acid ◦Hydroxybutyric acid ◦Acetone ◦These three substances are collectively known as the **ketone bodies** (also called acetone bodies or acetone).

Enzymes responsible for ketone bodies formation are associated with mitochondria.

◦**The main factors which control Ketogenesis in the liver**

◦Availability of the substrate (Long Chain Fatty Acids) : from increased production by lipolysis with increased delivery of FA to the liver.

◦The level of Malonyl Co A in the liver, with its influence to inhibit the Carnitine Palmitoyl Transferase I (CPT I)

◦The Glucagon / Insulin Ratio : a high ratio increases lipolysis and activation of oxidative ketogenesis , a low ratio counteracts ketogenesis

◦ Lipid Concentrations in the Blood:

◦ TOTAL LIPIDS 360-820 mg/dL

◦ TRIGLYCERIDES (TGs) 51-322 mg/dL

◦ PHOSPHOLIPIDS (PL) 125-275 mg/dL

◦ CHOLESTEROL: TOTAL 150-320 mg/dL

◦ HDL-CHOLESTEROL 30-84 mg/dL

◦ LDL-CHOLESTEROL 97-200 mg/dL

◦ FREE FATTY ACIDS 6-16 mg/dL

6)BIOSYNTHESIS OF PHOSPHOLIPIDS:

Biosynthesis of Phospholipids

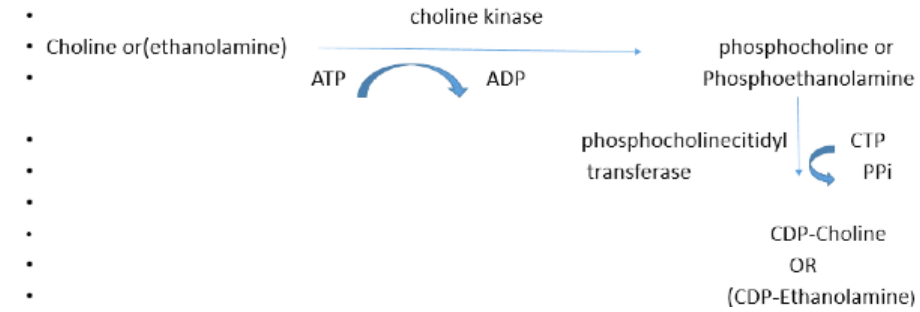
BIOSYNTHESIS OF PHOSPHOTIDYL CHOLINE(LECITHIN)

OR

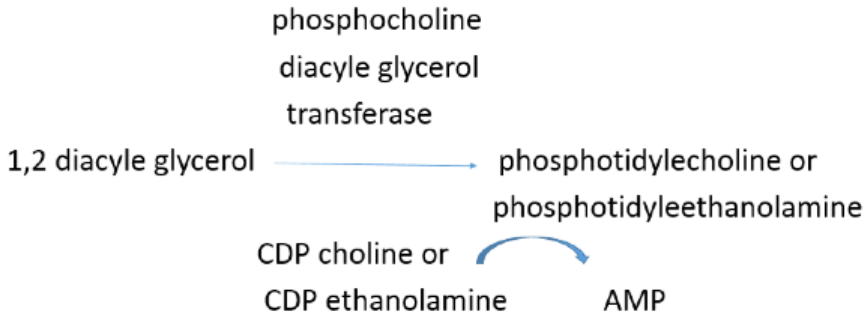
BIOSYNTHESIS OF PHOSPHODIDYLE ETHANOLAMINE (CEPHALINE)

Substrates required are

- Choline or Ethanolamine
- 1,2 Diacyle glycerol
- Two steps are required for biosynthesis
- **STEP 1**
- Activation of choline and ethanolamine:

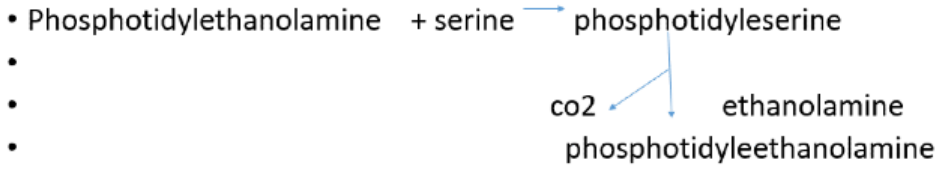


STEP 2:



Synthesis of phosphotidyleserine

- Methylation of phosphotidylecholine will give phosphotidylethanolamine.



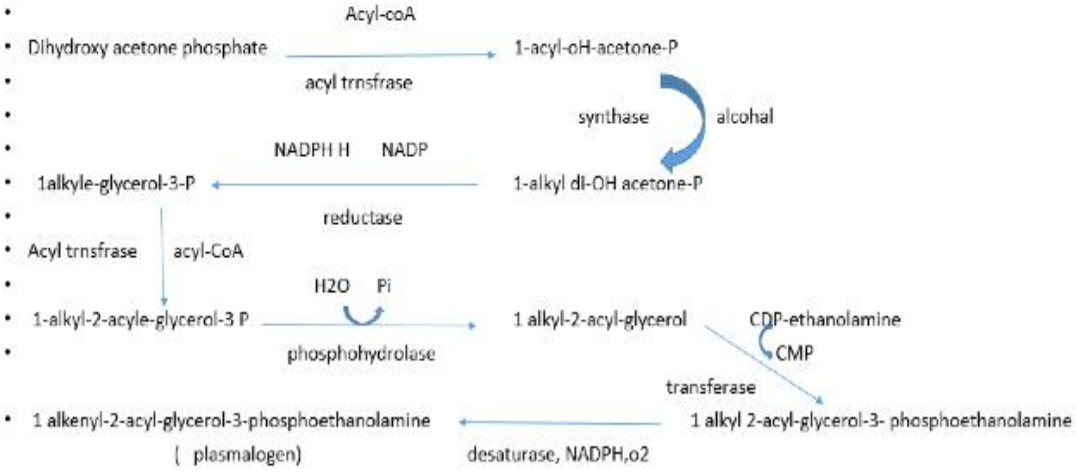
Synthesis ofphosphotidyleinositol

- CTP Phosphotidate transferase
- 1,2 diacylephosphotidate → CDP –diglyceride
- CTP → Ppi
- CDP-diglyceride
- Inositol → inositol transferase
- CMP
- phosphotidyleinositol

Synthesis of cardiolipin

- Cardiolipin is phospholipid present in mitochondria .
- Chemicaly it is diphosphotidyl glycerol.
- CDP-diglyceride
- \+ αGlycerol-P → Phosphotidyle glycerol-P
- CMP
- H2o → Pi
- phosphotidyl glycerol
- \+ CDP-diglycride
- cardiolipin

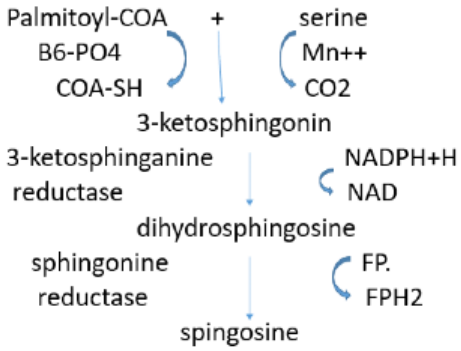
Biosynthesis of plasmalogens



BIOSYNTHESIS OF SPHINGOMYELIN

- Sphingomylins is a molecule in sphingosine is attached to the long chain fattyacids and phosphocholine or phosphoethanolamine.
- Spingomylin_____ fattyacids combination is called as ceramide.
- Sphingomylinis therefore called as ceramide phosphocholine or
- phosphoethanolamine.
- **STEPS OF BIOSYNTHESIS**
- Synthesize amino alcohol (sphingosine)
- Synthesize ceramide
- Finally synthesize sphingomyelin.

a) SYNTHESIS OF SPHINGOSINE



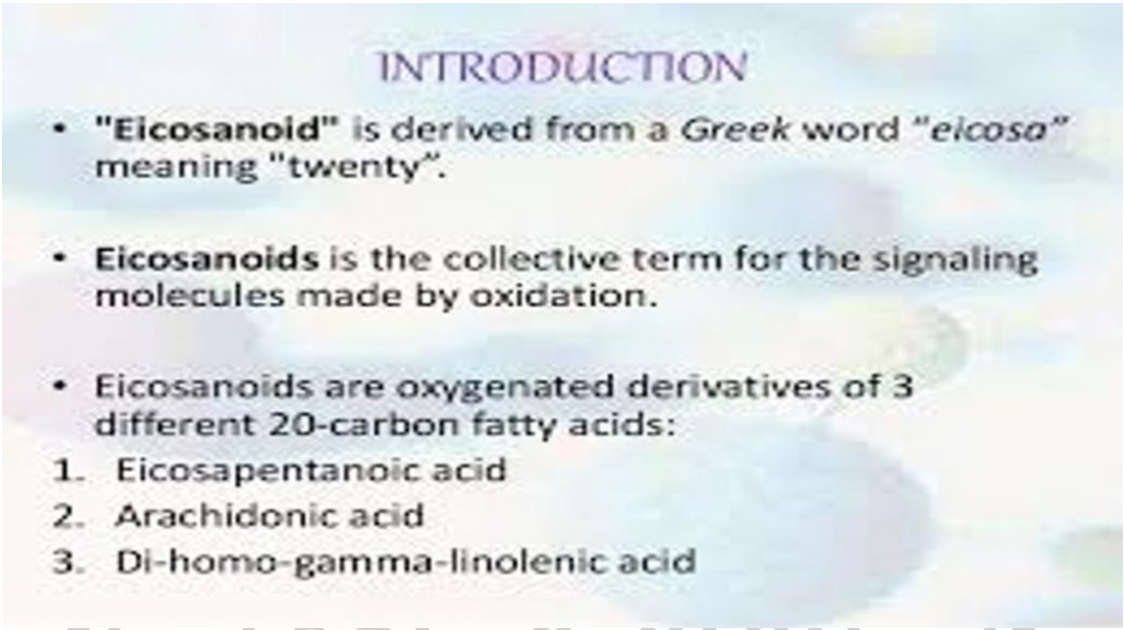
b) SYNTHESIS OF CERAMIDE

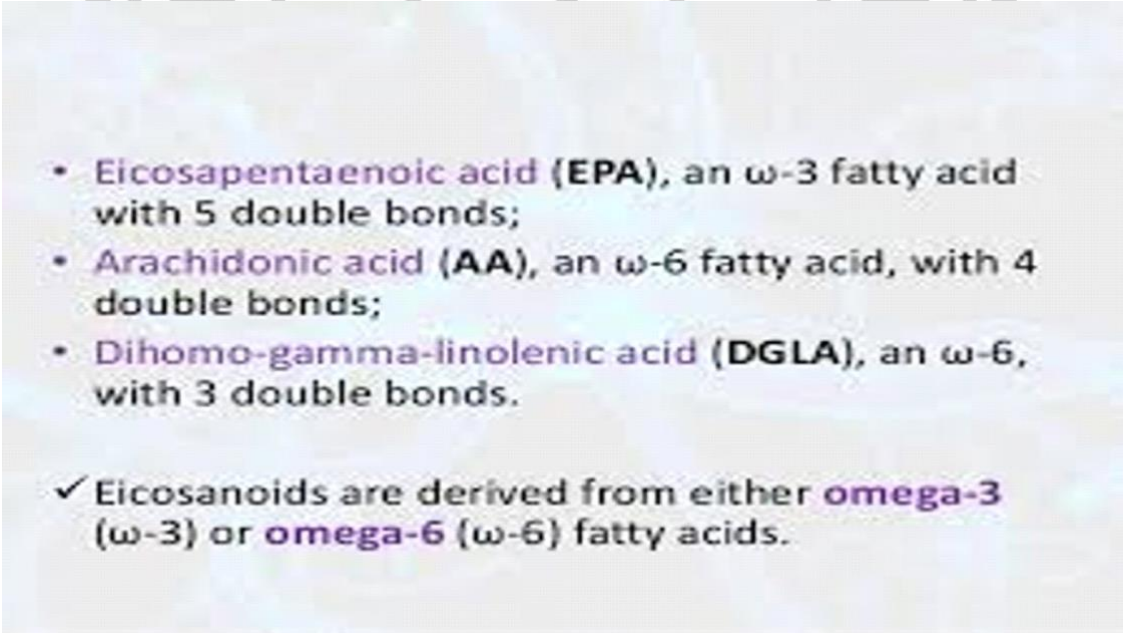
- Sphingosine combines with acyle- CoA to form ceramide.
- Acyle CoA is a long chain fatty acid.
- Acyle transferase
- Sphingosin ceramide
- Acyle Co-A Co-A
- C) SYNTHESIS OF SPHINGOMYELIN
- Ceramide reacts with CDP-Choline , it gives CMP and sphingomyelin.
- It can also be synthesized by the reaction of ceramide with phosphotidylcholine.

CATABOLISM OF LECITHIN

- There are three steps;
- It is degraded in the body by phospholipase A2,which hydrolyzes ester bond and form free fatty acids and lysolecithin.
- Lysolecithin is hydrolyzed by lysophospholipase (phospholipase B) and liberate free fatty acids and glycerol phosphoryle choline (glycerol phosphocholine).
- Glycerol phosphocholine is hydrolyzed by glyceryl phosphocholine hydrolase to form nitrogenous base (choline) and α glycerol –P.

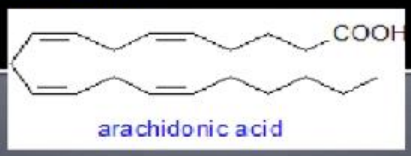
7) EICOSANOIDS:





Eicosanoids

Prostaglandins and related compounds are collectively known as Eicosanoids. Most are produced from Arachidonic acid, a 20-carbon polyunsaturated fatty acid (5,8,11,14-eicosatetraenoic acid).



arachidonic acid

Eicosanoids- Classification

Eicosanoids are classified in to two main groups-

1) Prostanoids
2) Leukotrienes and Lipoxins

Prostanoids are further sub classified in to three groups-

a) Prostaglandins(PGs)
b) Prostacyclins(PGIs)
c) Thromboxanes (TXs)

Prostanoids

All Prostanoids are considered to be derivatives of a cyclic saturated fatty acid called Prostanoic acid.

Prostaglandins(PGs)

PGs can be divided into four major groups.- PG-E, PG-F, PG-A and PG-B groups. PG-C and PG-D groups have also been recognized.

PG- G and PG-H, considered as Primary PGs, are intermediates in the synthesis of other prostaglandins.

Characteristic features of Structure of prostaglandins

1) A trans double bond is present between 13^{th} and 14^{th} carbon atom

2) An alpha oriented -OH group is present at 15^{th} position

3) Differences in the 4main groups are due to difference in structure of cyclopentane ring.

Series of prostaglandins

There are 3 series of prostaglandins-

Series-1 contain one double bond at 13-14 position (Trans)
Series-2 have two double bonds at 13-14 (trans) and 5-6 (Cis)
Series-3 – have three double bonds at 13-14 (trans) , 5-6 (Cis) and 17-18 (Cis) positions.

Characteristic features of prostaglandins

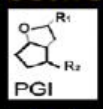
1) Act as local hormones.
2)Show the effects near the site of synthesis(autocrine and Paracrine effects)
3) Are not stored in the body
4) Have a very short life span and are destroyed within seconds or few minutes
5) Production increases or decreases in response to diverse stimuli or drugs
6) Are very potent in action. Even in minute (nanogram concentration), biological effects are observed.

Functions of Prostaglandins

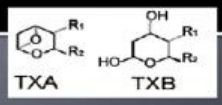
They have various roles in inflammation, fever, regulation of blood pressure, blood clotting, immune system modulation, control of reproductive processes, tissue growth, and regulation of the sleep/wake cycle.

Prostacyclins and Thromboxanes- Chemistry and Functions

1)Prostacyclins(PGI) contain another ring between 6 th and 9th carbon atoms



2) Thromboxanes (TX) have a six membered Oxane ring.
There are three series for thromboxanes as well as for Prostacyclins.



Functions of Prostcyclins

They are synthesized in heart and vascular endothelial cells.
-Inhibit platelet and leukocyte aggregation
-Decrease T-cell proliferation ,lymphocyte migration and secretion of IL-1α and IL-2
-Induce vasodilatation and production of cAMP
-prevent clot formation.

Synthesis of Prostaglandins

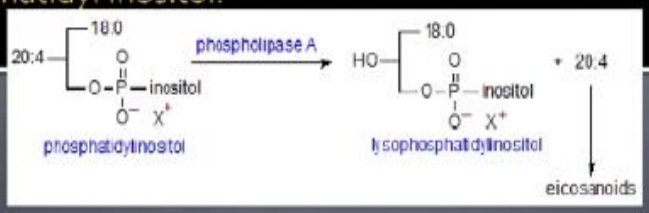
Series -1 Eicosanoids are synthesized from dihomo-γ-linolenic acid (DGLA)
Series-2 from Arachidonic acid and
Series-3 are synthesized from Eicosa pentaenoic acid.

The major source of Arachidonic acid is through its release from cellular stores.
Within the cell, it resides predominantly at the C–2 position of membrane phospholipids and is released from there upon the activation of PLA2

Steps of synthesis of Prostaglandins

Pathway is also called Cyclo-oxygenase pathway or cyclic pathway

-The prostanoid signaling cascade begins with an external stimulus, most often the binding of a ligand to a cell surface receptor that activates phospholipase A2. This enzyme releases Arachidonic acid from its esterified form in membrane phospholipids such as phosphatidylethanolamine and phosphatidyl Inositol.

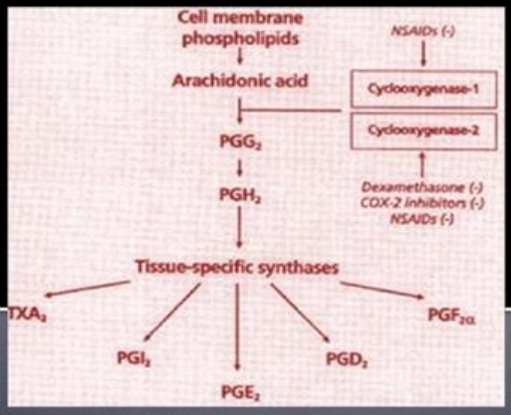


Steps of synthesis of Prostanoids

-Arachidonate is converted to PGH_2 by one of the isoforms of PGH synthase (PGHS-1 or -2), enzymes localized to the endoplasmic reticulum membrane and the nuclear envelope.

- PGH_2 is in turn metabolized to the prostanoid lipid signals (PGD_2, PGE_2, $PGF_{2\alpha}$, PGH_2, PGI_2, or TXA_2) by one of the secondary enzymes that are named for the individual prostanoid produced. The type of prostanoid produced is determined by which downstream enzyme is present; usually one downstream enzyme predominates in a given cell.

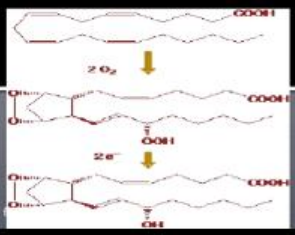
Steps of synthesis of Prostanoids



PGH synthase Isoforms

Each PGHS isoform catalyzes two separate reactions.
1) The first reaction (Arachidonate to PGG2) involves insertion of two molecules of oxygen and Cyclization of the fatty acid backbone. This step is catalyzed by the cyclo-oxygenase activity of PGHS-1 or -2; it is these cyclo-oxygenase activities (also called COX-1 and COX-2) that are inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs).

2) The second step (PGG2 to PGH2) involves the reduction of the hydro peroxide on C15 to an alcohol and is catalyzed by the peroxidase activity of PGHS-1 or -2.



Significance of PGH synthase isoforms

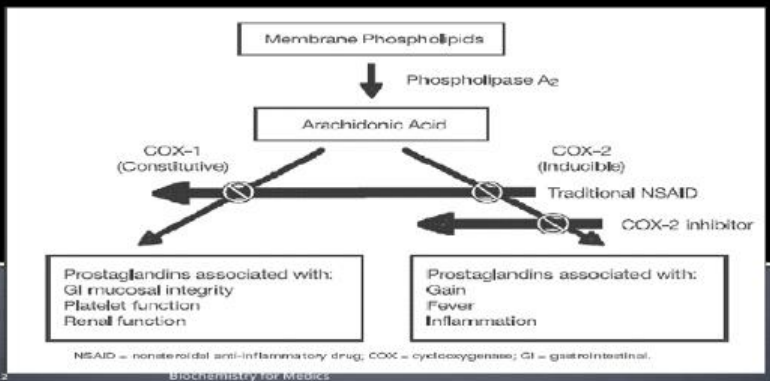
-There are two isoforms- PGH Synthase 1 and 2
- PGH Synthase 1 – Basal form(constitutive)
Many cells, including platelets and gastric mucosal cells, have moderate levels of the "basal" isoform, PGHS-1. Functions attributed to PGHS-1 include regulating hemostasis and vascular tone, renal function, and maintaining gastric mucosal integrity.
PGH Synthase 2- Inducible form
- present in a smaller number of cells, such as macrophages, vascular endothelial cells, and fibroblasts.
- have been implicated in cell proliferation, inflammation, carcinogenesis, and parturition.
- PGHS-2 are induced in response to cytokines or mitogens.

Inhibitors of Prostanoid synthesis

-Corticosteroids are anti-inflammatory because they prevent Phospholipase A_2 expression, reducing arachidonate release.

-Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and derivatives of ibuprofen, inhibit cyclooxygenase activity of PGH Synthase. They inhibit formation of prostaglandins involved in fever, pain, & inflammation.
They inhibit blood clotting by blocking thromboxane formation in platelets.
-Most NSAIDs inhibit both COX I & COX II.
- Selective COX-2 inhibitors have been developed, (such as Celecoxib and Rofecoxib.
- This selectivity has made the coxibs very useful for anti-inflammatory and antiproliferative therapy with reduced gastrointestinal side effects, but it also makes them ineffective as antiplatelet agents and consequently can increase cardiovascular risks

Selective V/S Non Selective COX inhibition



NSAID = nonsteroidal anti-inflammatory drug; COX = cyclooxygenase; GI = gastrointestinal.

Catabolism of Prostaglandins

1) All Arachidonic acid derivatives are quickly, in less than a few minutes, inactivated in the body by several complex reactions. "Switching off" of prostaglandin activity is partly achieved by a remarkable property of cyclooxygenase—that of self-catalyzed destruction; ie, it is a "suicide enzyme."

2)Furthermore, the inactivation of prostaglandins by 15-hydroxyprostaglandin dehydrogenase is rapid.(OH group preset at 15th position is changed to a keto group)

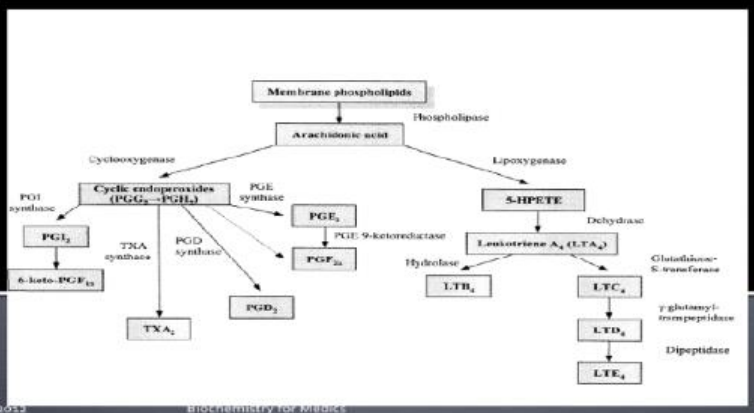
Leukotrienes

The Leukotrienes are identified as LTs.
-They are a family of conjugated trienes formed from eicosanoic acids in leukocytes, mastocytoma cells, platelets, and macrophages by the lipoxygenase pathway in response to both immunologic and nonimmunologic stimuli.

-Three different dioxygenases (dioxygenases) insert oxygen into the 5, 12, and 15 positions of Arachidonic acid, giving rise to hydroperoxides (HPETE).

- Only 5-lipoxygenase forms Leukotrienes.

Steps of synthesis of Leukotrienes



Functions of Leukotrienes

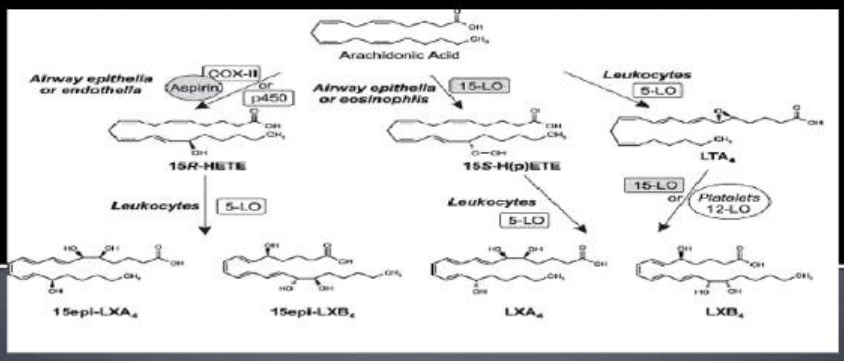
-Leukotrienes have roles in inflammation.
-They are produced in areas of inflammation in blood vessel walls as part of the pathology of atherosclerosis.
-Leukotrienes are also implicated in asthmatic constriction of the bronchioles.
-The peptidoleukotrienes, LTC_4, LTD_4 and LTE_4 are components of slow-reacting substance of anaphylaxis (SRSA). The subscript 4 in each molecule refers to the number of carbon-carbon double bonds present.

Lipoxins

Lipoxins are a family of conjugated tetraenes also arising in leukocytes.

They are formed by the combined action of more than one lipoxygenase.

Steps of Synthesis of Lipoxins



Physiological roles of Prostaglandins

1) Inflammation

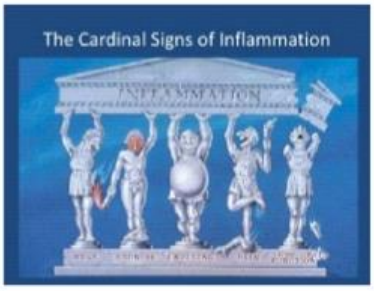
i) Fever- PGs induce fever by stimulating the thermoregulatory center in the brain.

ii) Pain- PGs sensitize pain receptors to stimulation, as a result increase pain perception.

iii) Swelling-There is vasodilatation and increased capillary permeability induced by PGS which is responsible for swelling of the inflamed tissue.

iv Erythema, wheal and Flare is also induced by PGs like **PGE and PGD2.**

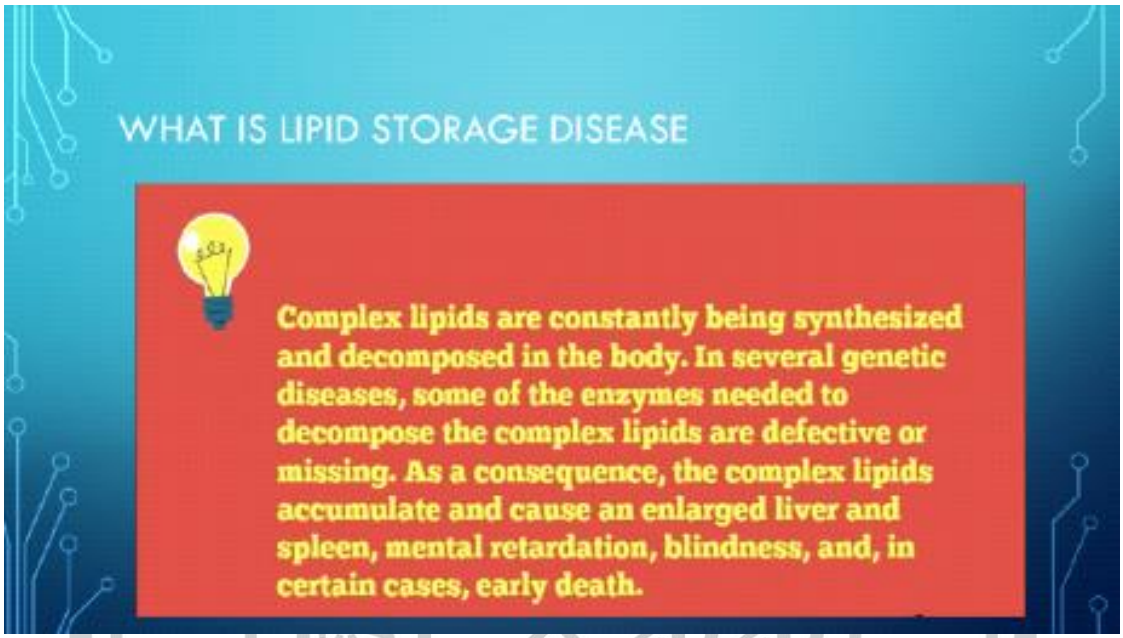
v) PGD2 is considered an important mediator of anaphylaxis

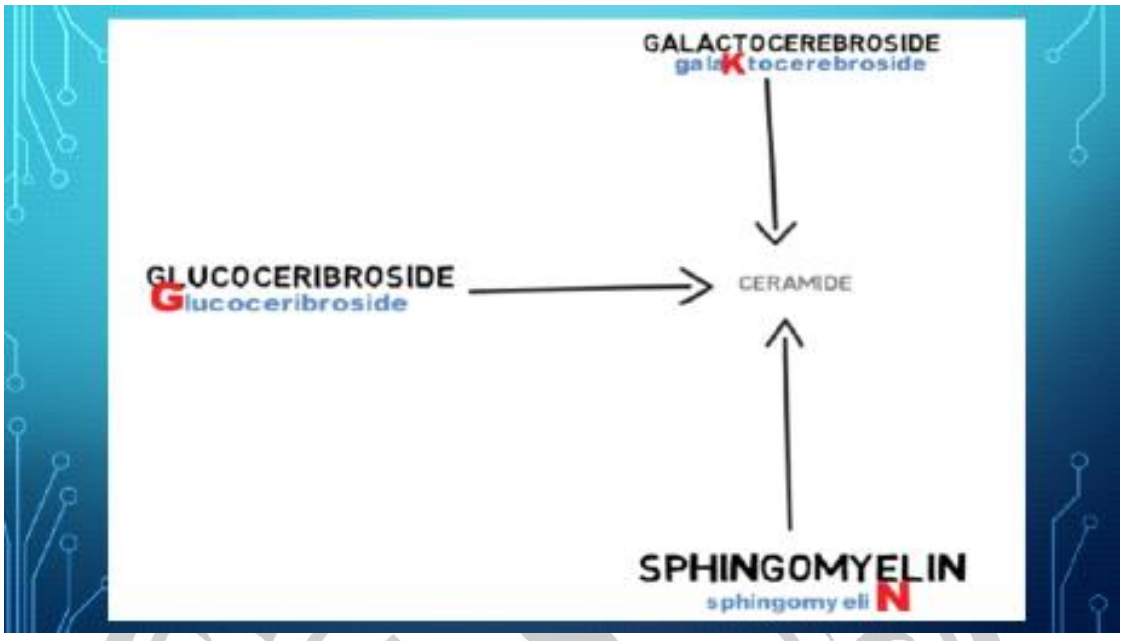


Effect on smooth muscle

Intestinal	Bronchial	Vascular	Uterine
PGE and PGF produce **contraction of the longitudinal smooth muscles** producing diarrhea, cramps and reflux of bile.	PGFs contract and PGEs relax bronchial smooth muscles.	PGEs cause vasodilatation. PGF2 α , and PG A2 cause vasoconstriction.	PGE1, PGE2 and PGF2α cause uterine contractions.
Clinical significance- Diarrhea and abdominal cramps are the commonly observed as side effects of PGs.	**Clinical significance-** PGE1 and PGE2 are therapeutically used as bronchodilators.	**Clinical significance-** Systemic blood pressure falls in response to PGEs and PGAs	**Clinical significance-** • PGE2 is used for the induction of labor at or near term. • In higher dosage PGEs are used as abortifacients in first and second trimester of pregnancy. • They are also responsible for causing dysmenorrhea.

8)LIPID STORAGE DISEASES:




GALACTOCEREBROSIDE
galaKtocerebroside
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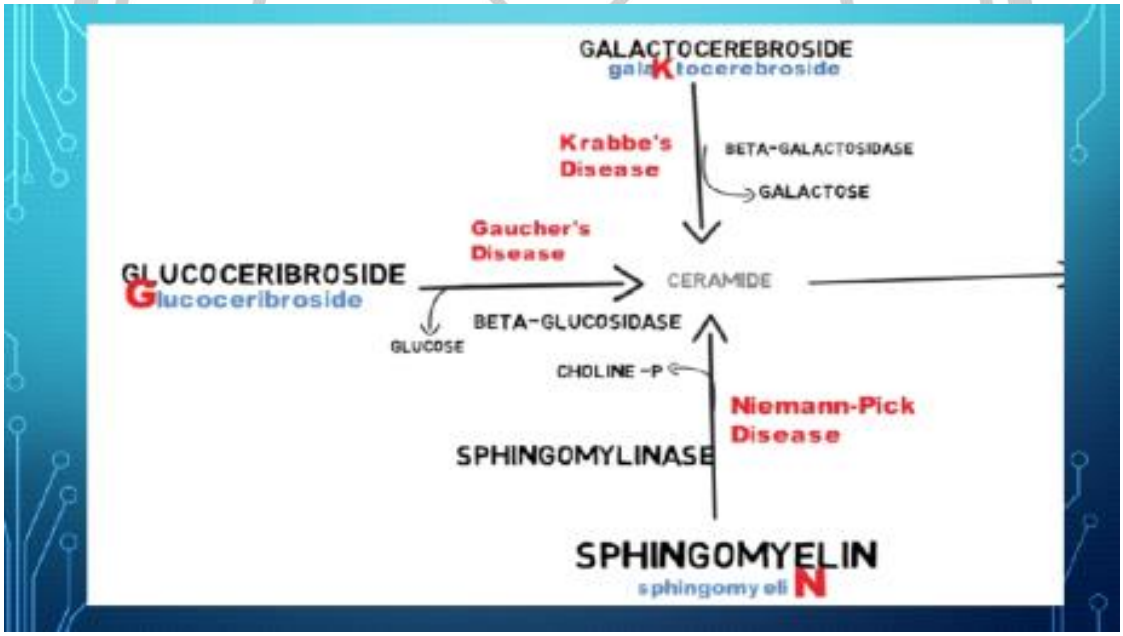
CERAMIDE
SPHINGOMYELIN
sphingomyeliN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Krabbe's
Disease
BETA-GALACTOSIDASE
GALACTOSE
Gaucher's
Disease
GLUCOCERIBROSIDE
Glucoceribroside
CERAMIDE
BETA-GLUCOSIDASE
GLUCOSE
CHOLINE -P
Niemann-Pick
Disease
SPHINGOMYLINASE
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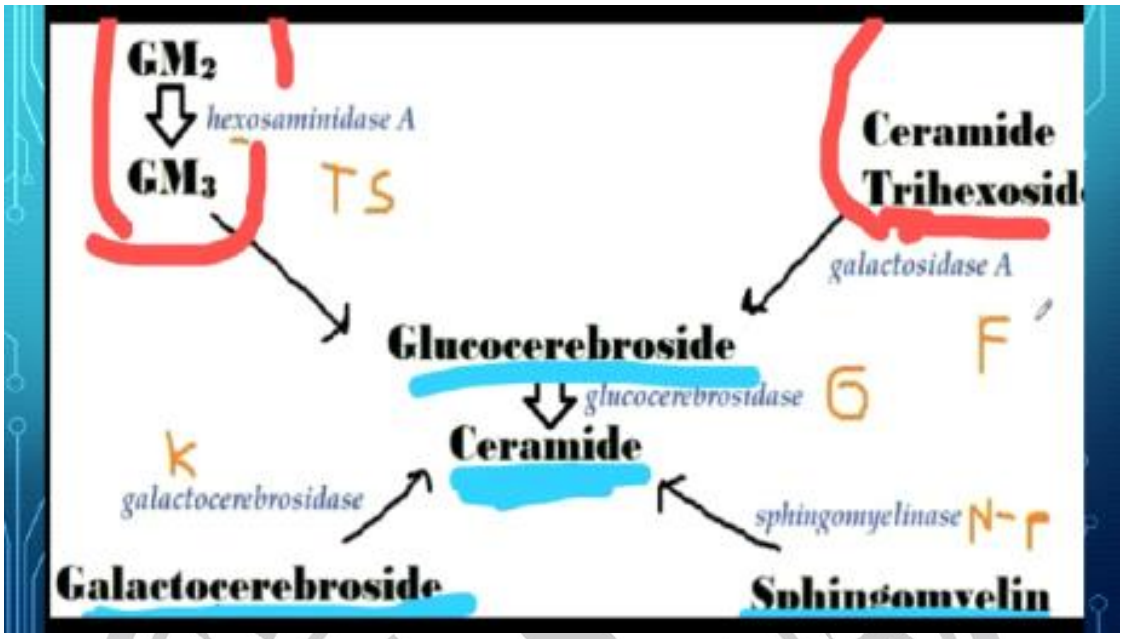


KRABBES DISEASE

Autosomal Recessive

Deficient Enzyme:Beta galactocerebrosidase

Accumulated substrate:Galctocerebroside

Microscopic feature:Globoid cells

Clinical feature:

1)Demyelination of cerebral hemisphere

2)Optic atrophy in 1st year of life

3)Severe mental impairment

GAUCHER DISEASE

Autosomal recessive

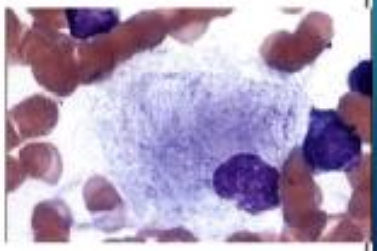
Deficient Enzyme: Beta glucocerebrosidase

Accumulated Substrate: Glucocerebroside

Microscopy: crumpled tissue paper

Types(Clinical Features):

1---- hepatosplenomegaly
Mild anemia
Aseptic necrosis of long-
. . ..bones



2---- INFANTILE GAUCHER
CNS involved
Death<1yr

3---- JUVENILE GAUCHER(less severe than type2)

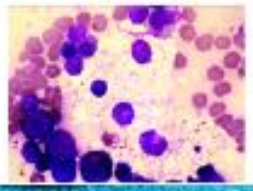
NEIMANPICK DISEASE

Autosomal Recessive

Deficient Enzyme: Sphingomyelinase

Accumulated substrate: Sphingomyelin

Microscopic feature: Histiocytes look foamy(as sphyngomyelin accumulates)



Clinical feature:

1)hepatosplenomegaly
2)anemia
3)neurodegeneration
4)cherry red spots on macula
5)death <3 years age

TAy sach disease

Autosomal Recessive

Deficient Enzyme: Hexoseaminidase A

Accumulated Substrate: GM2 Ganglioside

Microscope: lysosomes with onion skin

Clinical Features:

1)same like Neiman pick but no hepatosplenomegaly

*cherry red spot on macula

*CNS degeneration,blindness

FABRY'S DISEASE (FABRY'C)

X-Linked Recessive

Deficient Enzyme:**Alpha galactosidase A

Accumulted substrate:Trihexose *Ceramide

Clinical Features:

1)**Febrile Episodes

2)**Angiokeratoma

3)**Burning(peripheral neuropathy)

4)**Renal failure

5)**Youth death

6)**Cardiovascular disease

DISEASE	DEFICIENT ENZYME	ACCUMULATED SUBSTRATE	MICROSCOPY	CLINICAL FEATURES
KRABBES			Globoid cells	
GAUCHER				
NIEMAN PICK				
TAY SACH				
FABRYS				

9)BIOCHEMISTRY OF RED BLOOD CELLS

INTRODUCTION

Red blood cells (RBCs) are the **non-nucleated** formed elements in the blood. Red blood cells are also known as erythrocytes (erythros = red). Red color of the red blood cell is due to the presence of the coloring pigment called hemoglobin. RBCs play a vital role in transport of respiratory gases. RBCs are larger in number compared to the other two blood cells, namely white blood cells and platelets.

NORMAL VALUE

RBC count ranges between 4 and 5.5 million/cu mm of blood. In adult males, it is 5 million/cu mm and in adult females, it is 4.5 million/cu mm.

MORPHOLOGY OF RED BLOOD CELLS

NORMAL SHAPE

Normally, the RBCs are disk shaped and biconcave (dumbbell shaped). Central portion is thinner and periphery is thicker. The biconcave contour of RBCs has some mechanical and functional advantages.

Red Blood Cells

Advantages of Biconcave Shape of RBCs

1. Biconcave shape helps in equal and rapid diffusion of oxygen and other substances into the interior of the cell.
2. Large surface area is provided for absorption or removal of different substances.

3. Minimal tension is offered on the membrane when the volume of cell alters.

4. Because of biconcave shape, while passing through minute capillaries, RBCs squeeze through the capillaries very easily without getting damaged.

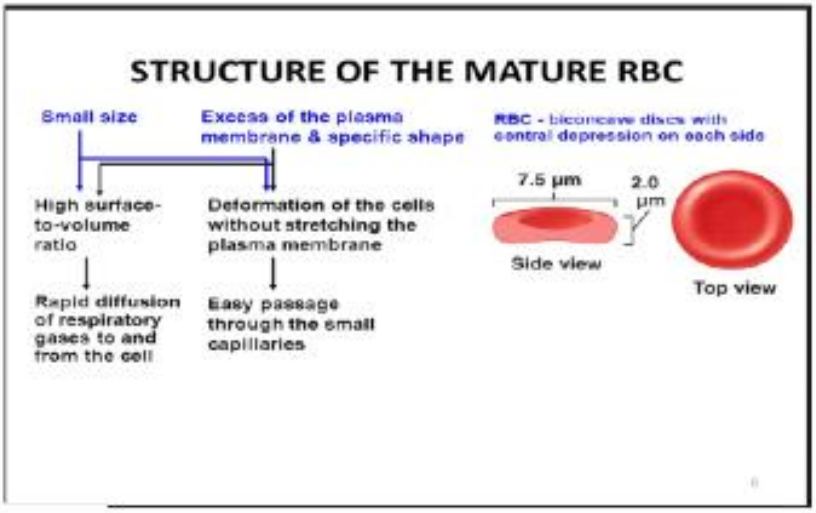
NORMAL SIZE

Diameter : 7.2 μ (6.9 to 7.4 μ).

Thickness : At the periphery it is thicker with 2.2 μ and at the center it is thinner with 1 μ (Fig. 9.1). This difference in thickness is because of the biconcave shape.

Surface area : 120 sq μ.

Volume : 85 to 90 cu μ.



NORMAL STRUCTURE

Red blood cells are nonnucleated.

Only mammal, which has nucleated RBC is camel. Because of the absence of nucleus in human RBC, the DNA is also absent. Other organelles such as mitochondria and Golgi apparatus also are absent in RBC. Because of absence of mitochondria, the energy is produced from glycolytic process. Red cell does not have insulin receptor and so the glucose uptake by this cell is not controlled by insulin.

RBC has a special type of **cytoskeleton,** which is made up of **actin** and **spectrin.** Both the proteins are anchored to transmembrane proteins by means of another protein called **ankyrin.**.

Absence of spectrin results in hereditary spherocytosis. In this condition, the cell is deformed, losses its biconcave shape and becomes globular (spherocytic). The spherocyte is very fragile and easily ruptured (hemolyzed) in hypotonic solutions

PROPERTIES OF RED BLOOD CELLS

ROULEAUX FORMATION

When blood is taken out of the blood vessel, the RBCs pile up one above another like the pile of coins. This property of the RBCs is called rouleaux (pleural = rouleau) formation (Fig. 9.2). It is accelerated by plasma proteins globulin and fibrinogen.

SPECIFIC GRAVITY

Specific gravity of RBC is 1.092 to 1.101.

PACKED CELL VOLUME

Packed cell volume (PCV) is the proportion of blood occupied by RBCs expressed in percentage. It is also called hematocrit value. It is 45% of the blood and the plasma volume is 55%

SUSPENSION STABILITY

During circulation, the RBCs remain suspended uniformly in the blood. This property of the RBCs is called the suspension stability.

LIFESPAN OF RED BLOOD CELLS

Average lifespan of RBC is about 120 days. After the lifetime the senile (old) RBCs are destroyed in reticuloendothelial system.

Determination of Lifespan of Red Blood Cells

Lifespan of the RBC is determined by radioisotope method. RBCs are tagged with radioactive substances like radioactive iron or radioactive chromium. Life of RBC is determined by studying the rate of loss of radioactive cells from circulation.

FATE OF RED BLOOD CELLS

When the cells become older (120 days), the cell membrane becomes more fragile. Diameter of the capillaries is less or equal to that of RBC. Younger RBCs can pass through the capillaries easily. However, because of the fragile nature, the older cells are destroyed while trying to squeeze through the capillaries. The destruction occurs mainly in the capillaries of red pulp of spleen

because the diameter of splenic capillaries is very small. So, the spleen is called **'graveyard of RBCs'.** Destroyed RBCs are fragmented and hemoglobinis released from the fragmented parts. Hemoglobin is immediately phagocytized by macrophages of the body,

particularly the macrophages present in liver **(Kupffer cells),** spleen and bone marrow.

Hemoglobin is degraded into iron, globin and porphyrin. Iron combines with the protein called apoferritin to form ferritin, which is stored in the body and reused later. Globin enters the protein depot for later use Porphyrin is degraded into bilirubin, which is excreted by liver through bile .

Daily 10% RBCs, which are senile, are destroyed in normal young healthy adults. It causes release of about 0.6 g/dL of hemoglobin into the plasma. From this 0.9 to 1.5 mg/dL bilirubin is formed.

FUNCTIONS OF RED BLOOD CELLS

Major function of RBCs is the transport of respiratory gases. Following are the functions of RBCs:

1. *Transport of Oxygen from the Lungs to the Tissues*

Hemoglobin in RBC combines with oxygen to form **oxyhemoglobin.** About 97% of oxygen is transported in blood in the form of oxyhemoglobin (Chapter 125).

2. *Transport of Carbon Dioxide from the Tissues to the Lungs*

Hemoglobin combines with carbon dioxide and form **carbhemoglobin.** About 30% of carbon dioxide is transported in this form

RBCs contain a large amount of the **carbonic anhydrase.** This enzyme is necessary for the formation of bicarbonate from water and carbon dioxide (Chapter 125). Thus, it helps to transport carbon dioxide in the form of bicarbonate from tissues to lungs. About 63% of carbon dioxide is transported in this form.

3. *Buffering Action in Blood*

Hemoglobin functions as a good buffer. By this action, it regulates the hydrogen ion concentration and thereby plays a role in the maintenance of acidbase balance

4. *In Blood Group Determination*

RBCs carry the **blood group antigens** like A antigen, B antigen and Rh factor. This helps in determination of blood group and enables to prevent reactions due to incompatible blood transfusion

VARIATIONS IN NUMBER OF RED BLOOD CELLS

PHYSIOLOGICAL VARIATIONS

A. *Increase in RBC Count*

Increase in the RBC count is known as **polycythemia.** It occurs in both physiological and pathological conditions. When it occurs in physiological conditions it is called physiological polycythemia. The increase in number during this condition is marginal and temporary. It occurs in the following conditions:

1. *Age*

At birth, the RBC count is 8 to 10 million/cu mm of blood. The count decreases within 10 days after birth due to destruction of RBCs causing **physiological jaundice** in some newborn babies. However, in infants and growing children, the cell count is more than the value in adults.

2. *Sex*

Before puberty and after menopause in females the RBC count is similar to that in males. During reproductive period of females, the count is less than that of males (4.5 million/cu mm).

3. *High altitude*

Inhabitants of mountains (above 10,000 feet from mean sea level) have an increased RBC count of more than 7 million/cu mm. It is due to **hypoxia** (decreased oxygen supply to tissues) in high altitude. Hypoxia stimulates kidney to secrete a hormone called **erythropoietin.**

4. Muscular exercise

There is a temporary increase in RBC count after exercise. It is because of mild hypoxia and contraction of spleen. Spleen stores RBCs Hypoxia increases the sympathetic activity resulting in secretion of adrenaline from adrenal medulla. Adrenaline contracts spleen and RBCs are released into blood.

5. Emotional conditions

RBC count increases during the emotional conditions such as anxiety. It is because of increase in the sympathetic activity as in the case of muscular exercise

6. Increased environmental temperature

Increase in atmospheric temperature increases RBC count. Generally increased temperature increases all the activities in the body including production of RBCs.

7. *After meals*

There is a slight increase in the RBC count after taking meals. It is because of need for more oxygen for metabolic activities.

conditions and exercise

B. *Decrease in RBC Count*

Decrease in RBC count occurs in the following physiological conditions:

1. *High barometric pressures*

At high barometric pressures as in deep sea, when the oxygen tension of blood is higher, the RBC count decreases.

2. *During sleep*

RBC count decreases slightly during sleep and immediately after getting up from sleep. Generally all the activities of the body are decreased during sleep including production of RBCs.

3. *Pregnancy*

In pregnancy, the RBC count decreases. It is because of increase in ECF volume. Increase in ECF volume, increases the plasma volume also resulting in hemodilution. So, there is a relative reduction in the RBC count.

PATHOLOGICAL VARIATIONS

Pathological Polycythemia

Pathological polycythemia is the abnormal increase in the RBC count. Red cell count increases above 7 million/ cu mm of the blood. Polycythemia is of two types, the primary polycythemia and secondary polycythemia.

Primary Polycythemia – Polycythemia Vera

Primary polycythemia is otherwise known as polycythemia vera. It is a disease characterized by persistent increase in RBC count above 14 million/cu mm of blood. This is always associated with increased white blood cell count above 24,000/cu mm of blood. Polycythemia vera occurs in **myeloproliferative disorders** like malignancy of red bone marrow.

Secondary Polycythemia

This is secondary to some of the pathological conditions (diseases) such as:

1. Respiratory disorders like emphysema.
2. Congenital heart disease.
3. Ayerza's disease (condition associated with hypertrophy of right ventricle and obstruction of blood flow to lungs).
4. Chronic carbon monoxide poisoning.
5. Poisoning by chemicals like phosphorus and arsenic.
6. Repeated mild hemorrhages.

All these conditions lead to hypoxia which stimulates the release of erythropoietin. Erythropoietin stimulates the bone marrow resulting in increased RBC count.

Anemia

Abnormal decrease in RBC count is called anemia. This

VARIATIONS IN SIZE OF RED BLOOD CELLS

Under physiological conditions, the size of RBCs in venous blood is slightly larger than those in arterial blood. In pathological conditions, the variations in size of RBCs are:

1. Microcytes (smaller cells)
2. Macrocytes (larger cells)
3. Anisocytes (cells with different sizes).

MICROCYTES

Microcytes are present in:

i. Iron-deficiency anemia

ii. Prolonged forced breathing

iii. Increased osmotic pressure in blood.

MACROCYTES

Macrocytes are present in:

i. Megaloblastic anemia

ii. Decreased osmotic pressure in blood.

ANISOCYTES

Anisocytes occurs in pernicious anemia.

VARIATIONS IN SHAPE OF RED BLOOD CELLS

Shape of RBCs is altered in many conditions including different types of anemia.

1. *Crenation:* Shrinkage as in hypertonic conditions.

2. *Spherocytosis:* Globular form as in hypotonic conditions.

3. *Elliptocytosis:* Elliptical shape as in certain types of anemia.

4. *Sickle cell:* Crescentic shape as in sickle cell anemia.

5. *Poikliocytosis:* Unusual shapes due to deformed cell membrane. The shape will be of flask, hammer or any other unusual shape.

VARIATIONS IN STRUCTURE OF RED BLOOD CELLS

PUNCTATE BASOPHILISM

Striated appearance of RBCs by the presence of dots of **basophilic materials** (porphyrin) is called punctate basophilism. It occurs in conditions like **lead poisoning.**

RING IN RED BLOOD CELLS

Ring or twisted strands of basophilic material appear in the periphery of the RBCs. This is also called the **Goblet ring.** This appears in the RBCs in certain types of anemia.

HOWELL-JOLLY BODIES

In certain types of anemia, some nuclear fragments are present in the ectoplasm of the RBCs. These nuclear fragments are called HowellJolly bodies

Erythropoiesis

DEFINITION

Erythropoiesis is the process of the origin, development and maturation of erythrocytes. Hemopoiesis or hematopoiesis is the process of origin, development and maturation of all the blood cells.

SITE OF ERYTHROPOIESIS

IN FETAL LIFE

In fetal life, the erythropoiesis occurs in three stages:

1. *Mesoblastic Stage*

During the first two months of intrauterine life, the RBCs are produced from **mesenchyme** of yolk sac.

2. *Hepatic Stage*

From third month of intrauterine life, **liver** is the main organ that produces RBCs. **Spleen** and **lymphoid organs** are also involved in erythropoiesis.

3. *Myeloid Stage*

During the last three months of intrauterine life, the RBCs are produced from red **bone marrow** and **liver.**

Erythropoiesis

IN NEWBORN BABIES, CHILDREN AND ADULTS

In newborn babies, growing children and adults, RBCs are produced only from the red bone marrow.

1. *Up to the age of 20 years:* RBCs are produced from red bone marrow of all bones (**long bones** and all the **flat bones**).
2. *After the age of 20 years:* RBCs are produced from **membranous bones** like vertebra, sternum, ribs, scapula, iliac bones and skull bones and from the ends of long bones. After 20 years of age, the shaft of the long bones becomes yellow bone marrow because of fat deposition and looses the erythropoietic function

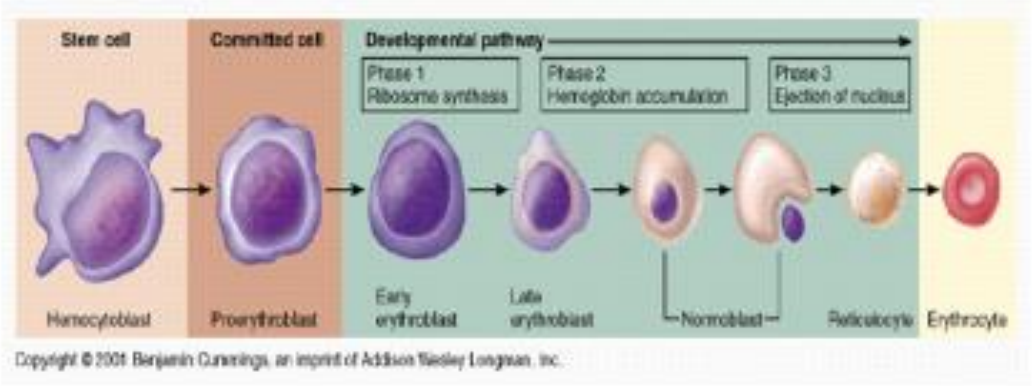
In adults, liver and spleen may produce the blood cells if the bone marrow is destroyed or fibrosed. Collectively bone marrow is almost equal to liver in size and weight. It is also as active as liver. Though bone marrow is the site of production of all blood cells, comparatively 75% of the bone marrow is involved in the production of leukocytes and only 25% is involved in the production of erythrocytes.

But still, the leukocytes are less in number than the erythrocytes, the ratio being 1:500. This is mainly because of the lifespan of these cells. Lifespan of erythrocytes is 120 days whereas the lifespan of leukocytes is very short ranging from one to ten days. So the leukocytes need larger production than erythrocytes to maintain the required number.

STAGES OF ERYTHROPOIESIS

Various stages between CFU-E cells and matured RBCs are

1. Proerythroblast
2. Early normoblast
3. Intermediate normoblast.
4. Late normoblast
5. Reticulocyte
6. Matured erythrocyte.



FACTORS NECESSARY FOR ERYTHROPOIESIS

Development and maturation of erythrocytes require variety of factors, which are classified into three categories:

1. General factors
2. Maturation factors
3. Factors necessary for hemoglobin formation.

GENERAL FACTORS

General factors necessary for erythropoiesis are:

i. Erythropoietin
ii. Thyroxine
iii. Hemopoietic growth factors
iv. Vitamins.

10)NUCLEOTIDES

COMPOSITION ◦ Nucleotides are composed of:

◦ Nitrogenous base

◦ Pentose sugar

◦ Phosphate groups

NITROGENOUS BASES

◦ • Aromatic and heterocyclic

◦ • Derived from purine or pyrimidine

◦ **Important Purines** ◦ Adenine and guanine are the principal purines of both DNA and RNA. Double cyclic ring

◦ **Important Pyrimidines** ◦ Pyrimidines that occur in DNA are cytosine and thymine. Cytosine and uracil are the pyrimidines in RNA. Single cyclic

Sugar & Phosphate group

◦ **Sugar** ◦ • Pentoses (5-C sugars)

◦ **• Numbering of sugars is "primed".**

◦ **Phosphate Groups** ◦ • Mono-, di- or triphosphates

◦ • Phosphates can be bonded to either C2, C3 or C5 atoms of the sugar

◦ **Nucleosides** ◦ • Result from linking one of the sugars with a purine or pyrimidine base through an N-glycosidic linkage

◦ – Purines bond to the C1' carbon of the sugar at their N9 atoms

◦ – Pyrimidines bond to the C1' carbon of the sugar at their N1 atoms

Nucleotides ◦ ◦ Result from linking one or more phosphates with a nucleoside onto the 5' end of the molecule

◦ **PHOSPHATE LINKAGE** ◦ 5' Phosphate ◦ 3' Phosphate ◦ 2' Phosphate

◦**Nomenclature**

◦ **Nucleosides:**

◦ **– Purine nucleosides end in "-sine"** ◦ **• Adenosine, Guanosine**

◦ **– Pyrimidine nucleosides end in "-dine"** ◦ **• Thymidine, Cytidine, Uridine**

◦ **Nucleotides: ◦ – Start with the nucleoside name from above and add "mono-", "di-", or "triphosphate"**

◦ **• Adenosine Monophosphate, Cytidine Triphosphate, Deoxythymidine Diphosphate**

◦ **• RNA (ribonucleic acid) is a polymer of ribonucleotides**

◦ **• DNA (deoxyribonucleic acid) is a polymer of deoxyribonucleotides**

◦ • Both deoxy- and ribonucleotides contain Adenine, Guanine and Cytosine ◦ – Ribonucleotides contain Uracil ◦ – Deoxyribonucleotides contain Thymine

◦**Types of Nucleic Acids**

◦ • DEOXY RIBONUCLEIC ACID – DNA

◦ • RIBO NUCLEIC ACID – RNA (mRNA ◦ – tRNA ◦ – rRNA)

◦ **DNA structure** ◦ • B-DNA, A-DNA , Z-DNA forms

◦ • B-DNA is most common

◦ • Antiparallel

◦ • stands complementary

◦ – Very important for information transfer

◦ – Each strand a template for the other.

◦ • right handed

◦ • major and minor groove

◦ **One Strand of DNA**

◦ • The backbone of the molecule is alternating phosphates and deoxyribose sugar

◦ • The teeth are nitrogenous bases.

◦ **Chargraff's Rule:**

◦ • Adenine and Thymine always join together

◦ • Cytosine and Guanine always join together

◦ **RNA**

◦ • codes for protein

◦ • single stranded

◦ • Ribose instead of deoxyribose

◦ • Thymine (T) replaced by Uracil

◦ 1. Nucleotides are constantly ◦ undergoing turnover!

◦ 2. There are many **enzymes** involved; ◦ Nucleotidases ◦ Nucleoside phosphorylases ◦ Deaminases ◦ Xanthine oxidases

◦ **Purine Degradation**

◦ • Purine Nucleotides from ingested nucleic acids or turnover of cellular nucleic acids is excreted by humans as uric acid.

◦ • Humans excrete about 0.6 g uric acid every 24 hours.

◦ **Pyrimidine Degradation**

◦ • Pyrimidines are generally degraded to intermediates of carbon metabolism (for example, succinyl-CoA) and ammonia (NH_4^+).

◦ • NH_4^+ is packaged as urea through the urea cycle and excreted by humans

◦ • Defects in enzymes of pyrimidine degradation have been documented, resulting in increased levels of pyrimidines and neurological disorders

◦ **Functions of nucleic acids**

◦ • DNA is a basis of heredity

◦ • DNA is carrier of genetic information

◦ • DNA contains genes, the information needed to synthesize functional proteins and RNAs ◦ • DNA contains segments that play a role in regulation of gene expression

◦ • Ribosomal RNAs (rRNAs) are components of ribosomes, playing a role in protein synthesis

◦ • Messenger RNAs (mRNAs) carry genetic information from a gene to the ribosome

◦ • Transfer RNAs (tRNAs) translate information in mRNA into an amino acid sequence

11)ENZYMES

What are Enzymes?

◦Biological catalysts which speed up the rate of reaction without becoming part of the reaction

◦Enzymes increases the rate of reaction by lowering the activation energy barrier, thus allowing reactions to proceed without an input of energy

ENZYME NOMENCLATURE

◦ Recommended Name:

◦ Suffix “ase” attached to the susbtrate of the reaction

◦ For example; Glucosidase, Urease

◦ Systematic Name

◦ IUBMB divided enzymes into six classes

◦ The suffix “ase” is attached to describe complete chemical reaction and substrate name

◦ For example; Pyruvate decarboylase

Mechanism of Action of Enzyme

◦ • Active site – a region of an enzyme comprised of different amino acids where catalysis occurs

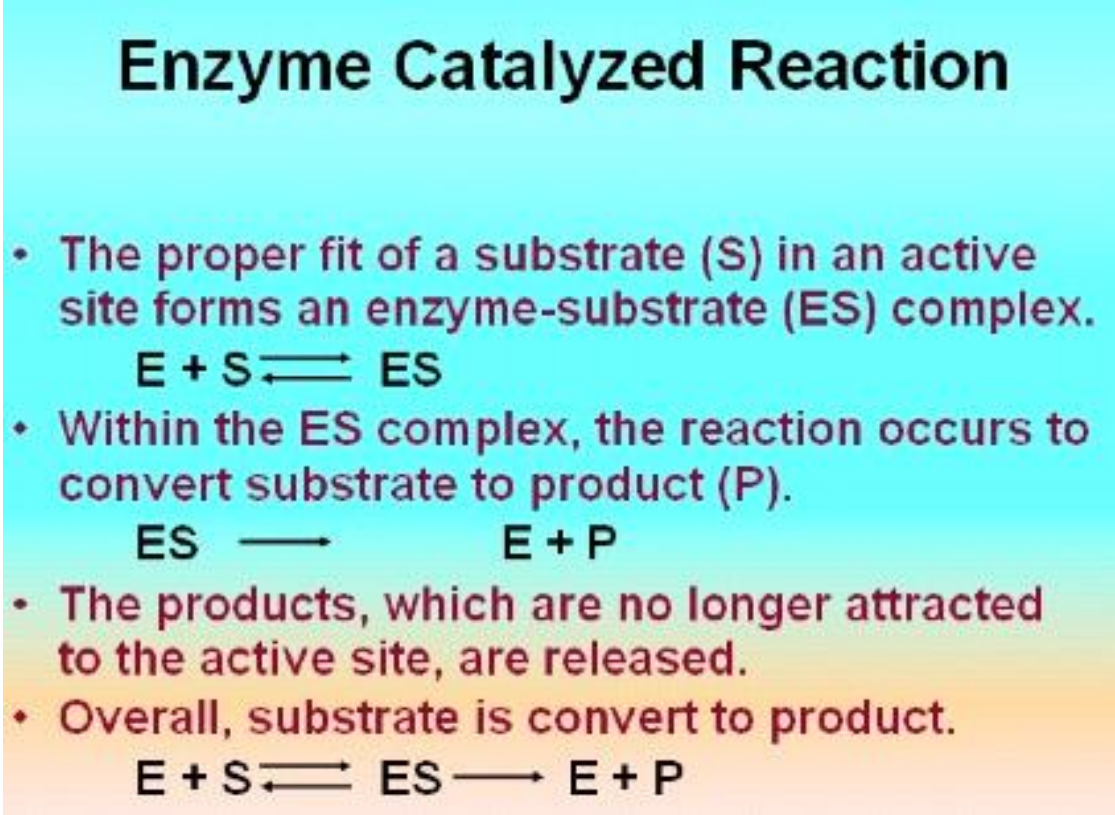
◦ • Substrate – the molecule being utilized and/or modified by a particular enzyme at its active site

◦ • Co-factor – organic or inorganic molecules that are required by enzymes for activity.

◦ Enzymes convert substrates into products

◦ • What is a substrate?

◦ – A substrate is the compound that is converted into the product in an enzyme catalyzed reaction



PHYSICAL COMPOSITION

◦ • Apoenzyme = the protein part of an enzyme without coenzymes or prosthetic groups that are required for the enzyme to have activity.

◦ • Coenzyme = small organic molecules which is dialyzable, thermostable and loosely attached to the protein part.

◦ • Prosthetic group = an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.

◦ • Cofactors= Inorganic molecules

◦ Cofactors

◦ • Non-protein molecules that help enzymes function.

◦ • Bind to active site to enhance enzymatic reactions.

◦ • Cofactors may be inorganic metals such as zinc, iron, or copper.

◦ **COENZYMES**

◦ • Heat stable, low mol wt organic compounds, non-covalently linked with enzymes, can be separated.

◦ APO + CO = Holoenzyme

◦ Act as intermediate or ultimate acceptor in group transfer

◦ **Important Coenzymes**

◦ • NAD+

◦ • NADP+

◦ • FAD

◦ • Coenzyme A

Classification

◦ 1)Oxidoreducatse

◦ 2)Transferase

◦ 3)Hydrolase

◦ 4)Lyase

◦ 5)Isomerase

◦ 6)Ligase

◦ **Factors Influencing Enzyme Activity**

◦ a) Enzyme concentration

◦ b) Temperature

◦ c) pH

◦ d) Substrate concentration

◦ e) Inhibitors

◦ **TYPES OF INHIBITORS**

◦ Reversible Inhibitor

◦ Competitive

◦ Non-Competitive

◦ Uncompetitive

◦ Irreversible Inhibitor

ISOENZYMES

◦ (1) Isozymes are physically distinct forms of the same enzyme.

◦ (2) Isozymes may differ from each other by differences in their amino acid sequences

◦ (3) The relative abundance of different isozymes varies for different tissues.

◦ Lactate dehydrogenase on electrophoresis gives 5 different bands and has 4 protomers

◦ **Medical Relevance**

◦ • Many diseases are caused by the absence, malfunction, or inappropriate expression of a particular enzyme—SOD

◦ • Enzymes serve as targets for a variety of drugs

◦ • Enzymes are sometimes administered in the treatment of disease

◦ • The presence or absence of specific enzymes can be used to diagnose specific diseases

12) INSULIN


Contents
• Introduction
• Structure
• Synthesis(site,process)
• Regulation of secretion
• Metabolic role


Contents
• Introduction
• Structure
• Synthesis(site,process)
• Regulation of secretion
• Metabolic role

- Is a polypeptide hormone produce by *ß-cells of langerhans of pancreas.*
- It has profound influence on metabolism of Carbohydrates, fat & proteins.
- It is considered as the anabolic hormone.

- The main function of insulin is to lower serum glucose and promote anabolism .
- Insulin is an essential growth factor required for normal development.

- It was the first hormone to be isolated, purified & synthesized.
- First hormone to be sequenced
- First hormone to be produced by recombinant DNA technology.

Structure

Structure

- Human Insulin contain 51 aminoacids, arranged in TWO Polypeptide chains.
- Chain A = 21 AA
- Chain B = 30 AA
- Two Interchain Disulfide bridge = A7-B7 & A20-B19.
- Intrachain Disulfide link in chain A = 6-11.

Synthesis of INSULIN

1)Site:Beta cells of langerhans of pancreas

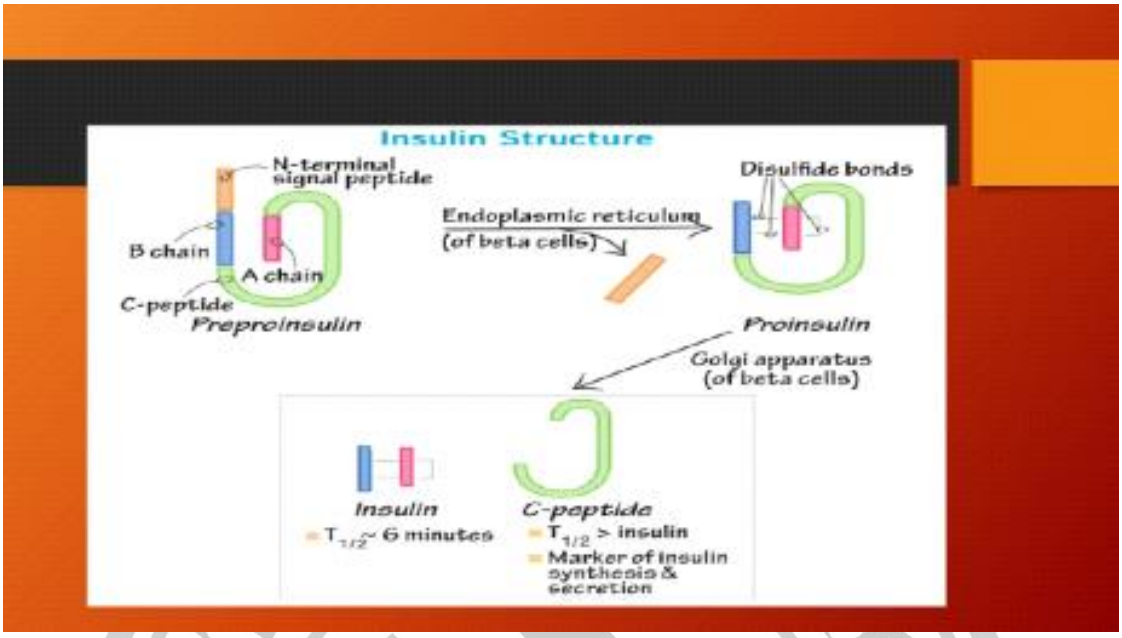
2)genes involved are located on chromosome 11.

Biosynthesis of Insulin – 3 major steps

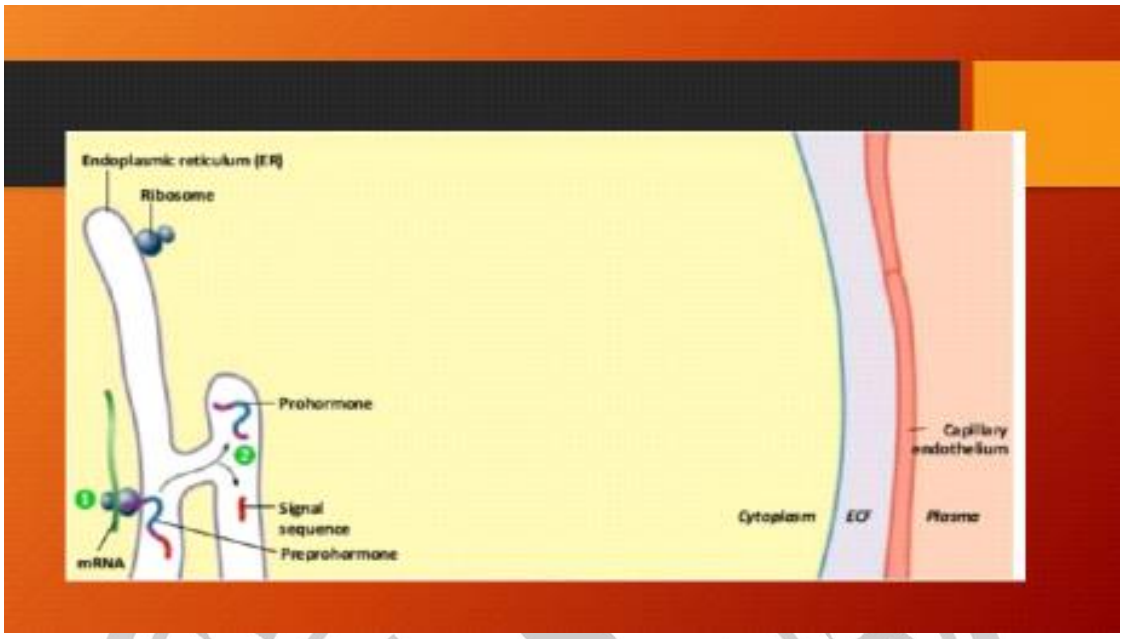
- Site; β- cells of Islets

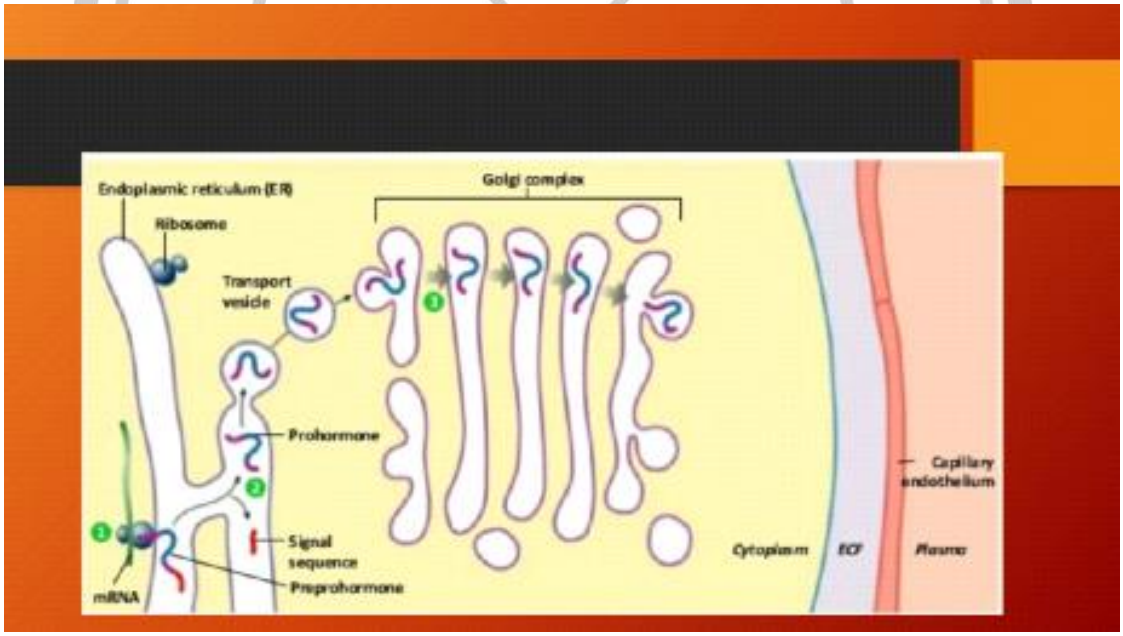
1. Synthesis of Preproinsulin.
2. Conversion of preproinsulin to proinsulin.
3. Conversion of proinsulin to insulin.

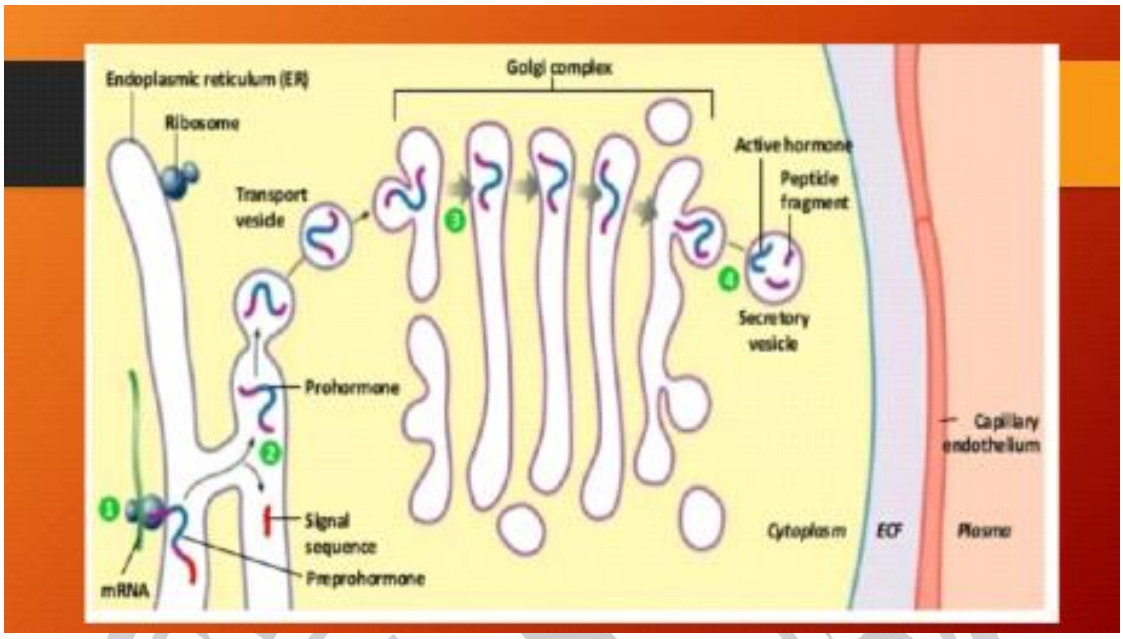
Insulin is synthesized by ribosomes of the rough ER as a larger precursor peptide that is then converted to the mature hormone prior to secretion

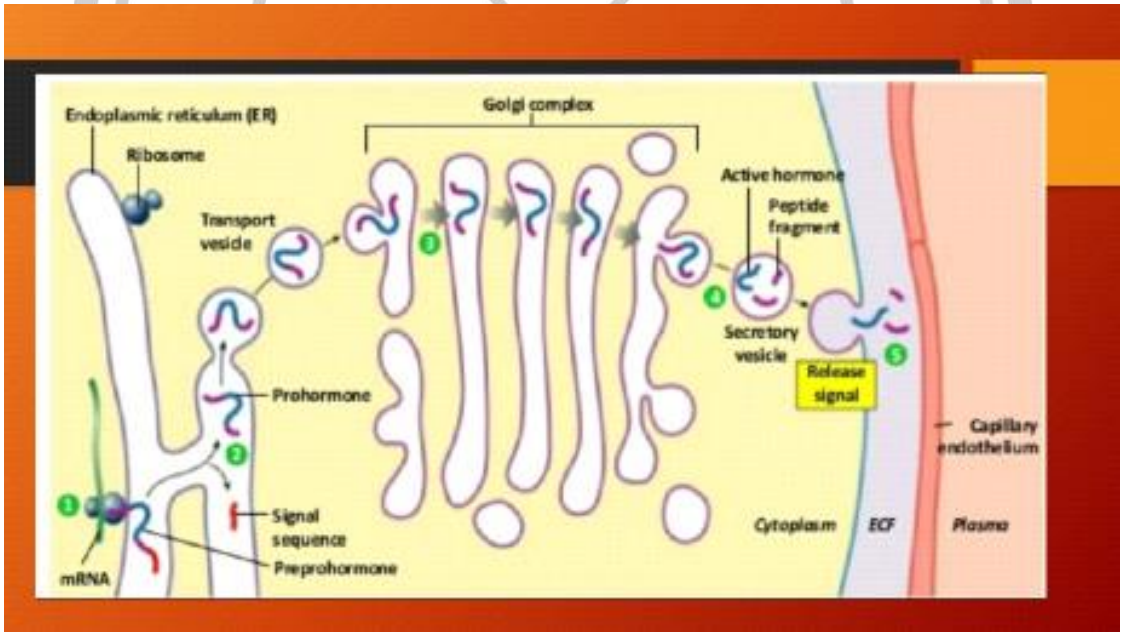

Insulin Structure
N-terminal signal peptide
B chain
A chain
C-peptide
Preproinsulin
Endoplasmic reticulum (of beta cells)
Disulfide bonds
Proinsulin
Golgi apparatus (of beta cells)
Insulin
$T_{1/2}$ ~ 6 minutes
C-peptide
$T_{1/2}$ > insulin
Marker of insulin synthesis & secretion

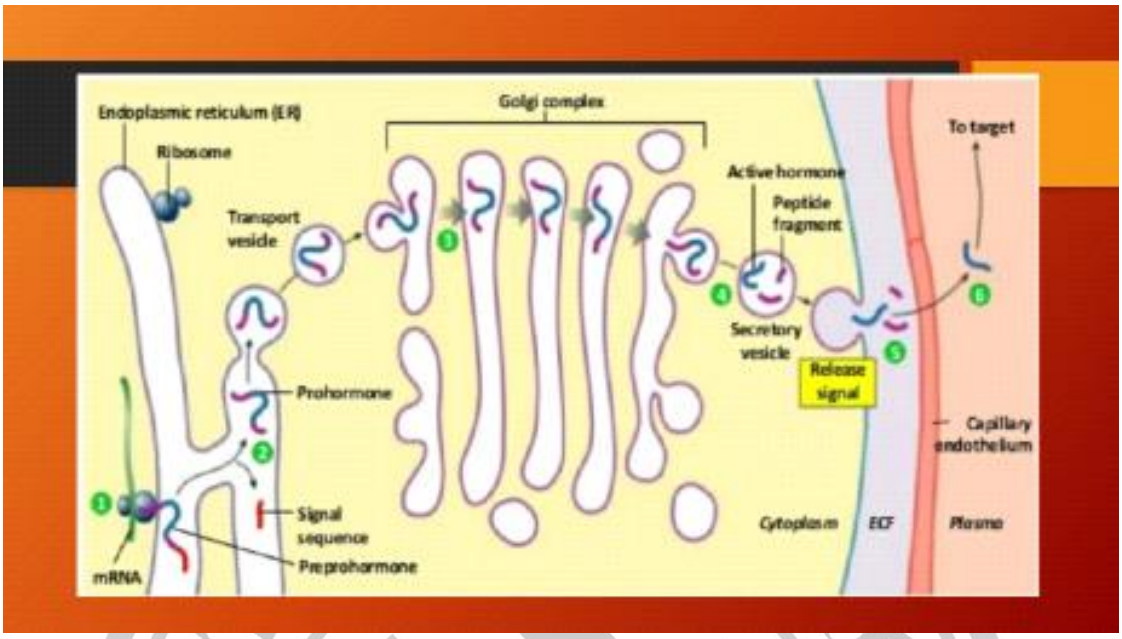

Endoplasmic reticulum (ER)
Ribosome
mRNA
Preprohormone
Capillary endothelium
Cytoplasm
ECF
Plasma


Endoplasmic reticulum (ER)
Ribosome
Prohormone
Signal sequence
Preprohormone
mRNA
Capillary endothelium
Cytoplasm
ECF
Plasma

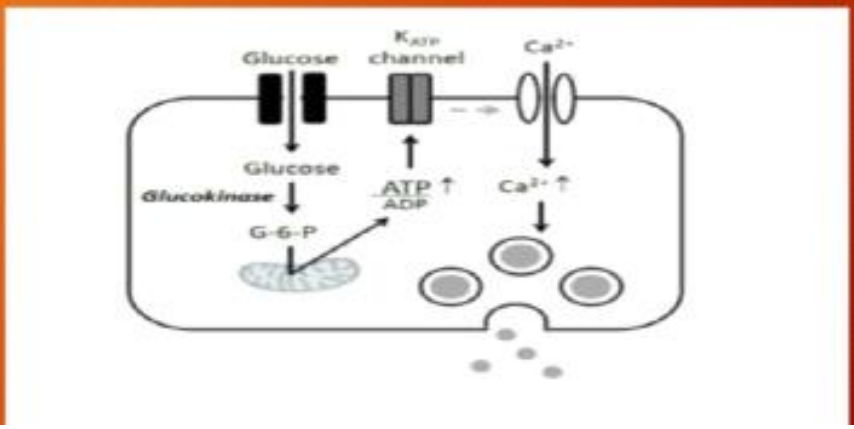

Endoplasmic reticulum (ER)
Golgi complex
Ribosome
Transport vesicle
Prohormone
Signal sequence
Preprohormone
mRNA
Capillary endothelium
Cytoplasm
ECF
Plasma


Endoplasmic reticulum (ER)
Ribosome
Golgi complex
Transport vesicle
Active hormone
Peptide fragment
Secretory vesicle
Prohormone
Capillary endothelium
Signal sequence
Preprohormone
mRNA
Cytoplasm
ECF
Plasma


Endoplasmic reticulum (ER)
Ribosome
Golgi complex
Transport vesicle
Active hormone
Peptide fragment
Secretory vesicle
Release signal
Prohormone
Capillary endothelium
Signal sequence
Preprohormone
mRNA
Cytoplasm
ECF
Plasma



Regulation of secretion



Regulation of secretion

ACTIVATORS

1)Increased glucose

2)Increased amino acids

3)Increased INCRETIN (GI peptide+ GLP-1)

4)Epinephrine(beta receptor)

- **INHIBITORS**

1)Stress

2)Infections

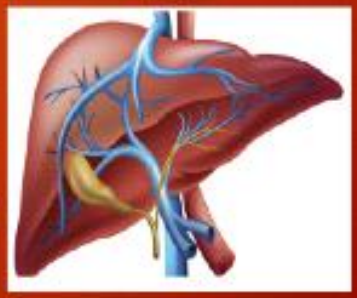
3)Fever

4)Hypoxia

5)Epinephrine(alpha receptor)

METABOLIC ROLE OF INSULIN

INSULIN (raised glucose, AA or incretin)



Stop new glucose formation:
1) Inhibit Gluconeogenesis
2) Inhibit Glycogenolysis

Decrease existing glucose:
(By converting to Glycogen)

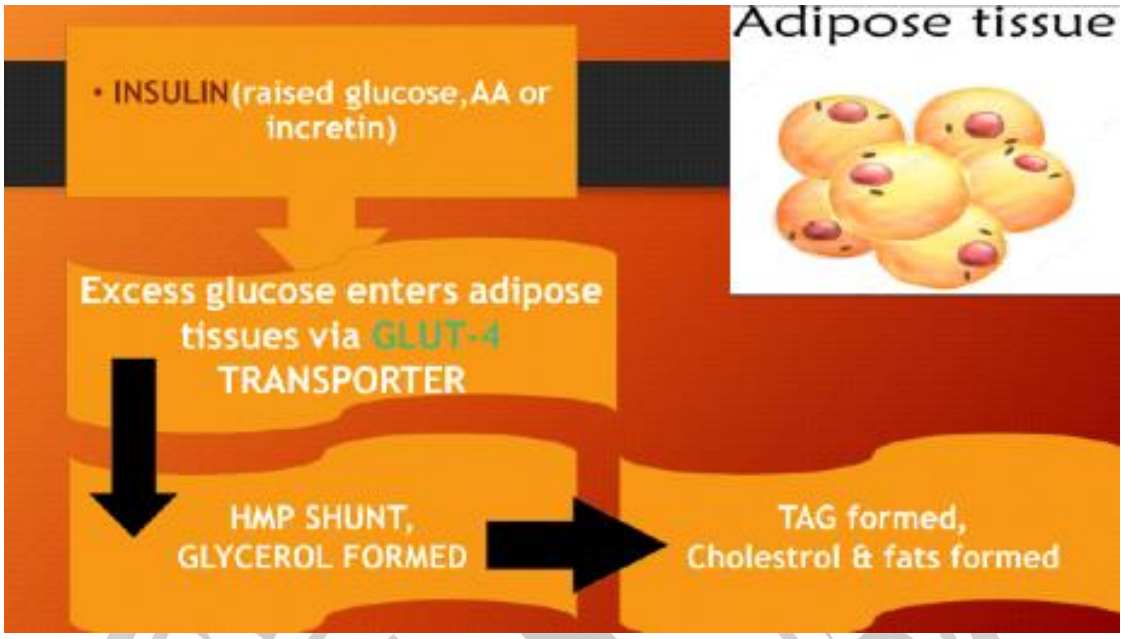
1) Activate Glycogenesis

INSULIN (raised glucose, AA or incretin)

SKELETAL MUSCLE

Excess glucose enters muscles via GLUT-4 TRANSPORTER

Glycogenesis:
Stored as glycogen



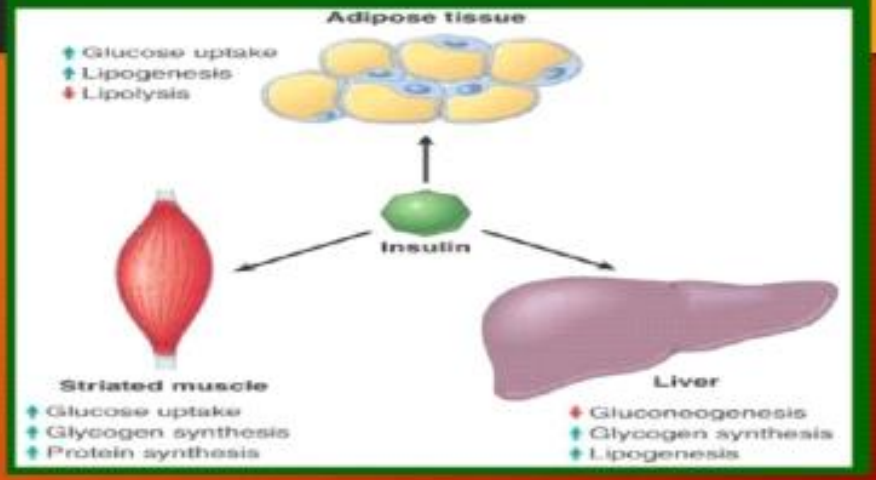
Effect on Amino Acids

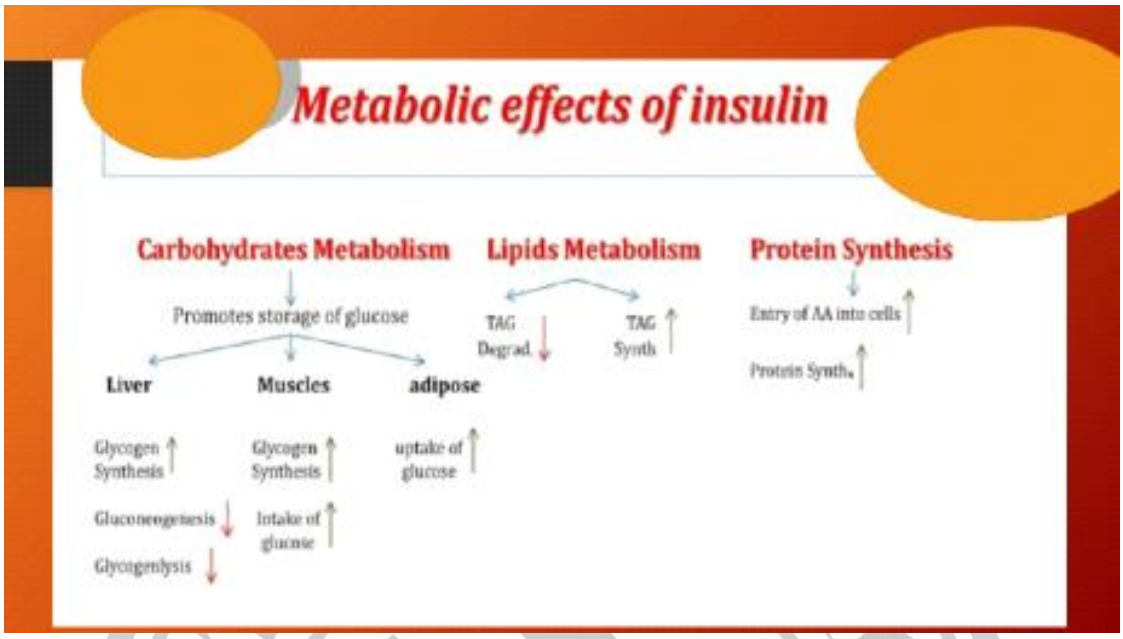
- Insulin causes Amino Acids entry to cells to form Proteins

Effect on fats

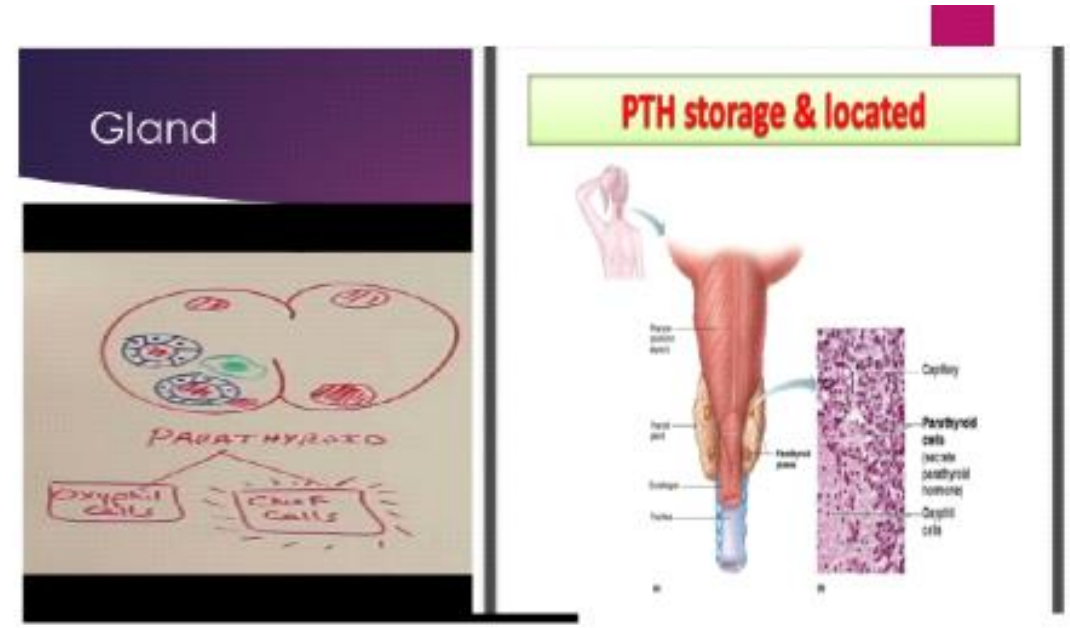
- Inhibit hormone sensitive LIPASE
- Converts excess glucose to TAG and fats

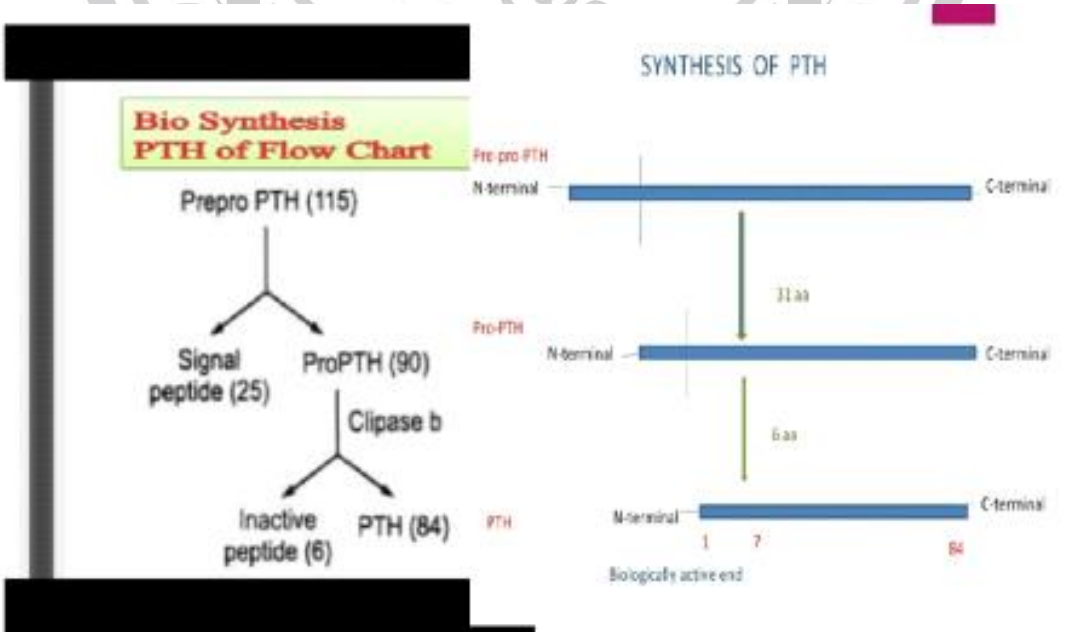
Role of Insulin



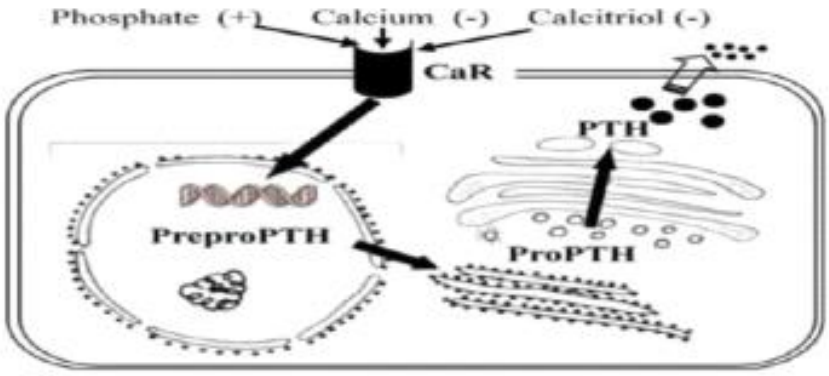

Metabolic effects of insulin
Carbohydrates Metabolism
Promotes storage of glucose
Liver
Muscles
adipose
Glycogen Synthesis
Gluconeogenesis
Glycogenlysis
Glycogen Synthesis
Intake of glucose
uptake of glucose
Lipids Metabolism
TAG Degrad.
TAG Synth
Protein Synthesis
Entry of AA into cells
Protein Synth.

13)PARATHROID HORMONE AND CALCIUM HOMEOSTASIS:

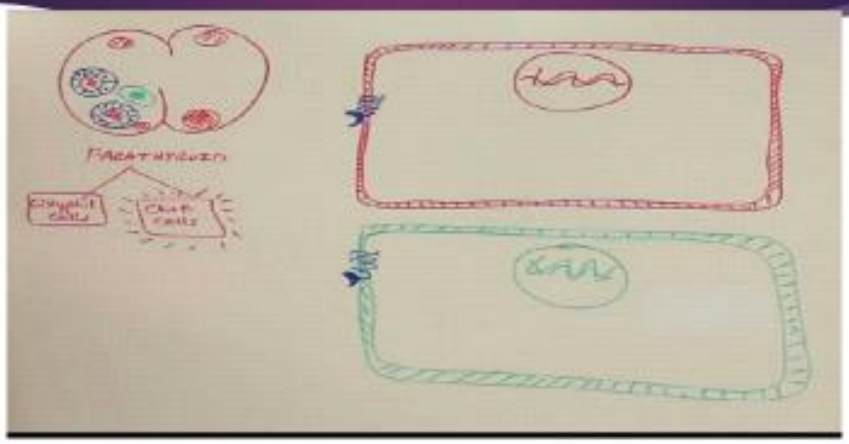


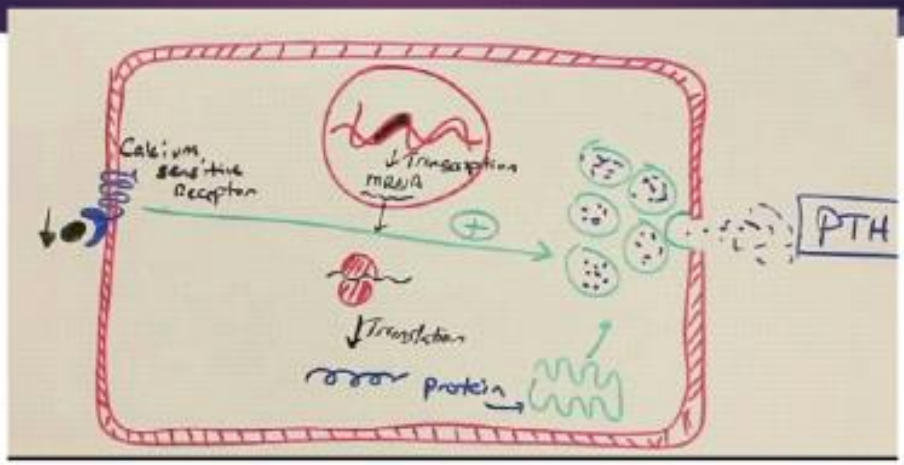


PTH secretion

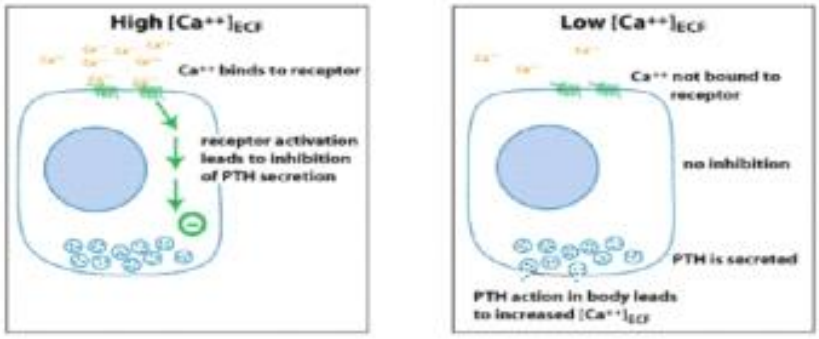


SYNTHESIS PTH



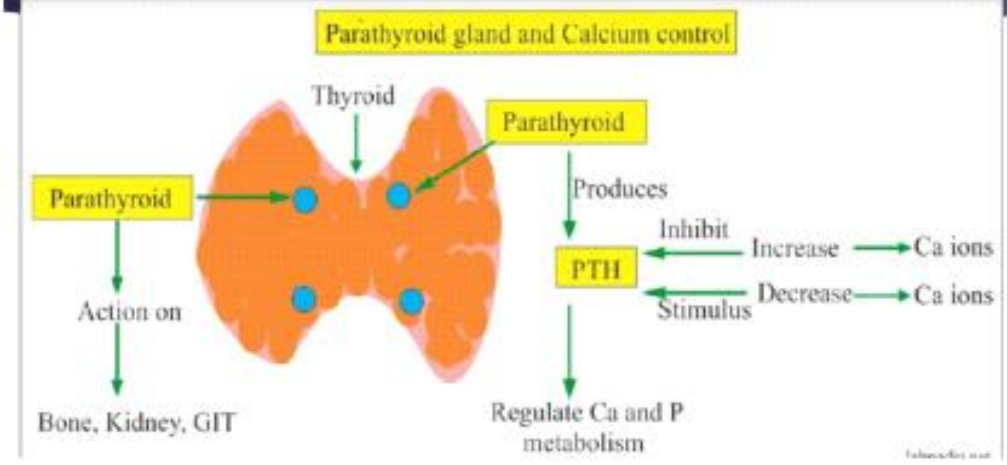


Difference between High (Ca^{2+}) and low (Ca^{2+})



Stimulus for PTH

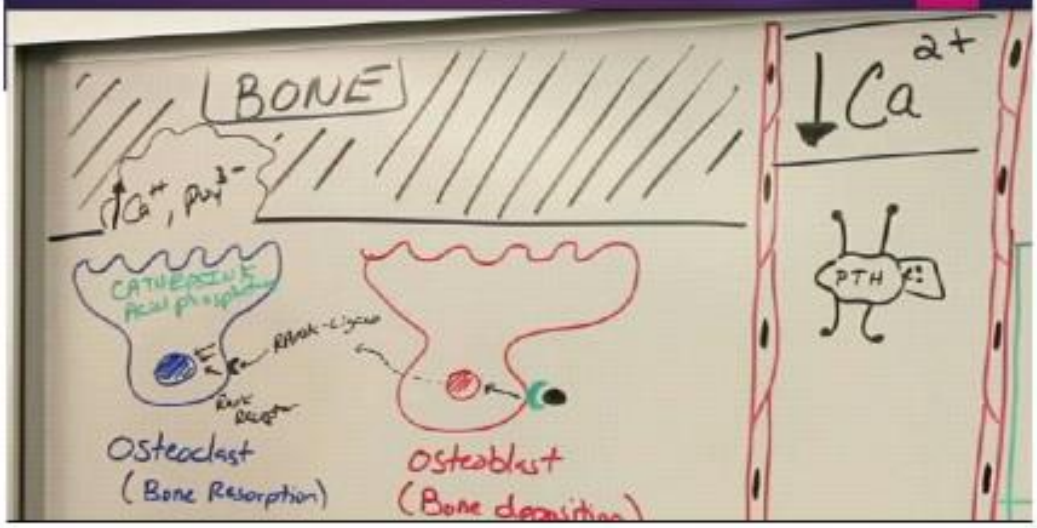
- **Low Ca levels**

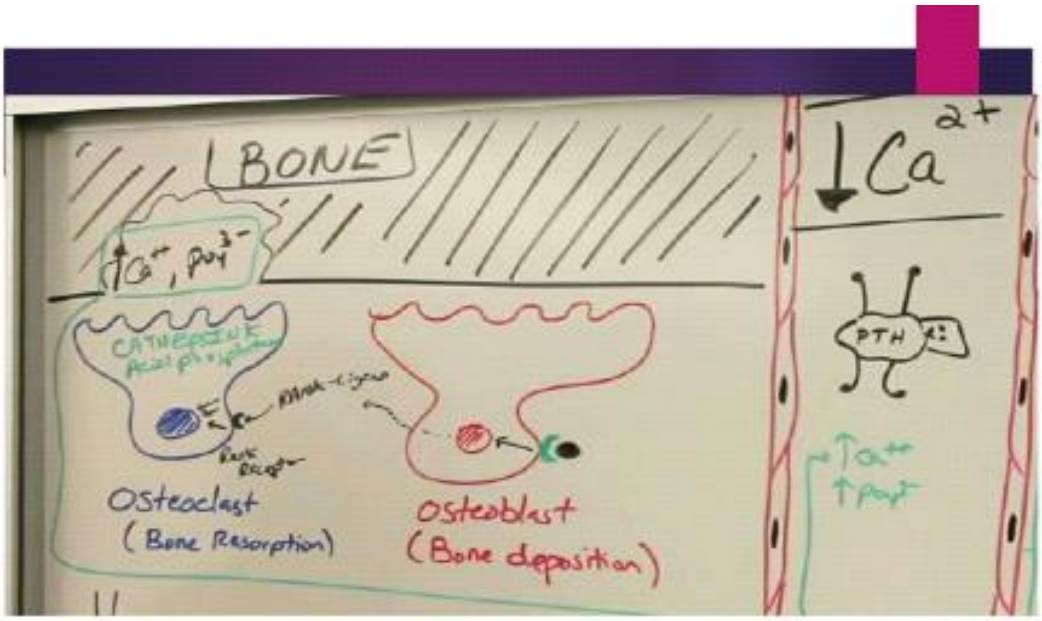


Functions of PTH

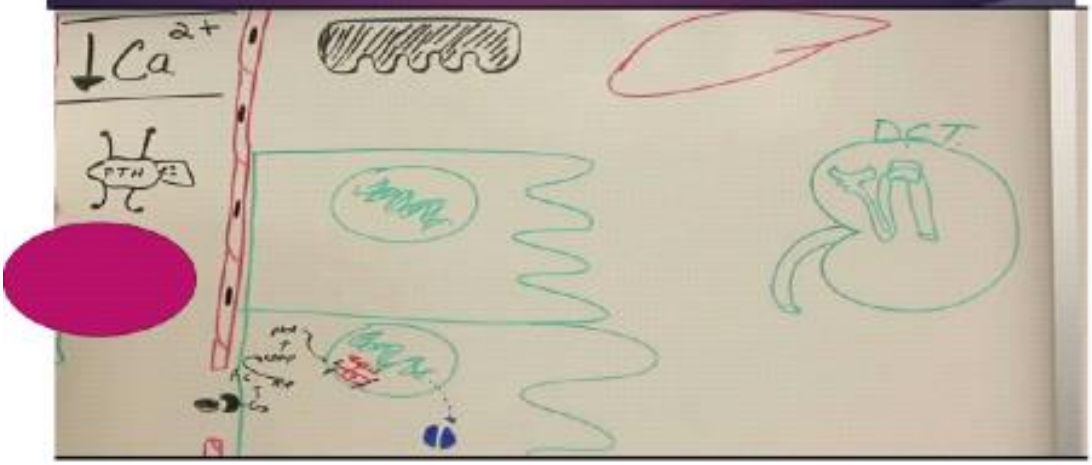
Bones, Kidneys ,Intestine

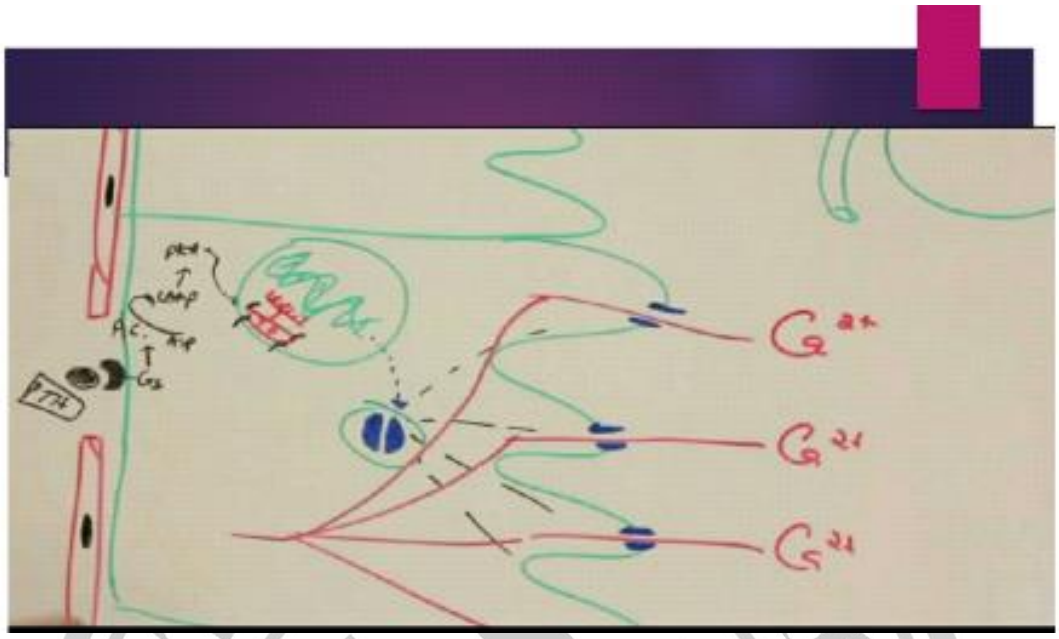
- ***Bones :*** parathyroid hormone stimulates the release of calcium from large calcium stores in the bones into the bloodstream. This increases bone destruction and decreases the formation of new bone.
- ***Kidneys :*** parathyroid hormone reduces loss of calcium in urine. Parathyroid hormone also stimulates the production of active vitamin d in the kidneys.
- ***Intestine:*** parathyroid hormone indirectly increases calcium absorption from food in the intestine, via its effects on vitamin D metabolism.

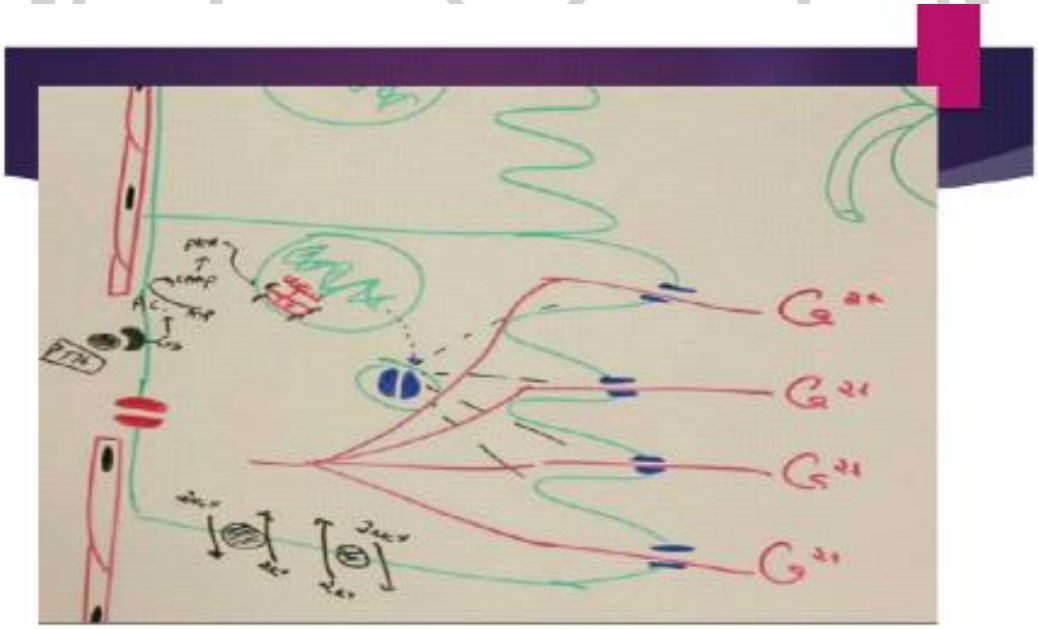


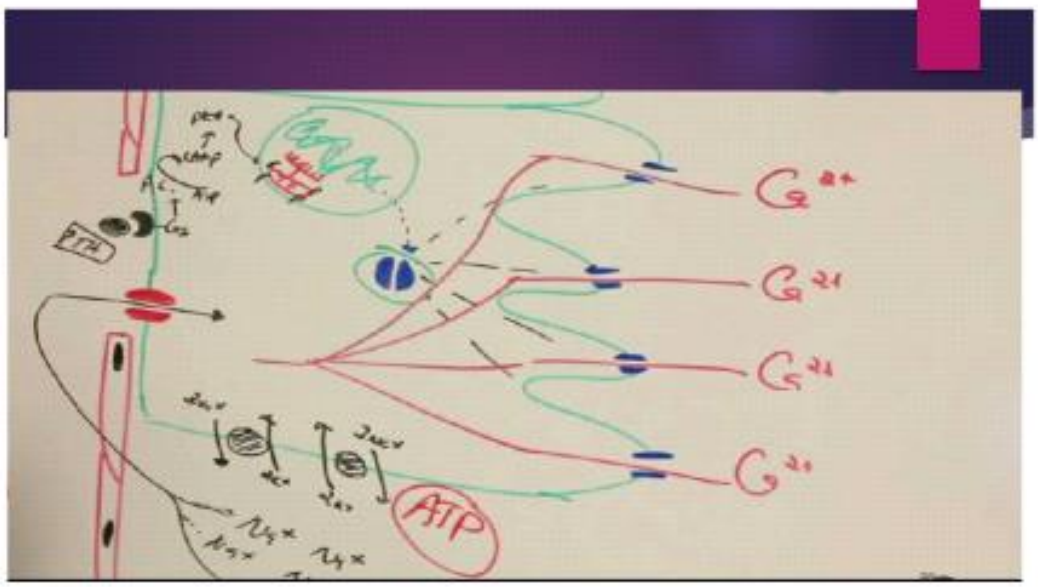

BONE
↓Ca 2+
PTH
Osteoclast
(Bone Resorption)
Osteoblast
(Bone deposition)

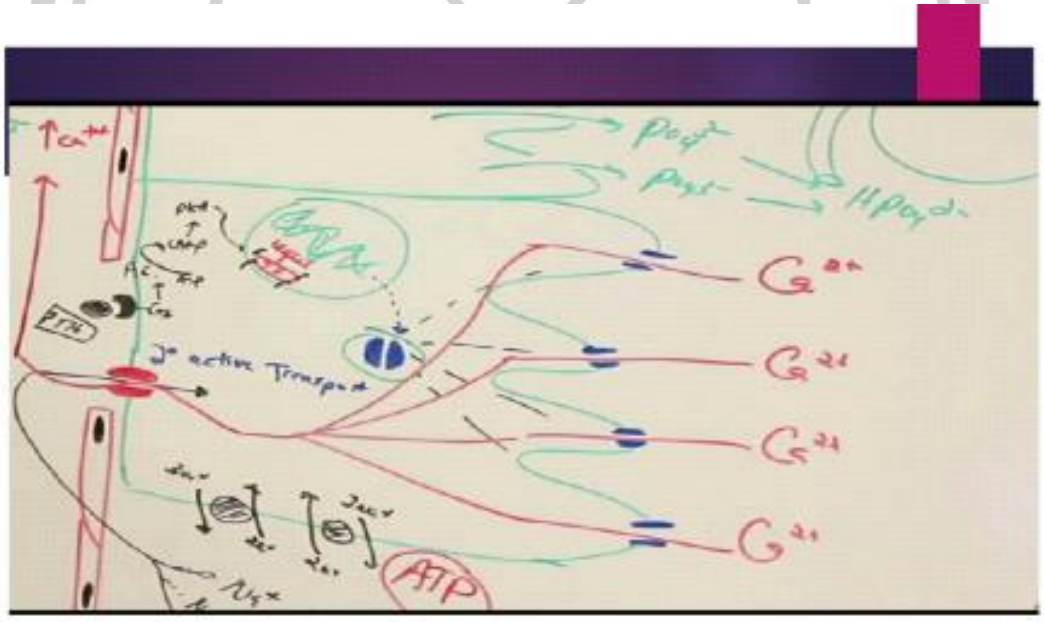
Kidney


↓Ca 2+
PTH
DCT

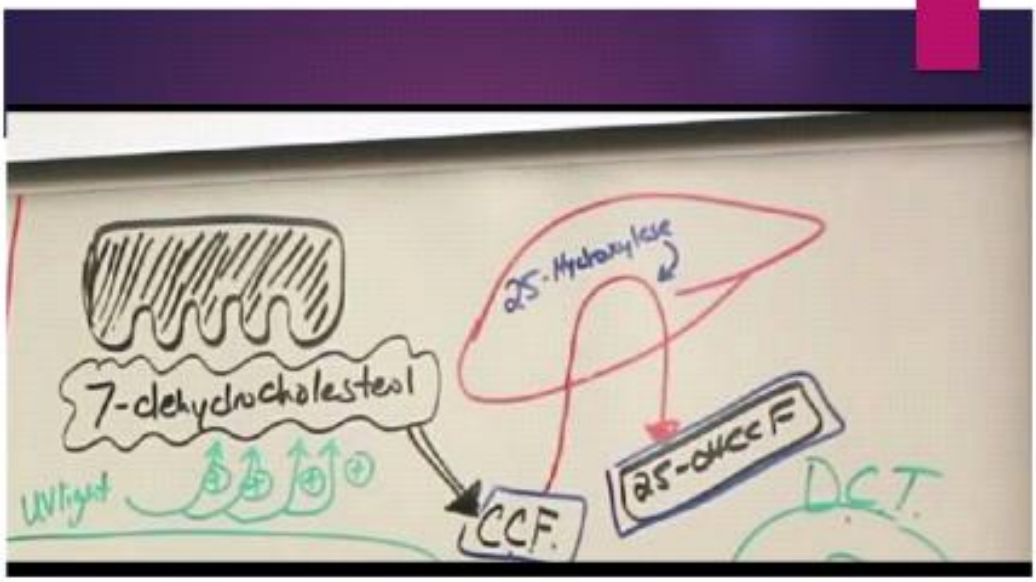

PTH
cAMP
AC
Gs
Gq
Gi
Gi

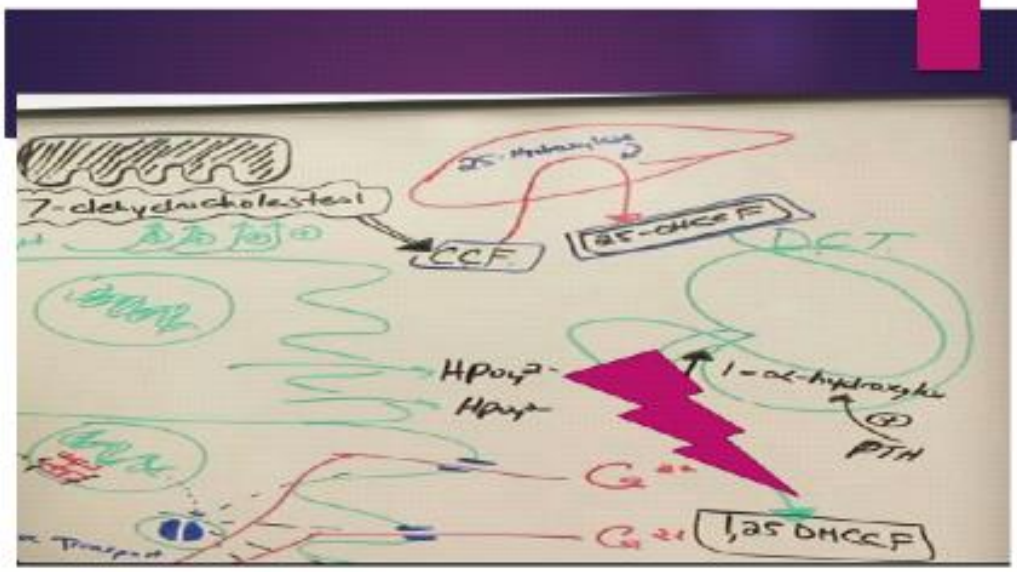

PTH
cAMP
AC
Gs
Gq
Gi
Gi

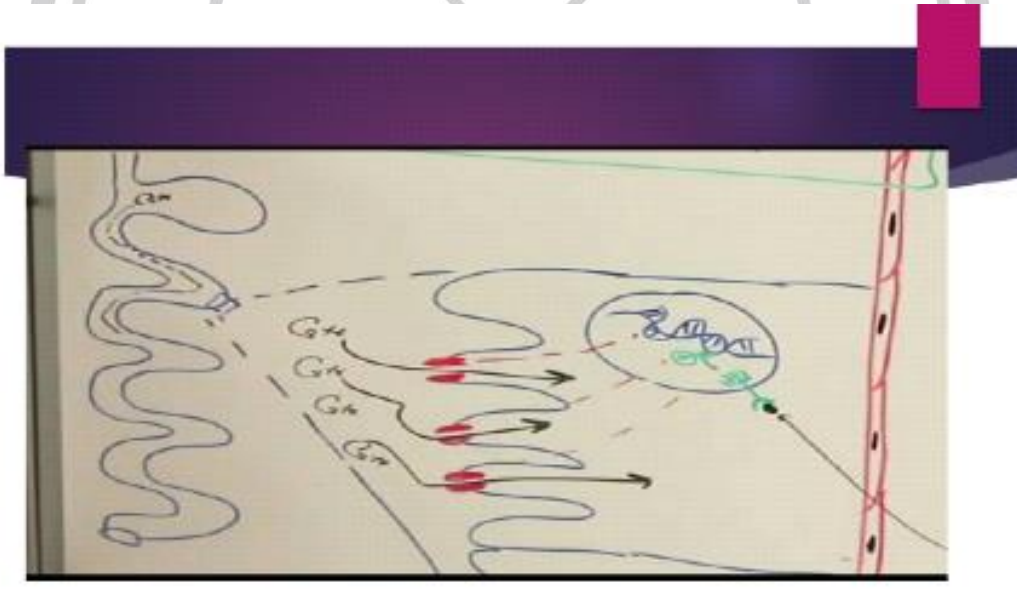

PTH
ATP


PTH
ATP

Indirect role via Vit D



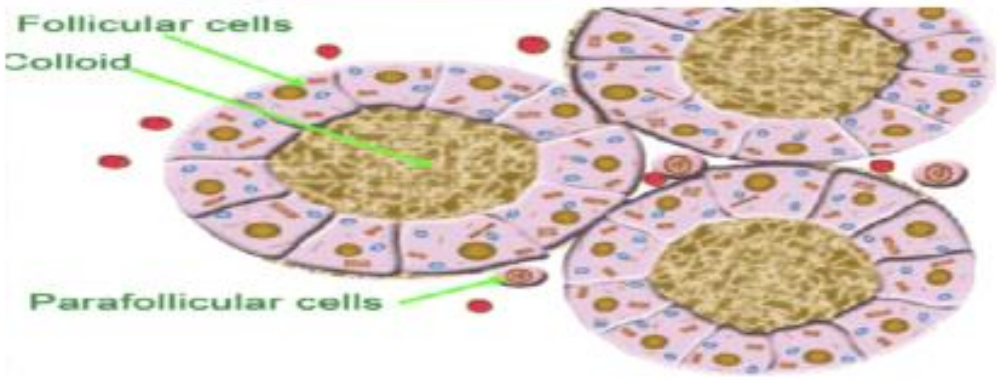

7-dehydrocholesterol
CCF
1,25 DHCCF
D.C.T.
PTH



Summary

- PTH increases blood calcium concentrations when calcium ion levels fall below normal. First, PTH enhances reabsorption of calcium by the kidneys; it then stimulates osteoclast activity and inhibits osteoblast activity. Finally, PTH stimulates synthesis and secretion of calcitriol by the kidneys, which enhances Ca2+ absorption by the digestive system.

2)Calcitonin



Stimulus

- High Ca levels

Role of Calcitonin in Ca homeostasis