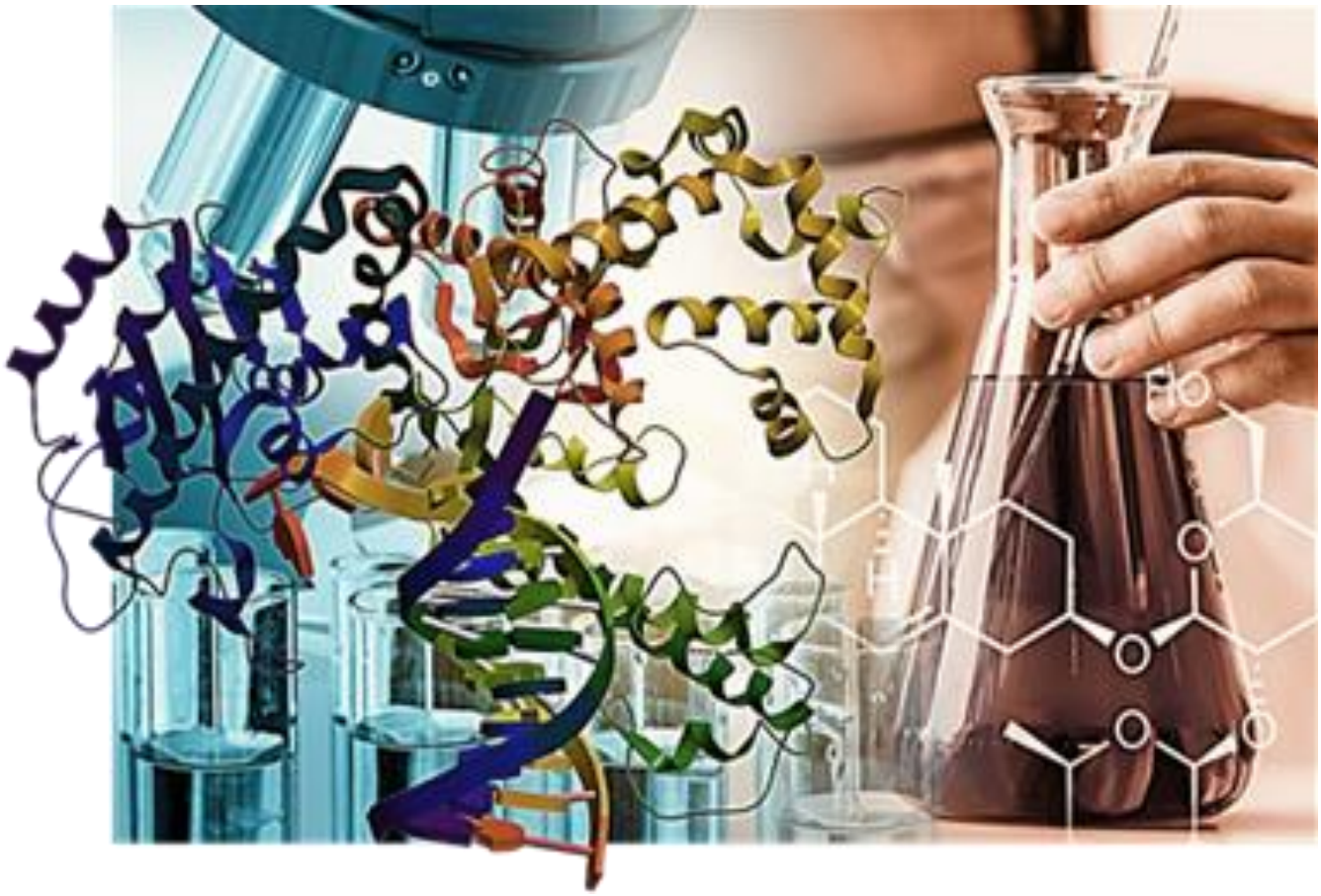


BIOCHEMISTRY- 1

FOR PHYSICAL THERAPY STUDENTS

By

Dr. Omayma Ashraf Hasan

Lecturer of medical biochemistry, Faculty of Medicine, SVU

2023-2024

List of contents:

Carbohydrate Chemistry

Definition:

Carbohydrates are **polyhydroxy aldehydes or ketones** or compounds which yield these on hydrolysis. The general molecular formula of carbohydrate is Cn(H2O)n. For example, glucose has the molecular formula C6H12O6.

Functions of Carbohydrates

1. Carbohydrates are the main sources of **energy** in the body. Brain cells and RBCs are almost wholly dependent on carbohydrates as the energy source.

2. Storage form of energy (starch and glycogen).

3. Excess carbohydrate is converted to fat.

4. Glycoproteins and glycolipids are components of cell membranes and receptors.

5. Structural basis of many organisms: Cellulose of plants; exoskeleton of insects, cell wall of microorganisms, mucopolysaccharides as ground substance

in higher organisms.

Classification:

According to number of sugar units:

I- **Monosaccharides**: have only one sugar unit are called (Greek, mono = one; saccharide = sugar). They cannot be further hydrolyzed into smaller units.

Monosaccharides are further classified according to:

a. **Type of the functional group**

Sugars having aldehyde group are called aldoses and sugars with keto group are ketoses and are sometimes (but not always) identified by the "**-ulose**" suffix. .

b. **Number of carbon atoms**

Depending on the number of carbon atoms, the monosaccharides are named as trioses (C3), tetroses (C4), pentoses (C5), hexoses (C6), heptose (C7) and so on.

Common monosaccharides:

No. of carbon atoms	*Generic name*	*Aldoses (with aldehyde group)*	*Ketoses (with keto group)*
3	Triose	Glyceraldehyde	Dihydroxy-acetone
4	Tetrose	Erythrose	Erythrulose
5	Pentose	Arabinose, Xylose, Ribose	Xylulose, Ribulose
6	Hexose	Glucose, Galactose, Mannose	Fructose
7	Heptose		Sedoheptulose

II. **Disaccharide**: when two monosaccharides are combined together with elimination of a water molecule.

III. **Oligosaccharides:** contain 3-10 monosaccharide units.

IV. **Polysaccharides:** when more than 10 sugar units are combined. (Greek, poly = many).

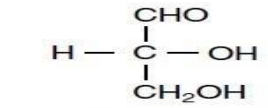
a. **Homopolysaccharides :** Polysaccharides having only one type of monosaccharide units.

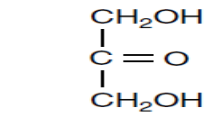
b. **Heteropolysaccharides:** those having different monosaccharide units**.**

Monosaccharides of biological importance

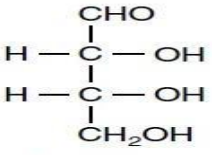
Trioses

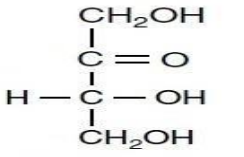
a**.** Aldotrioses: Glyceraldehyde "glycerose". b. Ketotrioses: Dihydroxyacetone.


D-Glycerose
(D-glyceraldehyde)

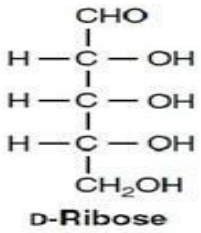

Dihydroxyacetone

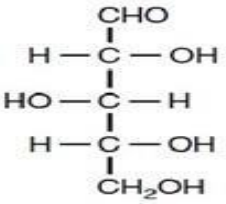
Tetroses:

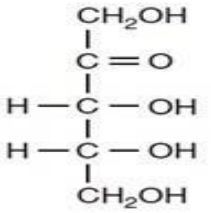

D-Erythrose

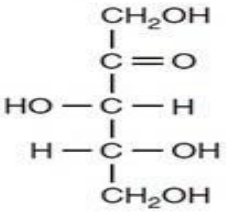

D-Erythrulose

Pentoses:


D-Ribose


D-Xylose

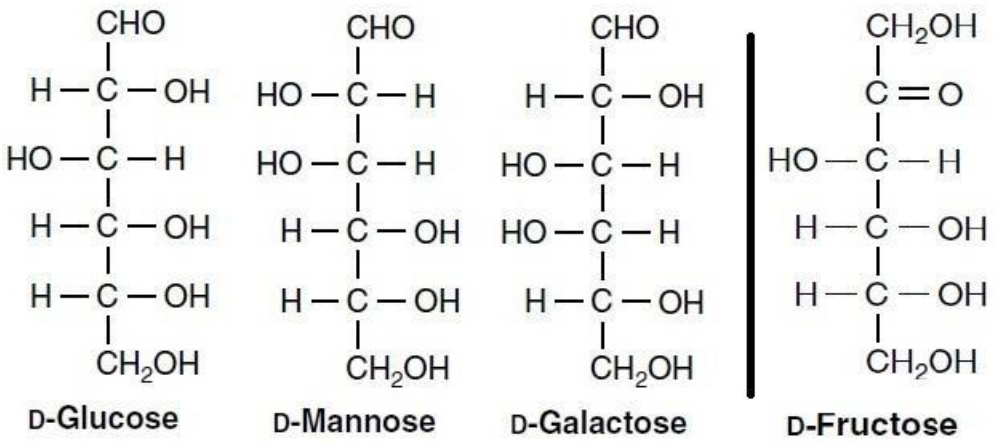

D-Ribulose


D-Xylulose

- Ribose has very important roles:

- It enters in the structure of nucleic acids (RNA and DNA.), and the biologically important nucleotides as ATP, GTP.AMP,…etc.
- Enters in the structure of coenzymes NAD, NADP and FAD.
  - **Ribulose** is an intermediate of HMP shunt pathway.
  - Xylose is seen in proteoglycans.
  - Xylulose is an intermediate of uronic acid pathway

Hexoses



Importance of glucose:

Found in fruits, fruit juices, hydrolysisof starch, maltose and lactose.

- It is the principal sugar of blood.
- Excess glucose level in the blood is called hyperglycemia and presence in urine (glucosuria) indicates diabetes mellitus
- Major source for cell energy specially neurons.
- Can be used in synthesis of other body carbohydrates e.g., glycogen, galactose, ribose, etc.

Importance of Fructose: “fruit sugar”:

Latin word for fruit —"fructus"

- Found in fruit juices, honey
- Released by the hydrolysis of inulin
- Main nutritional source of energy for the spermatozoa and is found in the seminal fluid
- Can be converted to glucose in the liver
- It is the sweetest sugar

Importance of Galactose:

- It can be converted into glucose in the liver.

- It is synthesized in mammary gland to make the lactose of milk (milk sugar)

- Enters in the structure of and glycoproteins.

Importance of **Mannose:** inters in the structure of many glycoproteins.

DERIVED CARBOHYRATES

Definition: They are derived from carbohydrates by various chemical reactions.

Types:

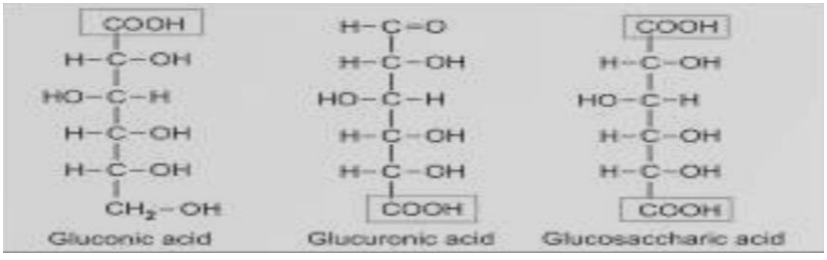
1) **Sugar acids:** formed by oxidation of sugars.

Examples, and biomedical importance:

a- Under mild oxidation conditions (hypobromous acid, Br2/H2O), the aldehyde group is oxidized to carboxyl group to produce aldonic acid. Thus, glucose is oxidized to gluconic acid, mannose to mannonic acid and galactose to galactonic acid.

b- Under moderate oxidation conditions, the last carbon becomes COOH group to produce **uronic acid**. Thus glucose is oxidized to glucuronic acid, mannose to mannuronic acid and galactose to galacturonic acid. The **glucuronic acid** is used by the body for conjugation with insoluble molecules to make them soluble in water for detoxification purpose and also for synthesis of heteropolysaccharides.

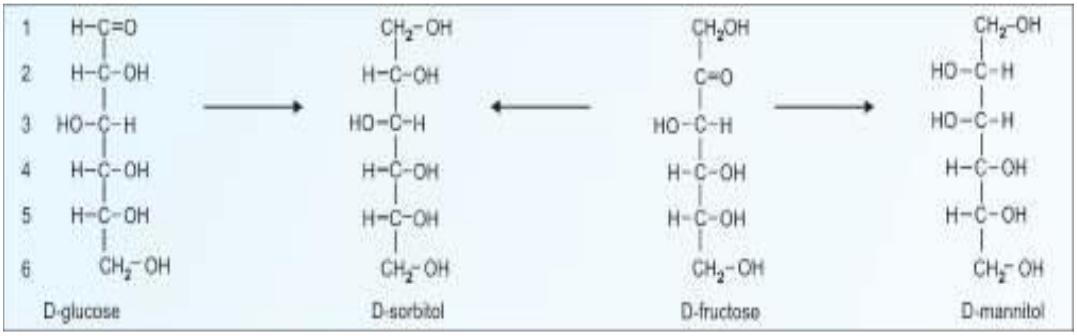
c- Under strong oxidation conditions (nitric acid + heat), the first and last carbon atoms are simultaneously oxidized to form dicarboxylic acids, known as saccharic acids (Fig. 6.12). Glucose is thus oxidized to glucosaccharic acid, mannose to mannaric acid and galactose to mucic acid. The mucic acid forms insoluble crystals, and is the basis for a test for identification of galactose.



2) **Sugar alcohols:** They results from reduction of sugars by reducing agents such as sodium amalgam. Aldose yields corresponding alcohol. But ketose forms two alcohols, because of appearance of a new asymmetric carbon atom in this process.

Examples, and biomedical importance

- Glucose is reduced to sorbitol; mannose to mannitol while fructose becomes sorbitol and mannitol. Galactose is reduced to dulcitol and ribose to ribitol. Sorbitol, mannitol and dulcitol are used to identify bacterial colonies. Mannitol is also used to reduce intracranial tension by forced diuresis. The osmotic effect of sorbitol and dulcitol produces changes in tissues when they accumulate in abnormal amounts,e.g. cataract of lens. Ribitol enters in the structure of riboflavin (vitamin B2).



3) Glycosides:

They are formed when the hydroxyl group of anomeric carbon of monosaccharide (carbon 1 in aldoses or carbon 2 in ketoses) condenses with another compound with a glycosidic linkage. The other compound may be may be:

a) Another monosaccharide to form disaccharide glycosides as maltose, lactose and sucrose.

b) Aglycone i.e. non-carbohydrate to form glycoside.

Examples

1. Sugar nucleotide as ATP, GTP and other nucleotides: aglycone here is purines and pyrimidines.
2. Cardiac glycosides: Aglycone here is steroid. Cardiac glycosides such as digitalis are used in treatment of cardiac disease.
3. Glycollplds: as cerebrosides.
4. Glycoprotein.
5. Disacharides

4) Formation of Esters

Hydroxyl groups of sugars can be esterified to form acetates, propionates, benzoates, phosphates, etc.

Sugar phosphates are of great biological importance. Metabolism of sugars inside the body starts with phosphorylation. Glucose-6-phosphate and glucose-1-phosphate are important intermediates of glucose metabolism.

5) Amino Sugars

Amino groups may be substituted for hydroxyl groups of sugars to give rise to amino sugars. Generally, the amino group is added to the second carbon atom of hexoses. Amino sugars enter in glycoproteins.

Examples and importance:

Glucosamine is seen in hyaluronic acid, heparin and blood group substances. Galactosamine is present in chondroitin of cartilage, bone and tendons.

Mannosamine is a constituent of glycoproteins.

6) Deoxy sugars

Oxygen of the hydroxyl group may be removed to form deoxy sugars.

Deoxy sugars will not reduce and will not form osazones.

Examples

- L-fucose (6-deoxyL-galactose) is present in blood group antigen and many other glycoproteins.
- Deoxyribose is an important part of nucleic acid.

Disaccharides

When two monosaccharides are combined together by glycosidic linkage, a disaccharide is formed.

The important disaccharides are:

1. Sucrose

It is the sweetening agent known as cane sugar. It is present in sugarcane and various fruits. Sucrose contains glucose and fructose. Sucrose is **not a reducing sugar**; and it will not form osazone. This is because the linkage involves first carbon of glucose and second carbon of fructose, and free reducing groups are not available.

Hydrolysis of sucrose (optical rotation +66.5°) will produce one molecule of glucose (+52.5°) and one molecule of fructose (–92°). Therefore, the products

will change the dextrorotation to levorotation, or the plane of rotation is inverted. Equimolecular mixture of glucose and fructose thus formed is called **invert sugar**. The enzyme producing hydrolysis of sucrose is called **sucrase or invertase.** Honey contains invert sugar. Invert sugar is sweeter than sucrose.

2. **Lactose**
   a) It is the sugar present in milk. On hydrolysis lactose yields glucose and galactose. Lactose consists of glucose and galactose linked by 1 - 4 galactosidic linkage. It is a reducing disaccharide. It can show mutarotation. And it can form characteristic osazone crystals
3. **Maltose: Also called malt sugar:**

It Is formed of 2 molecules of glucose linked together by a 1 - 4 glycosidic bond. Maltose is produced during digestion of starch and glycogen by amylase enzyme. It is a reducing agent (can reduce Benedict's reagent). It can show mutarotation. It can form characteristic osazone crystals.

4. **Isomaltose**

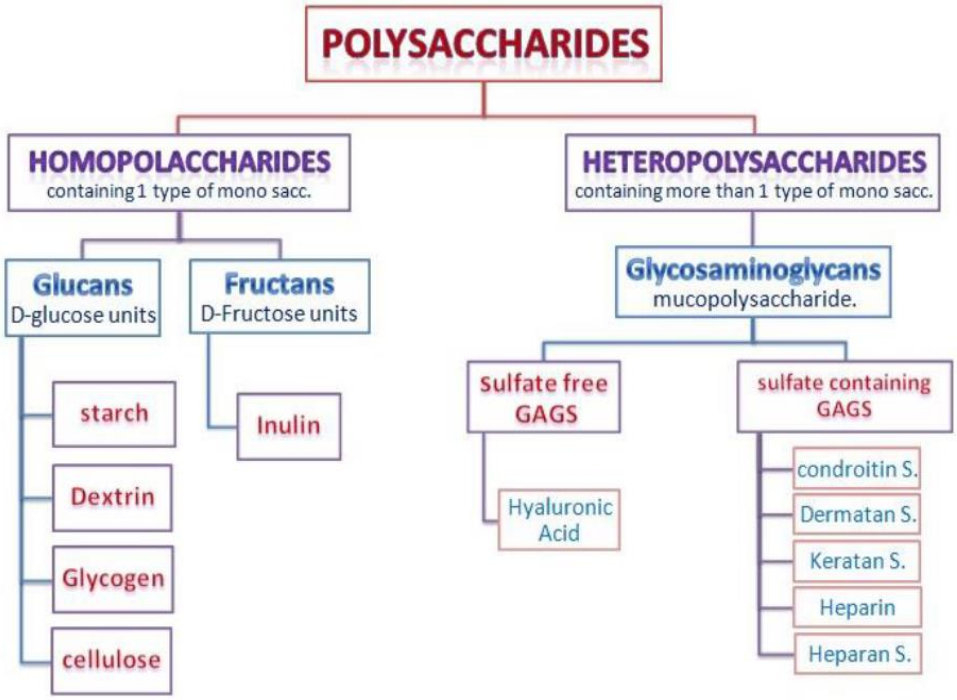
It is formed of 2 molecules of glucose linked together by a 1 - 6 glycosidic bond. Isomaltose is produced during digestion of starch and glycogen by amylase enzyme. It is a reducing agent (can reduce Benedict's reagent). It can show mutarotation. It can form characteristic osazone crystals.

Polysaccharides

Definition: carbohydrates, formed of more than 10 sugar units.

Classification:

A. Homopolysaccharides: They contain repeated sugar units and include: starch, dextrins, glycogen, cellulose, inulin and dextrans.
B. Heteropolysaccharides: They contain repeated different sugar units and include glycosaminoglycans



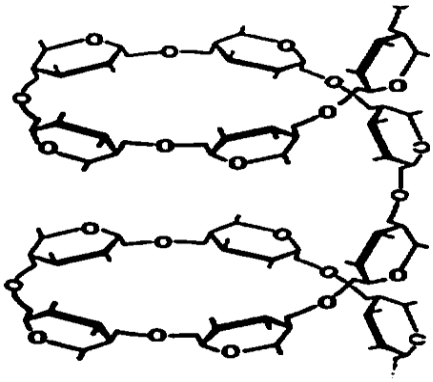
A) Homopolysaccharides:

1. Starch (also called glucosan or glucan):

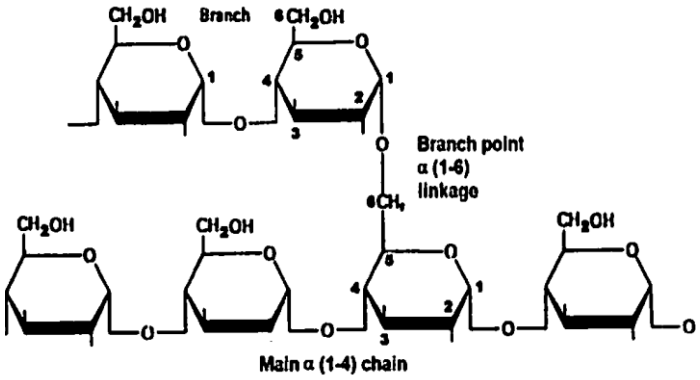
a) Structure: Starch granule is formed of inner (amylose) and outer (amylopectin) layers:

1) Inner layer: called amylose. It constitutes 15-20% of the granule and formed of non-branching helical structure of glucose units linked together by α 1 - 4 glycosidic bond.

2) Outer layer: called amylopectin. It constitutes 80-85% of the granule and formed of branched chain. Each chain is composed of 24-30 glucose units linked together by α 1 - 4 glycosidic bond and α1 - 6 glycosidic bond at the branching points.



Inner layer of starch (amylose).



Outer branching layer of starch amylopectin

b) Sources: cereals, potatoes, legumes and other vegetables.

c) Functions: starch is the main carbohydrate content in our diet.

d) Properties:

1) Starch gives blue color with Iodine. Amylopectin gives red color with Iodine.

2) Partial hydrolysis (digestion) by amylase enzyme gives variousforms of dextrins.

2. Dextrlns: These are hydrolytic products of starch. They are formed of glucose units but simpler than starch. They include amylodextrin, erythrodextrin and achrodextrin. They give red color with Iodine.

3. Glycogen: (also called animal starch):

a) Structure: similar to amylopectin of starch

I) Glycogen is a bomopolysaccharide formed of branched chains. Each branch is made of 12-14 glucose units linked together by α 1 - 4 glycosidic bond and α1 - 6 glycosidic bond at the branching points.

b) Location: Glycogen Is present mainly in liver and muscles.

c) Functions of glycogen:

1) Liver glycogen: It maintains normal blood glucose concentration especially during the early stage of fast (between meals). After 12-18 hours fasting, liver glycogen is depleted.

2) Muscle glycogen: It acts as a source of energy within the muscle Itself especially during muscle contractions.

d) Properties: It gives reddish violet color with iodine.

4. Cellulose:

a) Structure: It Is long straight nonbranching chains of β-glucose units linked together by β1-4 glycosidic bond.

b) Sources: Cellulose is the chief structural carbohydrate of the framework of plants as leafy vegetables, fruits, wood, cotton, etc.

d) Importance:

1) The presence of cellulose in diet is important because it increases the bulk of stool. This stimulates intestinal movement and prevents constipation.

2) Cellulose Is a constituent of dietary fibers. These fibers help in decreasing absorption of toxic compounds and reduce the incidence of cancer colon.

5. Dextrans;

a. **Structure:** It is a branched chain homopolysaccharide. Each branch is formed of glucose units, linked together by α 1-3 glycosidic bonds and by α 1-6 glycosidic bond at branching point.

b. **Sources:** dextran is synthesized from sucrose by certain bacteria.

c**. Functions**: Dextran is used as plasma substitute and prevents thrombosis.

6. Inulin:

a) Structure: It is a fructosan i.e. formed of repeated units of fructose linked together by β 1-2 bonds.

b) Sources: Onions, and roots of some plants.

c) Medical Importance: Inulin clearance is one of diagnostic tests for investigating glomerular filtration rate.

HOMOPOLYSACCHARIDES				
GLUCANS	Starch	Starch granule is formed of: 1) **Inner layer:** called **amylose. It** constitutes 15-20% of the granule and formed of non-branching helical structure of glucose units linked together by α 1 — 4 glycosidic bond. 2) **Outer layer:** called **amylopectin** constitutes 80-85% of the granule and formed of branched chain. Each chain is composed of 24-30 glucose units linked together by α 1 — 4 glycosidic bond and a 1 — 6 glycosidic bond at branching points.	- cereals, potatoes, legumes and other vegetables - In plants it synthesized by photosynthesis.	1) Starch gives blue color with iodine. Amylopectin gives red color with iodine. 2) Partial hydrolysis (digestion) by amylase enzyme gives various forms of dextrins
	Dextrins	- amylodextrin, erythrodextrin and achrodextrin.	- By hydrolysis of starch.	- They give red color with iodine.
	Glycogen	- Highly branched chain - Each branch is composed of 12-14 glucose units. - Similar to amylopectin	- The storage form of CHO in human and animals - in liver, muscles	- gives reddish violet color with iodine
	Cellulose	- long linear chains of (β-D-glucopyranose) linked together by β 1-4 glycosidic bond - **The presence of cellulose in diet is important because it:** - increases the bulk of stool. - This stimulates intestinal movement and prevents constipation.	- plants: vegetables, cotton	- give NO color with iodine - insoluble in water - **Cannot** be digested due to absence of digestive hydrolase enzyme that attacks β-linkage.
Fructans	Inulin	- Repeated units of fructose linked together by β1-2 bonds.	- Root of artichokes and other plants.	- Inulin clearance is one of diagnostic tests for investigation of GFR.

B) Heteropolysaccharides
Glycosamlnoglycans, GAGs (mucopolysaccharides):

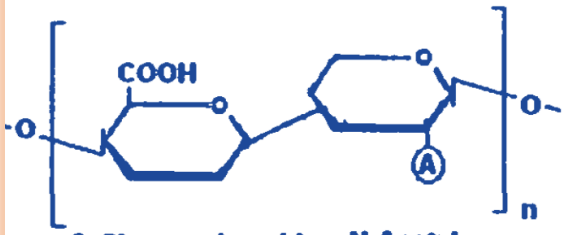
Definition: Mucopolysaccharides or **glycosamino glycans** (GAG) are heteropolysaccharides, containing uronic acid and amino sugars.

General features:

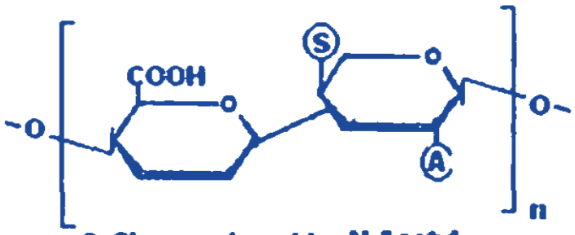
- They are formed of repeating disaccharide units (acidic sugar-amino sugar)n·
  a) The acidic sugar is either glucuronic acid or its eplmer, L-iduronlc acid.

b) The amino sugar is either glucosamine or galactosamlne in which the amino group is usually acetylated. The amino sugar may also be sulfated at carbon 4 or 6.

- The uronic acid and sulfate residues cause them to be very negatively charged.
- Because of the presence of these charged groups, they attract water molecules and so they produce viscous solutions. So, they can act as lubricants and cushion for other tissues.
- Most of GAGs are present extracellularly except heparin.
- Most of them form the structural components of connective tissue such as bone, elastin and collagen. Also, they are present in synovial fluid and the vitreous humor of the eye.
- Mucopolysaccharides in combination with proteins form mucoproteins.
- Mucopolysaccharides are excreted in urine in abnormal amounts in the group of lysosomal storage disorders known as **mucopolysaccharidoses**.
- Examples of mucopolysaccharides are hyaluronic acid, heparin, chondroitin sulfate, dermatan sulfate and keratan sulfate.



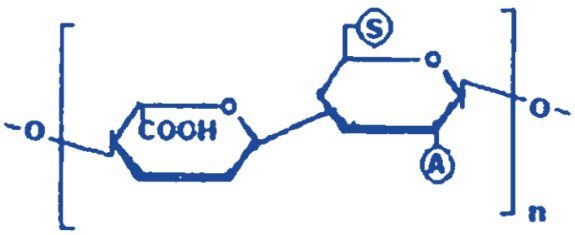
Hyaluronic acid



Chondroitin sulfate

Keratan sulfate

Heparin



Dermatan sulfate

Type	Structure	Site	Functions
Hyaluronic acid	Glucuronic acid	Cartilage	lubricant in joints
		Synovial fluid	makes cartilage **compressible**
	N-acetyl glucosamine NO Sulfate	Connective tissue	cell migration during **wound repair**
		Vitreous humor of the eye	cell migration during **morphogenesis**
Chondroitin 4- and 6 sulfate	Glucuronic acid	sage, tendons, ligaments and bones	Have role in compressibility of cartilage in weight bearing
	N-acetylgalactosamine with sulfate on either C4 or C6	Aorta, skin, cornea, umblical cord and in certain neurons	it binds collagen and hold fibers in strong network
Keratan sulfate	Galactose (no uronic acid), with sulfate on C6	Cornea	corneal transparency
	N-acetyl glucosamine with sulfate on C6	Found in Cartilage	
Dramatan sulfate	L-Induronic acid	Cornea	corneal transparency
	N-acetylgalactosamine with sulfate on C6	Sclera. Skin, blood vessels and heart valves	Maintaining the shape of the eye.
Heparin	Induronic acid with sulfate on C2	mast cells (intracellular compound) in the wall of blood vessels	anticoagulant
	Glucosamine with sulfate on C2 and C6		
Heparan sulfate		cell membrane	- act as receptors - cell adhesion and cell-cell interaction
		basement membrane of the kidney	Determining the charge selectiveness of glomerular filtration.

Conjugated Carbohydrates

They include:

1- Glycolipids: see lipids chemistry.

2- proteoglycans and Glycoproteins.

3- Fibronectin .

4- Laminin.

I. Proteoglycans and Glycoproteins:

Where the carbohydrate chains are attached to a polypeptide chain. If the carbohydrate content is less than 10%, it is generally named as a glycoprotein. If the carbohydrate content is more than 10% it is a mucoprotein. (But some authors use these words as synonyms).

A. Proteoglycans:

These are chains of glycosaminoglycans e.g. hyaluronic acid, chondroitin sulfate, keratan sulfate, dermatan sulfate, heparin and heparan sulfate attached to protein molecule. They serve as a ground substance and associated with structure elements of tissues as bone. elastin and cartilage (see functions of glycosaminoglycans) . The carbohydrate part is presented in very long unbranched chains (more than 50 monosaccharide molecules) attached to protein core .

B. Glycoproteins (mucoproteins):

1. **Structure:** They consists of:

a) Protein core.

b) Carbohydrate chains which are branched short chain(from 2-15 monosaccharide units) such chains are usually called oligosaccharide chains. They include :

1) Hexoses: Galactose and mannose.

2) Acetylhexosamines: Nacetylglucosamine

3) Pentoses: Arabinoe and xylose .

4) Methyl pentose: L-fucose.

5) Sialic acid .

6) They contain **no** uronic acids or sulfate groups.

2. **Functions :**

a) Glycoproteins are components of extracellular matrix.

b) They are components of mucins of gastrointestinal and urogental tracts, where they act as protective biologic lubricants.

c) Glycoproteins are components of cell membrane as:

1) Blood group antigens (A, B, AB).

2) Cell surface receptors: e.g. for hormones.

3) Glycophorin: It is glycoprotein present in human red cell membrane. It prolonged the life span of the lipid membrane.

d) Plasma proteins: present in plasma are glycoproteins.

e) Most enzymes and protein hormones glycoproteins

	Glycoproteins	proteoglycans
Definition	Are proteins that contain oligosaccharide chains.	chains of glycosaminoglycans attached to protein molecule
1- Structure CHO component	Oligosaccharide units.	Glycosaminoglycans.
Ptn component	Protein core	Protein
Types of sugar	Contain no uronic acid	Contain uronic acid
	Pentoses: as arabinose and xylose.	Sugaramines as glucosamines.
	Methylpentoses: L-fucose	
Sulfate group	Contain no sulfate	Contain sulfate.
Size of CHO component	2-15 units.	More than 50 units.
Repeating structure	Little or non.	Repeating disaccharides.
Shape	Usually branched	Linear, unbranched.
2- Function	- Extracellular matrix. - Mucin. - Blood group antigens e.g. A, B and AB. - Cell receptors. - Glycophorins. - Plasma proteins. - Some hormones. - Enzymes. - Antibodies.	- ground substance and support tissues as cartilage, bone and tendons - cell membrane

Chemistry of Lipids

Lipids may be **defined as** compounds which are relatively insoluble in water, but freely soluble in non-polar organic solvents, such as benzene, chloroform, ether, hot alcohol, acetone, etc.

Fatty acids: R.COOH

- Fatty acids are water-insoluble "long chain hydrocarbons".
- They are mostly monocarboxylic i.e. having one carboxyl group at the end of the chain (-COOH).
- They are mostly aliphatic (i.e. not branched). A few branched chain fatty acids arepresent in animals and plants.
- Fatty acids may be Saturated: (no double bonds) or Unsaturated: (containing one or more double bonds).
- Fatty acids may be Essential: cannot be synthesized in the body or Nonessential: can be synthesized in the body.
- Fatty acids occur mainly as esters in natural fats and oils.
- Fatty acids may also present as free fatty acids (FFA) in the plasma carried on PP.
- Short chain F.A : less than 10 C , long chain F.A: more than 10 C.

Polyunsaturated fatty acids (PUFA, essential fatty acids)

Definition: They are fatty acids containing **more than one** double bond.

- In polyunsaturated F.A. each 2 double bonds are separated by methylene group **(-CH2)**

Classification:

- **PUFA** are classified according to the position of the 1**st** double bond in relation to **ω** carbon into ω3, ω6, ω7 & ω9 F.A.

▪ ω3 PUFA:

PUFA having the 1st double bond at carbon **3** in relation to ω carbon

Examples:

- α Linolenic acid (18:3) —> Parent FA, is the precursor of other members of this group in the body
- Cervonic acid (22 : 6)

- Clupanodonlc acid: 22:5. It is present in fish oils. It is a component of phospholipids in brain.

▪ ω6 PUFA:

1. Linoleic acid (**18:2** Δ9'12, ω6) —> Parent FA
2. γ Linolenic acid (18:3)
3. Arachidonic acid (**20:4** Δ5'8'11'14, ω6). It is present in peanut oil. It is a component of phospholipids in animal. It is a precursor of **eicosanoids.**

Essential and nonessential fatty acids:

A. Nonessential fatty acids:

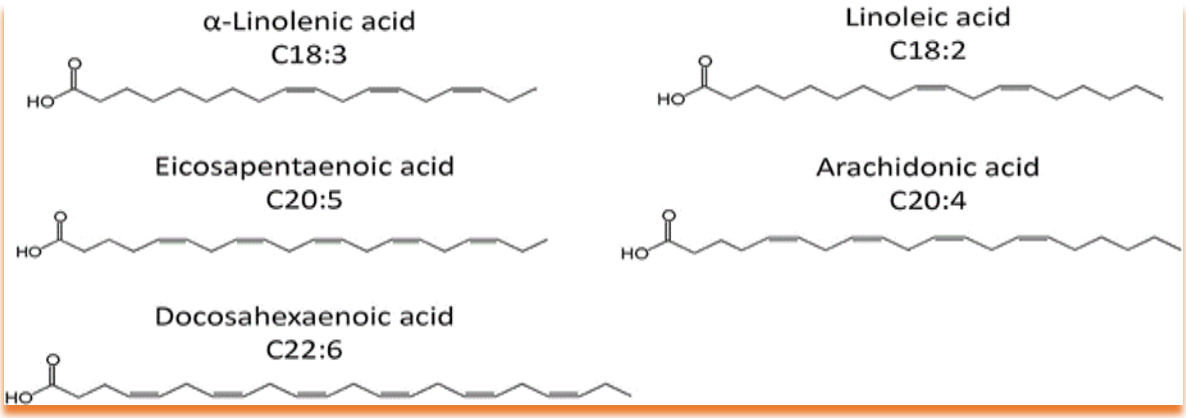
1. These are fatty acids which can be synthesized in the body. Thus, they are not necessary to be obtained from the diet.
2. They include all saturated and monounsaturated fatty acids as palmitoleic and oleic acid.
3. They can be synthesized from acetyl COA (active acetate) derived from glucose oxidation.

B. Essential fatty acids:

a) These are fatty acids that cannot be synthesized in the body. They must be obtained from the diet.

b) They include fatty acids that contain more than one double bond (polyunsaturated fatty acids) e.g. α -lenoleic, lenolenic, arachidonic acids.

c) The human body has enzyme system that can form only one double bond at the ninth carbon atom.



Sources:

a) Vegetable oils e.g. corn oil, soya bean oil, safflower oils, sunflower, linseed oil and cotton seed oil.

b) Fish oils: shark liver oils, which particularly contain the ω**3** polyunsaturated fatty acids.

Importance:

a) Normal growth.

b) They enter in the structure of phospholipids and cholesterol esters.

c) They enter in the structure of cell membranes and are required for the fluidity of membrane structure.

d) They protect against atherosclerosis and coronary heart disease by decreasing free cholesterol and LDL.

e) Arachidonic acid (20C) is a precursor of a group of eicosanoid

EICOSANOIDS

Definition: They are 20 C compounds (Greek, eikosi = twenty), derived from arachidonic acid.

Types:

1. Prostanoids, containing:

a. Prostaglandins (PGs);

b. Prostacyclins (PGIs);

c. Thromboxanes (TXs)

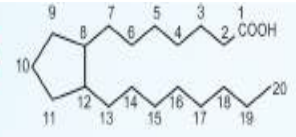
2. Leukotrienes (LTs)

1. Prostanoids

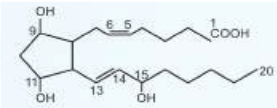
a. Prostaglandins (PG): PGs were originally isolated from prostate tissue and hence the name. But they are present in almost all tissues. They are the most potent biologically active substances

Chemical Structure

All prostaglandins are considered to be derived from the 20 C cyclic saturated fatty acid, prostanoic acid. The five-carbon ring is saturated. All naturally occurring PGs have an OH group at C15.



Prostanoic acid



Prostanglandin-F2

Classification of Prostaglandins

According to the attachment of different substituent groups to the ring, PGs are named with capital letters such as A, B, E and F. PGF is designated as alpha to denote the projection of the OH group in naturally occurring prostaglandins.

In the same series, depending on number of double bonds on the side chains they are denoted by a subscript after the capital letter, e.g. PGE1, PGE2, PGE3, etc.

1. Series 1 contains 1 double bond at 13–14.
2. **Series 2** have 2 double bonds at 13–14 (trans) and 5–6. This is the most common variety.
3. Series 3 have 3 double bonds, 13–14 , 5–6 and 17–18.

Biosynthesis of Prostaglandins

- Prostaglandins are derived from the PUFA, the three series being derived from the following fatty acids.

1 series (1 double bond)—from Linoleic acid
2 series (2 double bonds)—from Arachidonic acid
3 series (3 double bonds)—Eicosa penta-enoic

- Naturally occurring PGs belong to the 2 series.
- PGs are not stored as such; the precursor fatty acids are stored in membrane as phospholipids. The arachidonic acid is released by the action of **phospholipase A2**on phospholipids (Fig.14.3).
- Synthesis is catalyzed by prostaglandin H synthase

(PGHS). It contains two separate enzyme activities, **cyclo-oxygenase** and peroxidase.

- PGG2 and PGH2 are formed as intermediates during the synthesis of other PGs. Specific enzymes convert PGH2 to other prostaglandins.

Regulation of Synthesis

- The **phospholipase** (PL) is activated by epinephrine, thrombin, angiotensin II, bradykinin and vasopressin. Steroids inhibit PL and prevent release of arachidonic acid from membranes.

- **Cyclo-oxygenase** is activated by catecholamines and inhibited by non-steroid anti-inflammatory drugs (NSAIDs). **Aspirin** acetylates serine at the active site and irreversibly inhibits the cyclo-oxygenase.
- Cyclo-oxygenase is a "**suicide**" **enzyme**, self catalyzed destruction rapidly inactivates the enzyme.

Functions

1. PGs in general inhibit gastric secretion and increase intestinal motility. The inhibitory effect on gastric secretion is used therapeutically in treatment of acid peptic disease.
2. The PGF2 stimulates the uterine muscles. Hence PGF2 may be used for medical termination of pregnancy. Yet another use is in inducing labor and arresting postpartum hemorrhage.
3. The PGF is a constrictor of bronchial smooth muscle; but PGE is a potent bronchodilator. PGE series are used in aerosols for relieving bronchospasm.
4. The PGE2 and D2 produce inflammation by increasing capillary permeability. Erythema and wheal are produced at the site of injury. aspirin; cortisol have anti-inflammatory effect through inhibition of PG synthesis.

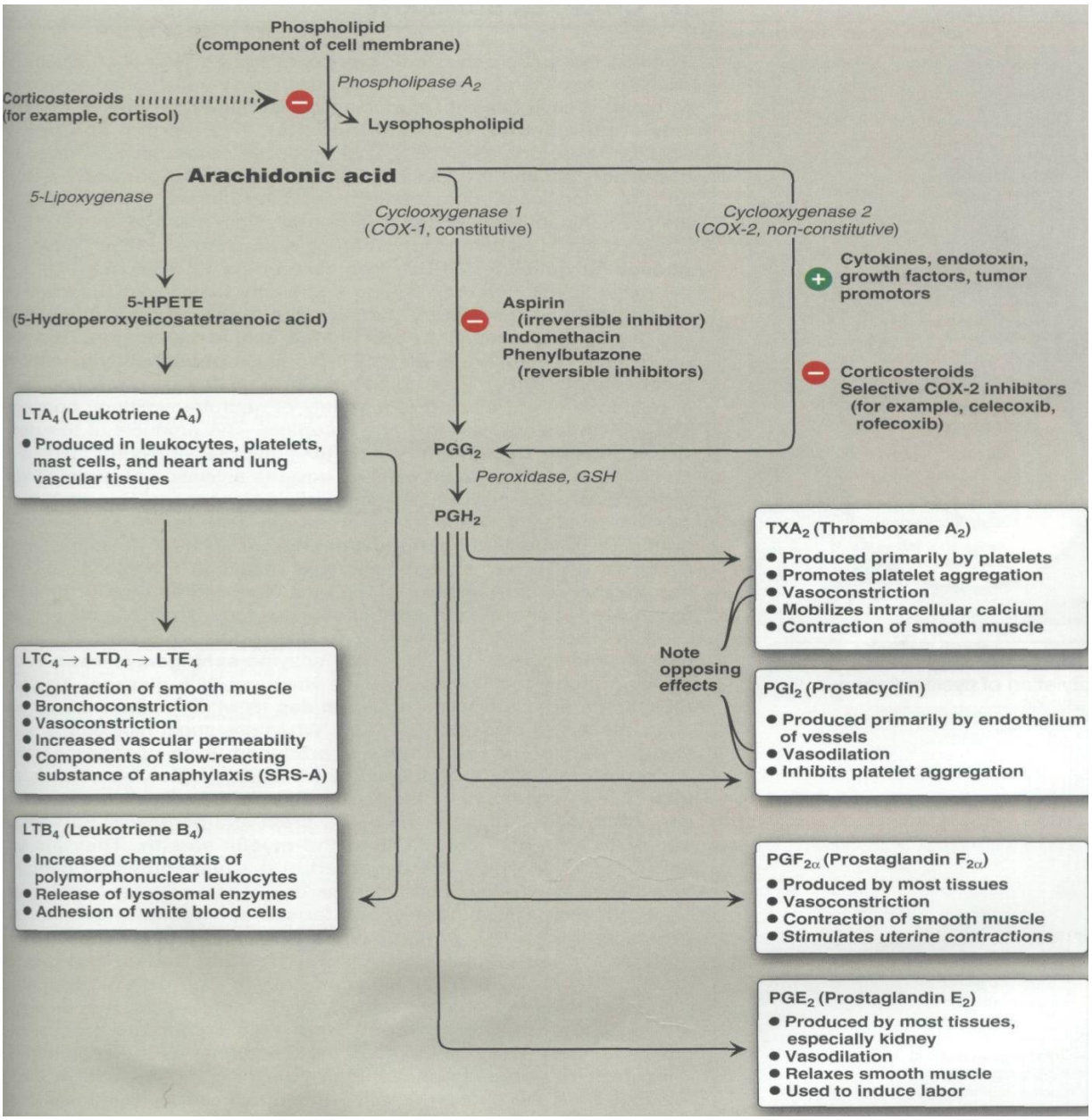
b) Prostacyclnes: They cause vasodilatation and inhibit platelets aggregation.

c) Thromboxanes: They cause aggregation of platelets.

2. Leukotriens (L T):

a) They are present in leucocytes, platelets and mast cells.

b) They cause chemotaxis i.e. Collection of white blood cells at the site of inflammation.



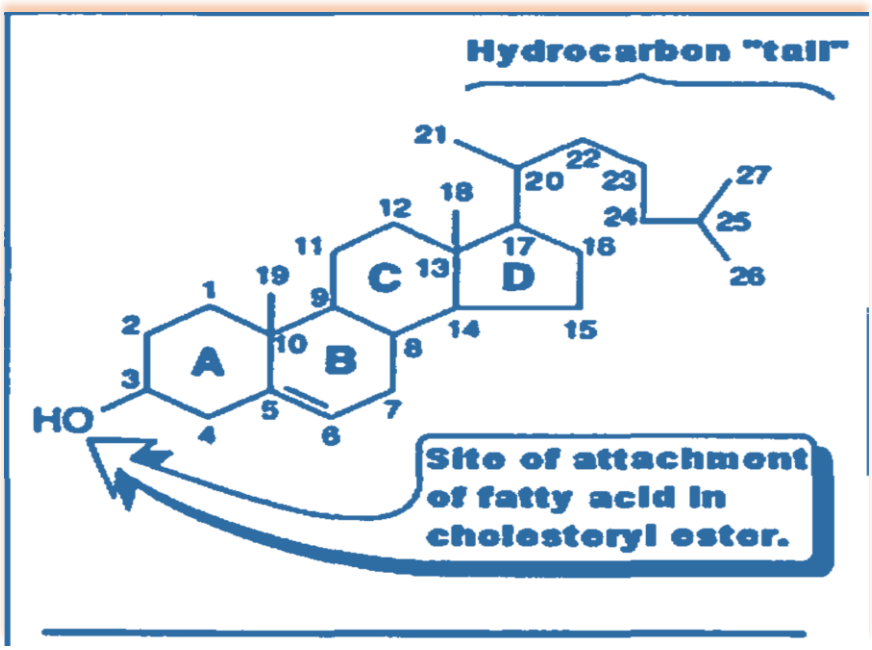
Synthesis, types, and functions of eicosanoids

METABOLISM OF CHOLESTEROL

STRUCTURE

- Cholesterol is an animal sterol.

- It is a solid alcohol having OH group at C**3**



SOURCES OF CHOLESTEROL

A. Endogenous: Cholesterol is formed

in the body almost in all nucleated cells from Acetyl-COA (about 700 mg/day).

B. Exogenous: Cholesterol occurs only in food of animal origin such as egg yolk, meat, liver and brain. Diet supplies about 400 mg/day.

SYNTHESIS OF CHOLESTEROL

A. Location:

1. Intracellular location: Cytosol.

2. Organ location: a) Liver is the major

b) Other tissues e.g. intestine, adrenal cortex, gonads and skin.

B. Precursor: Acetyl CoA.

C. Steps:

1. Formation of acetoacetyl CoA: by condensation of two molecules acetyl CoA:

2. Conversion of acetoacetyl CoA to mevalonate

3. Conversion of mevalonate to cholesterol.

REGULATION OF CHOLESTEROL SYNTHESIS

HMG COA reductase the key enzymes for cholesterol synthesis. It is present in two forms: **active dephosphorylated** and **inactive Phosphorylated**.

It is Regulated through:

1. Feedback inhibition: Cholesterol acts as feedback inhibitor of HMG COA reductase enzyme. Thus, it decreases more cholesterol synthesis.

2. Feedback regulation: Cholesterol (either synthesized by the cell or reaching it from diet) inhibits HMG COA reductase gene. this **decreases** transcription and synthesis of HMG CoA reductase.

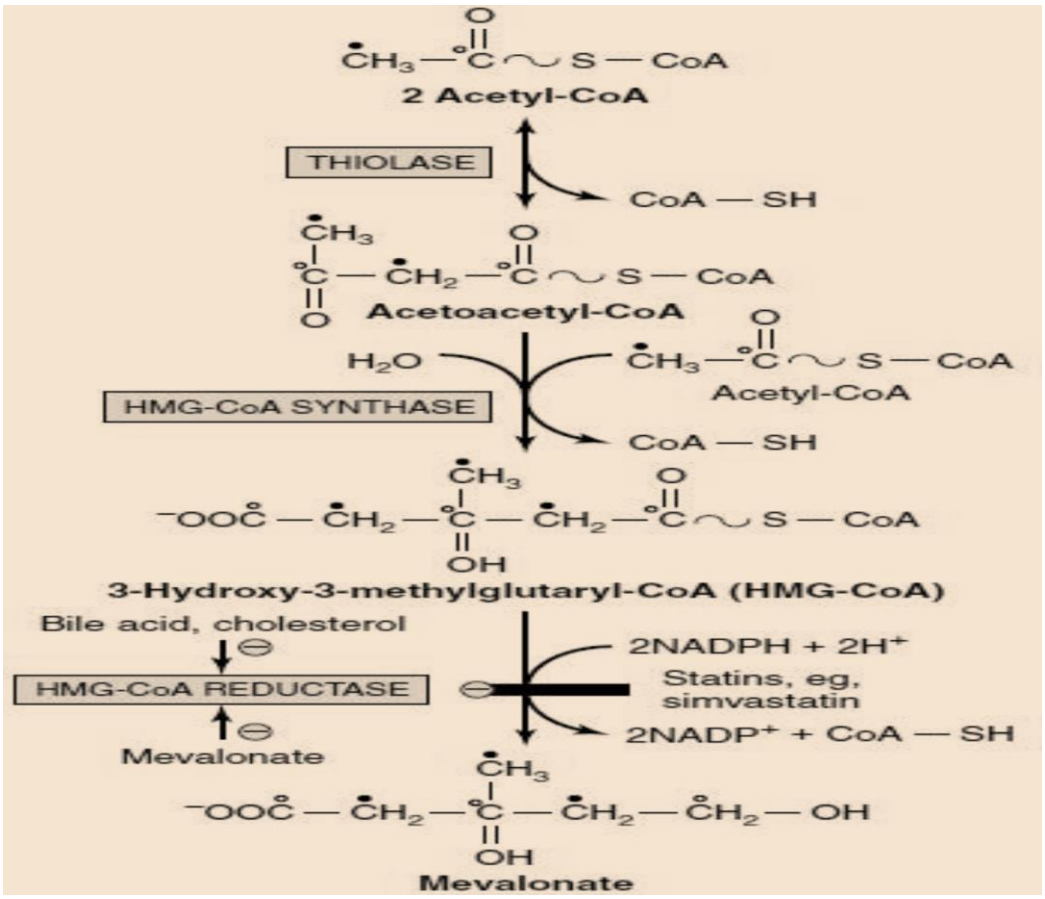
3. Hormonal regulation:

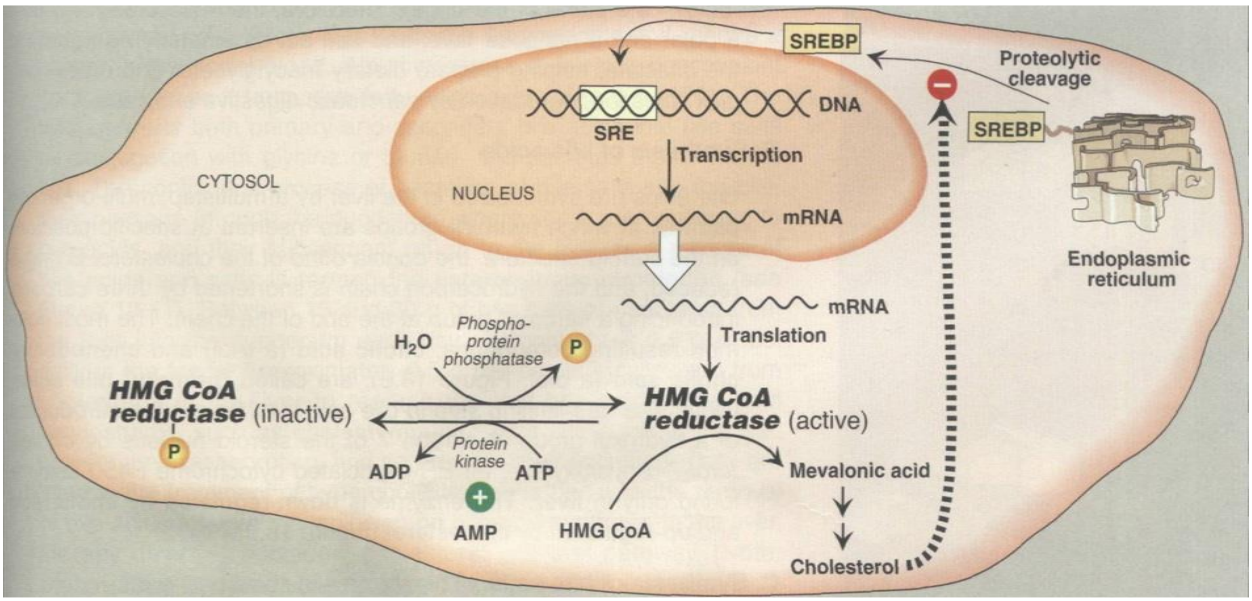
a) Glucagon: Inhibits HMG CoA reductase.

b) Insulin: Stimulates HMG CoA reductase.

4. Inhibition by drugs:

Lovastatin and **mevastatin** are drugs, which inhibit HMG CoA reductase by reversible competitive inhibition. They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.


2 Acetyl-CoA
THIOLASE
CoA — SH
Acetoacetyl-CoA
H2O
HMG-CoA SYNTHASE
Acetyl-CoA
CoA — SH
3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)
Bile acid, cholesterol
HMG-CoA REDUCTASE
2NADPH + 2H+
Statins, eg, simvastatin
2NADP+ + CoA — SH
Mevalonate
Mevalonate


SREBP
Proteolytic cleavage
DNA
SRE
Transcription
SREBP
CYTOSOL
NUCLEUS
mRNA
Endoplasmic reticulum
mRNA
Translation
H2O
Phospho-protein phosphatase
P
HMG CoA reductase (inactive)
HMG CoA reductase (active)
Protein kinase
ADP
ATP
Mevalonic acid
AMP
HMG CoA
Cholesterol

FUNCTIONS OF CHOLESTEROL:

A. Cholesterol enters in the structure of every body cell (e.g. cell membrane).

B. Cholesterol Is the precursor of:

1. VItamin D3

2. Steroid hormones:

a) Estrogens and progesterone (ovaries).

b) Testosterone and androgens (testes).

c) Glucocorticolds and mineralocorticoids (Adrenal

cortex).

3.bile acids: (Liver)

EXCRETION OF CHOLESTEROL:

About one gram of cholesterol is excreted daily. It is secreted as cholesterol, bile acids and coprostanol (Some cholesterol is synthesized by intestinal cells and modified by bacteria before excretion. Bacterial enzymes reduce cholesterol intocoprostanol, which is excreted into feces).

PLASMA CHOLESTEROL:

A. Cholesterol present in plasma is either free or esterified (cholesteryl ester).

1. Total plasma cholesterol: 140 -220 mg/dl.

2. Free plasma cholesterol: 26 - 126 mg/dl.

B. Hypercholesterolemia:

Definition: It is increased plasma cholesterol concentration above 220

mg/dl.

Causes:

a) Diet rich in carbohydrate, cholesterol and saturated fatty acids.

b) Hypothyroidism as thyroxin stimulates conversion of cholesterol to bile acids.

c) Diabetes mellitus.

d) Kidney affection (nephrotic syndrome) unknown mechanism.

e) Obesity.

f) Obstructive jaundice due to decreased excretion of cholesterol and bile acids.

g) Familial hypercholesterolemia.

c. Hypocholesterolemia:

Definition: It is decreased plasma cholesterol concentration below 140 mg/dl

Causes :

a) Prolonged fasting which causes decreased secretion of Insulin (decreased activation of HMG-CoA reductase).

b) Diet rich In unsaturated fatty acids and poor in saturated fatty acids, carbohydrate and cholesterol.

c) Liver diseases, as liver Is the site where most plasma cholesterol is synthesized.

d) Hyperthyroidism.

e) Chronic Infection as tuberculosis.

TRANSPORT* OF *CHOLESTEROL:

A. Cholesterol is hydrophobic. It Is transported in plasma in the more soluble lipoprotein forms: LDL, VLDL and HDL (see plasma lipoproteins).

B. Free cholesterol Is removed from tissues by H~L and transported to be excreted by the liver.

C. Cholesterol ester is the storage form of cholesterol: It is formed in both

tissues and plasma.

1. In tissues (liver), cholesterol is esterified by ACAT enzyme (acyl CoA

cholesterol acyl transferase):

Cholesterol + Acyl CoA…… Cholesteryl ester+ CoASH

2. In plasma, cholesterol is esterified by LCAT enzyme (lecithin cholesterol acyl transferase). LCAT is associated with HDL.

Cholesterol + lecithin ……. Cholesteryl ester+ lysolecithin

PLASMA LIPIDS

Total plasma lipid is 400—600 mg/dL. Out of this, 40% is cholesterol; 30% is phospholipids; 20% is triglycerides.

Since lipids are insoluble in water, they need the help of carriers in plasma. Therefore, they are complexed with proteins to form **lipoproteins.** The protein part of lipoprotein is called **apolipoprotein**. Apolipoproteins are synthesized by liver. Failure of liver to synthesize apolipoproteins leads to accumulation of fat In liver and this condition is called fatty liver.

Methods of separation of plasma lipoproteins:

1. by ultra centrifugation
2. electrophoresis

Classification of Lipoproteins

Depending on the density (**by ultra centrifugation**) or on the electrophoretic mobility, the lipoproteins in plasma are classified into five major types:

1. **Chylomicrons—**contains apoprotein B-48.

2. **Very low density lipoproteins** (VLDL) or pre-beta lipoproteins. Main apoprotein is B-100.

3. **Intermediate density lipoproteins** (IDL) or broadbeta lipoproteins

4. **Low density lipoproteins** (LDL) or beta-lipoproteins. Major apoprotein in LDL is B-100.

5. **High density lipoproteins** (HDL) or alpha-lipoproteins. Major apoprotein in HDL is apo-A.

Free fatty acids (FFA) or non-esterified fatty acids (NEFA) are complexed with albumin. FFAs are not generally included in the classification of lipoproteins,

because they are loosely bound to the protein.

	Chylomicron	*VLDL*	*IDL*	*LDL*	*HDL*	*FFA (*)*
Density g/L	<0.95	0.95–1.006	1.006–1.019	1.019–1.063	1.063–1.121	1.28–1.3
Diameter (nm)	500	70	30	25	15	-
Electrophoretic mobility	origin	pre-beta	broad beta	beta	alpha	albumin
% Composition						
Protein	2	10	20	20	30-60	99
TAG	80	50	30	10	10	0
Phospholipids	10	20	20	20	20-30	0
Cholesterol	10	20	30	50	10-30	0
FFA	0	0	0	0	0	1
Apoproteins	A,B-48,C-II,E	B-100, C-II,E	B-100, E	B-100	A-I, C, E	Albumin
Transport function	TAG from gut to muscle and adipose tissue	TAG from liver to muscle and adipose tissue		Cholesterol from liver to peripheral tissues	Cholesterol from peripheral tissues to liver	FFA from adipose T to muscle and liver

(*) Free fatty acids are not generally included in the lipoproteins. They are seen in circulation, weakly bound to albumin.

Apoprotein	*Component of*	*Functions*	*Molecular weight*	*Blood level mg/dL*	*Site of production*
apo A-I	HDL	Activation of LCAT; ligand for HDL receptor; Anti-atherogenic	28,000	150	Intestine; liver
apo A-II	HDL	Inhibits LCAT; stimulates lipase	17,000	30	Intestine; liver
apo B-100	LDL; VLDL	Binds LDL receptor	550,000	100	Liver
apo B-48	Chylomicrons	48% size of B-100. Major structural apoprotein of chylomicrons	250,000	–	Intestine
apo C-I	Chylo; VLDL	Activation of LCAT; anti atherogenic	7,000	10	Liver
apo E	LDL; VLDL; chylomicron	Arginine rich; ligand for hepatic uptake	30,000	2	Liver
apo Lp(a)	Lp(a)	Attached to B-100; impairs fibrinolysis; highly atherogenic		< 30	Liver

Chylomicrons metabolism:

1-Site of synthesis: intestinal mucosal cells

2-Functions: transport dietary lipids from intestine to peripheral tissues.

3-Structure:

a-Main lipids: triacylglycerols. Chylomicrons contains also cholesterol, phospholipids and fat-soluble vitamins.

b-Proteins: (2%), apo 848 and receives apo Cll and apo E from HDL.

4-catabolism: Main sites of metabolism of chylomicrons are adipose tissue and skeletal muscle.

The half-life of chylomicrons in blood is about 1 hour. TG are hydrolyzed by lipoprotein lipase (which is activated by apo Cll). The remaining parts are chylomicron remnants, which are then taken up by the liver. Hepatocyte receptors can recognize apoB48 and apo E.

Disorders of chylomicron& metabolism:

a) Deficiency of lipoprotein lipase: Leads to hyperlipoproteinemla.

b) The disease is called familial lipoprotein lipase deficiency; it is characterized by marked increase of plasma chylomicrons, especially after fatty meal.

VLDL metabolism:

1-Site of synthesis: Liver.

2-Functions: transport lipids mainly TG from liver to peripheral tissues.

3-Structure:

a-Main lipids: triacylglycerols. It contains also cholesterol, phospholipids.

b-Proteins: (10%), apo 8 100 and receives apo Cll and apo E from HDL.

4-catabolism: TG are hydrolyzed by lipoprotein lipase (that is activated by apo Cll). The remaining parts are IDL, which are then converted into LDL by transferring phospholipids, apo Cll and apo E to HDL.

Disorders of VLDL metabolism:

Fatty liver: This is an accumulation of abnormal amounts of fat in liver. It occurs when there is excess triacylglycerols synthesis in the liver, which is then excreted in the form of VLDL.

LDL METABOLISM:

1-SITE OF SYNTHESIS: circulation from VLDL.

2-FUNCTION: LDL particles provide cholesterol to peripheral tissues.

3·STRUCTURE:

a- Lipid contents: cholesterol, cholesterol esters and phospholipids.

b- Protein contents: (22%), apo B1oo.

4·**CATABOLISM**:

LDL apo B 100 are recognized by tissue receptors. After binding with receptors, the LDL are internalized by endocytosis. Inside cells LDL are separated from receptors and hydrolyzed by lysosomal enzymes releasing cholesterol, amino acids, fatty acids and phospholipids.

*If the cell contains oversupply of cholesterol from LDL, HDL or chylomicron remnants, the cholesterol amount can be decreased by:

a-Inhibition of HMG-CoA reductase + Inhibition of cholesterol synthesis.

b-Stimulation of ACAT enzyme + Cholesterol ester.

c-lnhibition of synthesis of LDL receptors + inhibition of LDL uptake by

cells.

However, the effect of cholesterol-induced suppression of LDL receptor synthesis is to decrease the rate at which LDLs and IDLs are removed from the serum. This can lead to excess circulating levels of cholesterol. The excess cholesterol tends to be deposited within the arteries, leading to atherosclerosis.

LDL and Clinical Applications

The LDL concentration in blood has positive correlation with incidence of cardiovascular diseases. A fraction of cholesterol is taken up by macrophages; this is not a regulated pathway. Increased levels of LDL or modification of LDL by glycation (as seen in diabetes mellitus) or oxidation increases the fraction of cholesterol taken up by are taken up by macrophages or scavenger cells. This is

the starting event of atherosclerosis leading to myocardial infarction.

When these cells become engorged with cholesterol, foam cells are formed, that get deposited in the sub-endothelial space triggering formation of atheromatous plaque. Procoagulant changes are induced in the endothelium resulting in increased chances of thrombosis and coronary artery disease.

Since LDL-cholesterol is thus deposited in tissues, the LDL (low density lipoprotein) variety is called “bad cholesterol”.

Insulin and tri-iodothyronine (T3) increase the binding of LDLs to liver cells, whereas glucocorticoids have the opposite effect. The effects of insulin and T3 on hepatic LDL binding may explain the hypercholesterolemia and increased

risk of atherosclerosis that have been shown to be associated with uncontrolled diabetes or hypothyroidism.

Disorders of LDL metabolism

Type II familial hypercholesterolemia: LDL receptors are deficient in tissues and liver. Usually associated with atherosclerosis.

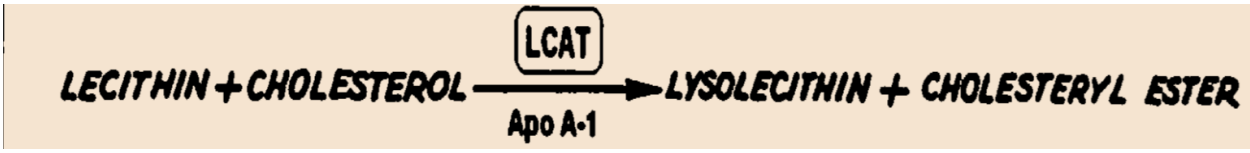
Metabolism of high-density lipoproteins (HDL), the good cholesterol:

1. **Site of synthesis**: liver.

2. **Functions:**

a) Act as reservoir of apo C-11 that is transferred to chylomicrons and VLDL to activate lipoprotein lipase enzyme.

b) Remove free (unesterified) cholesterol from extrahepatic tissue and esterifying it, using a plasma enzyme called: lecithin, cholesterol acyl transferase (LCAT). The apo A-1 OF HDL activates LCAT.



c) HDL particles carry cholesterol esters to:

1) VLDL and LDL.

2) Liver where the HDL is hydrolyzed and cholesterol released.

3. **Structure:**

a) Lipids: Mainly phospholipids together with esterified &

unesterified cholesterol.

b) Proteins (50%): Include Apo A-1, Apo C and Apo E.

4**. Catabolism:**

a) A newly secreted HDL are disc shaped particles containing mainly unesterified cholesterol and phospholipids.

b) HDL Is converted into spherical particles by accepting unesterified cholesterol from peripheral tissues (surface of cell membranes).

c) Once the free cholesterol is taken up, It is immediately esterified by

LCAT. The resulting cholesterol ester is very hydrophobic, so it remains in HDL and cannot be transferred to peripheral tissues.

d) The liver takes up HDL particles, where the cholesteryl esters are hydrolyzed. The released cholesterol may undergo:

1) Binding with apoproteins to form lipoproteins.

2) Converting to bile acids.

3) Secreted into the bile to be removed from the body.

Hormones of the Adrenal gland

Classification:

- The hormones of adrenal cortex (steroid hormones) which have a biological activity can be

classified into 3 classes: glucocorticoids, mineralocorticoids and androgens.

All contain steroid ring and derived from cholesterol.

Glucocorticoids and mineralocorticoids contain 21 carbon atoms and have 2 carbon side chain at C-17.

Androgens contain 19 carbon atoms and have keto or hydroxyl group at C-17.

Glucocorticoids and androgens are synthesized in zona fasciculata and zona reticularis, while mineralocorticoids are synthesized in the subcortical zona glomerularis of adrenal cortex.

- Hormones of adrenal medulla: Catecholamines (epinephrine and norepinephrine)

Steroid hormones

BIOSYNTHESIS:

A. There is a common metabolic pathway for the biosynthesis of all steroid hormones.

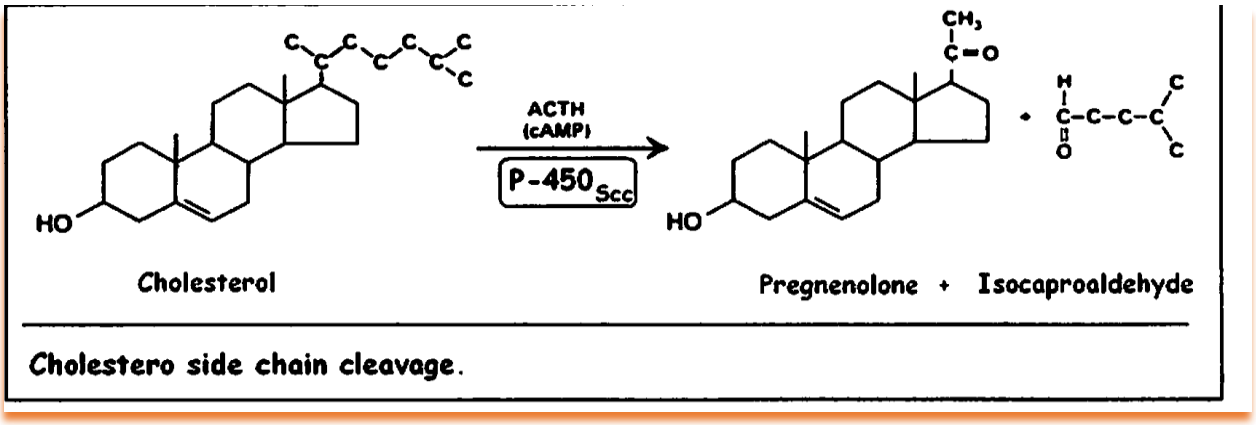
B. Synthesis and secretion of steroid hormones occur in the adrenal cortex (cortisol, aldosterone, and androgens), ovaries and placenta (estrogens and progestins), and testes (testosterone).

C. Synthesis involves shortening the hydrocarbon chain of cholesterol, and hydroxylation of the steroid nucleus. The cholesterol substrate can be newly synthesized, taken up from lipoproteins, or released from cholesteryl esters stored in the cytosol of steroidogenic tissues. An important control point is the movement of cholesterol into mitochondria. This process is mediated by StAR (steroidogenic acute regulatory protein).

D. The first step is the conversion of cholesterol into pregnenolone:

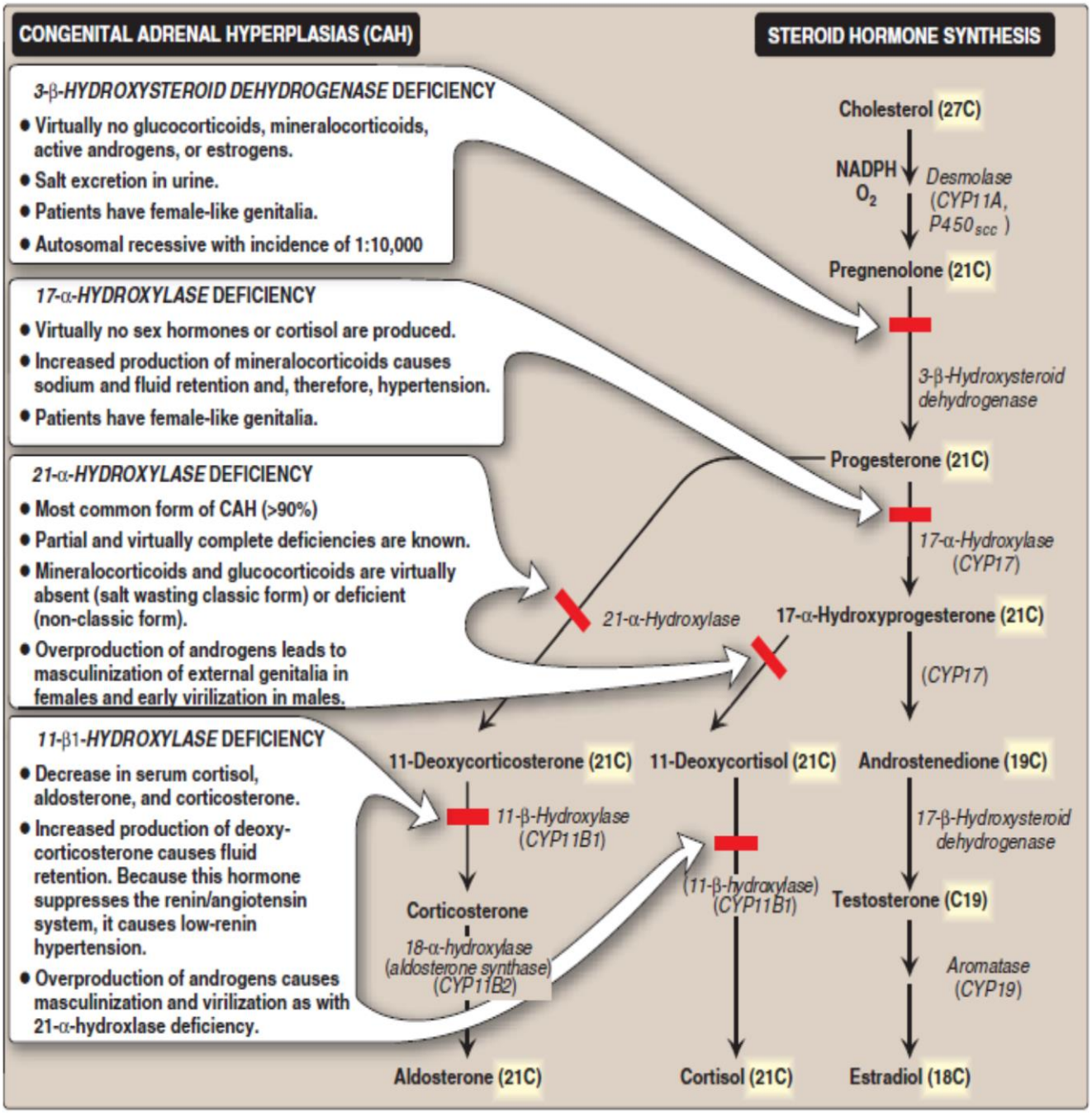
1. This reaction is the rate limiting step in steroidogenesis and occurs in the mitochondria.

2. It needs an enzyme called: cytochrome P-450 side chain cleavage enzyme (P-450 sec).
3. It requires NADPH and molecular oxygen



E. Pregnenolone is next oxidized and then isomerized to progesterone, and 17-hydroxypregnenolone, both are further modified by a series of hydroxylation and oxidation reactions to other steroid hormones.
F. 17-hydroxypregnenolone Is converted - 'into dehydroepiandrosterone (DHEA) which Is the main androgen produced by adrenal cortex. DHEA is then converted by -a sulfotransferase- to DHEA sulphate, which is then secreted in the blood. The major steroid hormones secreted by the human adrenal cortex. are: cortisol, corticosterone, aldosterone and DHEA sulphate.
G. Adrenal cortex can synthesize very small amount of testosterone from DHEA. Also small amount of estrogens can be synthesized from aromatization of testosterone.

Synthesis of steroid hormones and related Abnormalities (congenital adrenal hyperplasia)



PLASMA TRANSPORT of steroid hormones:

A. **Glucocorticoids:**

1. Cortisol circulates in plasma in free form (8%) and in association of protein (92%).
2. The free cortisol is the biologically active form of the hormone. The cortisol binding protein is called: transcortin or corticosteroid binding globulin (CBG).
3.Very small amount of cortisol is bound to albumin.

A. **Mineralocorticoids:** do not have specific plasma transport protein.

Functions of glucocorticoids

Metabolic effects of glucocorticoids

A. Increase glucose production in liver by stimulating gluconeogenesis (stimulate synthesis of enzymes of gluconeogenesis and increase the delivery of amino acids (the gluconeogenic substrate) from peripheral tissues).

B. Increase hepatic glycogen deposition by prompting the activation of

glycogen synthetase.

C. Promote lipolysis (in extremities) but can cause lipogenesis in other sites (hoe and trunk) especially at higher than physiological levels.

D. Promote protein and RNA metabolism. This is an anabolic effect at physiologic levels, but can be catabolic in certain conditions and at higher than physiologic levels.

EFFECTS ON HOST DEFENSE MECHANISMS:

A. Suppress the immune response.

B. Suppress the inflammatory response by:

1. Decreasing the number of circulating leukocytes and the migration of tissue leukocytes.

2. Inhibiting fibroblast proliferation.

OTHER EFFECTS:

A. Necessary for maintenance of normal blood pressure and cardiac output.

B. Required for maintenance of normal water and electrolyte balance. Perhaps by restraining ADH release and by increasing angiotensinogen (Na'). These effects contribute to the effect on blood pressure.

C. Necessary, with the hormones of the adrenal medulla, in allowing the organism to respond to stress.

Actions of mineralocorticoids

Aldosterone's primary effect is on the kidney tubules, where it stimulates sodium uptake and potassium excretion. [Note: An effect of aldosterone is an increase in blood pressure. Competitive inhibitors of ACE are used to treat renin-dependent hypertension.]

REGULATION (CONTROL) OF SECRETION:

A. **Glucocorticoids:**

1. When the body is stressed, corticotropin releasing hormone (CRH) Is released by the hypothalamus, which stimulates anterior pituitary to produce ACTH.

2. ACTH binds with receptors in the cell membrane of zona fasiculata and zona reticularls. This leads to activation of adenylate cyclase and conversion of ATP into cyclic AMP.

3. Cyclic AMP will stimulate formation and secretion of glucocorticoids.

B. **Mineralocorticoids:**

1. When the body is subjected to hypotension, (decreased renal perfusion pressure), anoxia or kidney trauma, the kidney responds by secreting renin hormone.

2. Renin will activate angiotensinogen into angiotensin I and II.

3. Angiotensin II will stimulate zona reticularis to produce mineralocorticoids (aldosterone) which act on distal convoluted tubules of the kidney causing Na• and water reabsorption and K• excretion.

Hormones of gonads

I. The gonads have 2 functions which are production of germ cells and sex hormones.

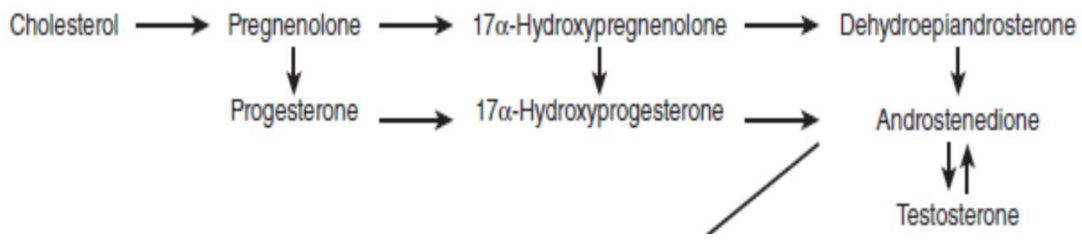
A. In males: testes produce spermatozoa and testosterone
B. In females: Ovaries produce ova and the steroid hormones estrogens and progesterone.

Male sex hormones (androgens):

- Androgens are produced by the Leydig cells and the sterol cells of the testes.
- Ovaries produce also androgens in small amounts.
- Many androgens are produced by the testes, but the most active members are testosterone and its metabolite dihydrotestosterone (DHT)
- Sertoll cells also produce an androgen binding protein (ABP) which binds testosterone and dihydrotestosterone. ABP is secreted into the lumen of seminiferous tubules and in this position, it binds testosterone (produced by Leydig cells) and transports it in very high concentration to the site of spermatogenesis. This explains why testosterone when given as a drug does not support spermatogenesis.

Biosynthesis of androgens:

1. Testosterone is synthesized from cholesterol by a pathway similar to that described for steroidogenesis in adrenal cortex.



Plasma transport of testosterone:

- Testosterone and DHT circulate in plasma In free form- (2%) and In association with proteln(98%).
- The free testosterone Is the biologically active form of hormone.
- The testosterone binding protein is called: testosterone-estrogen-binding globulin {TEBG) or sex hormone binding globulin (SHBG) which is produced by the liver.

Regulation (control) of secretion:

1. LH stimulates steroidogenesis and testosterone production by binding to receptors on the cell membrane of the Leydig cells (by a mechanism similar to that of ADTH in the adrenal cortex).
2. FSH binds to the sterol cells and promotes the synthesis of androgen-binding protein (ABP) which binds testosterone and secreted in the lumen of seminiferous tubules. This will stimulate spermatogenesis.

Actions of TESTOSTERONE

- Stimulates spermatogenesis.
- Promotes development of male secondary sex characteristics.
- Promotes anabolism.
- Masculinization of the fetus

Metabolic products of Testosterone in Peripheral Tissues

The most significant metabolic product of testosterone is DHT, since in many tissues, including prostate, external genitalia, and some areas of the skin, this is the active form of the hormone. The plasma content of DHT in the adult male is about one-tenth that of testosterone. About 50 to 100 μg of DHT are secreted by the testes. The rest is produced peripherally from testosterone in a reaction catalyzed by the NADPH-dependent **5 α-reductase.**

Some estradiol is formed from the peripheral aromatization of testosterone, particularly in males.

Testosterone is metabolized in liver. Its metabolites conjugate with sulfate or glucuronate before excretion in urine.

Hypogonadism:

1. This is a condition of deficiency of testosterone synthesis .

2. It may be due to:

a) Primary hypogonadism: due to absence or disease of the testes.

b) Secondary hypogonadism: due to defective secretion of LH *&/* or FSH •

3. It Is characterized by Impotence, obesity and muscular wasting (due to loss of the protein anabolizing effect of testosterone).

FEMALE SEX HORMONES (ESTROGENS and PROGESTINS):

A. **Estrogens are produced by:**

1. Follicles and corpus luteum of the ovary.

2. Placenta which produces increased amount in the second and third trimesters of pregnancy.

B. Three types of estrogens are present:

1. Estradiol (E2) and estrone (E1) are produced by the ovary.

2. Estriol (E3) is produced by the placenta.

Progestins (progesterone) are produced and secreted by corpus luteum.

Biosynthesis of estrogens and progesterone:

1. It is similar to those of male hormones.

2. Estrogens are formed by the aromatization of androgens in a complex

process that involves 3 hydroxylation steps by theca and granulosa cells of the ovaries

Plasma transport of estrogens and progesterone:

1. Estrogens are bound to testosterone-estrogen binding globulin (TEBG), and progesterone is bound to corticosteroid binding globulin (CBG).

2. Only the free (unbound) hormones have biological activity.

Regulation (control) of secretion:

1. Combined action of FSH and LH is required for the synthesis of estradiol.

2. LH is required for ovulation and synthesis of progesterone.

Metabolism and excretion:

1. **Estrogens:** The liver converts estradiol and estrone to estriol which conjugates with sulfate or glucuronic acid before excreted in urine or bile.

2. **Progesterone:** The liver converts progesterone into a compound called pregnandiol which conjugates with sulfate or glucuronic acid and excreted in urine.

Functions:

1. **Estrogens:**

- Estrogens stimulate the growth of the cells of uterus, vagina, graafian follicles of the ovary and the mammary gland.
- Estrogens are responsible for the development of &secondary sexual characters e.g. female voice, female pattern of hair and fat distribution.
- Estrogens induce, in the uterus and mammary gland, the synthesis of progesterone receptors.
- Estrogens are responsible for the maintenance of the menstrual cycle.
- Estrogens are required for the development of mammary gland.

Progesterone:

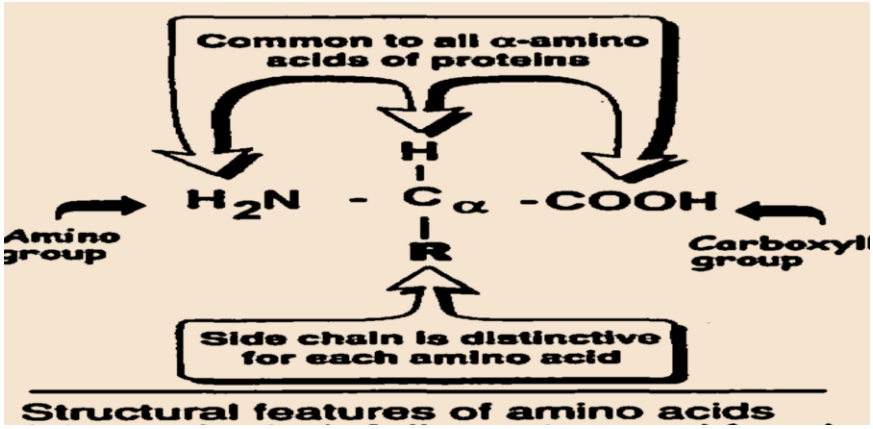
- Progesterone is necessary for the implantation of fertilized ovum in the uterus.
- Progesterone inhibits uterine contraction during pregnancy (maintain pregnancy).
- Progesterone stimulates the growth of the secretory glands of uterus and mammary gland.
- Progesterone Is responsible for the maintenance of menstrual cycle (luteal phase).
- Progesterone antagonizes the action of estrogens in various tissues.

PROTEIN CHEMISTRY

All proteins are polymers of amino acids. Proteins are composed of a number of amino acids (more than 50 amino acids) linked by peptide bonds.

CLASSIFICATION OF AMINO ACIDS

I. Based on Structure

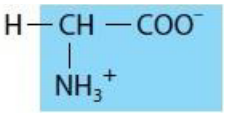
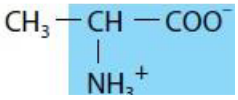


Structural features of amino acids

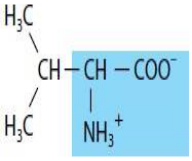
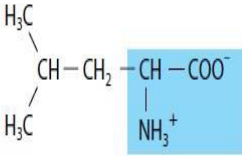
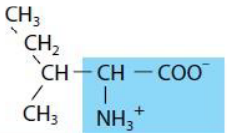
A. Aliphatic amino acids

1. **Mono-amino mono-carboxylic acids** (neutral amino acids):

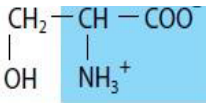
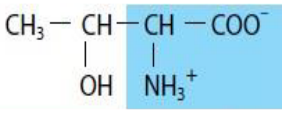
• Simple amino acids: Glycine, Alanine

Glycine  Alanine 

• Branched chain amino acids: Valine, Leucine, Isoleucine

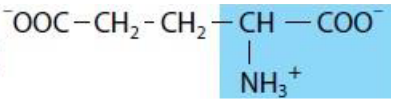
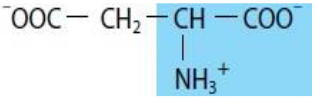
Valine  Leucine  Isoleucine 

• Hydroxyamino acids: Serine, Threonine.

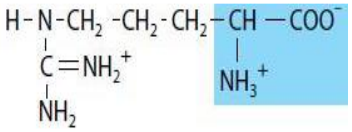
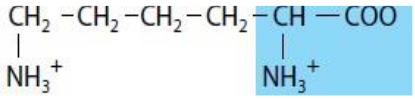
Serine  Threonine 

• Sulfur-containing amino acids: Cysteine, Methionine

2. Monoamino dicarboxylic acids (acidic amino acids): Aspartic acid, Glutamic acid.

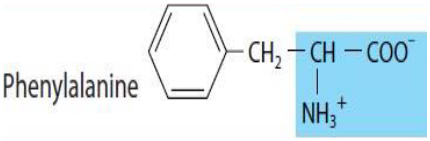
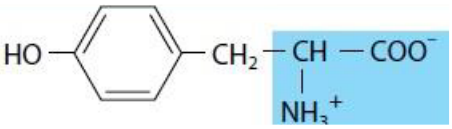
Glutamic acid  Aspartic acid 

1. **Diamino monocarboxylic acids (basic amino acids:** Lysine, Arginine

Arginine  Lysine 

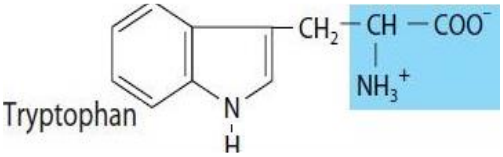
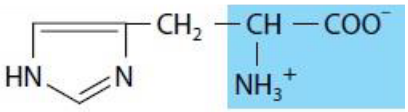
B. Aromatic amino acids:

Phenylalanine, Tyrosine

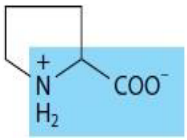
Phenylalanine  Tyrosine 

C. Heterocyclic amino acids:

Tryptophan, Histidine

Tryptophan  Histidine 

D.Imino acid: Proline

Proline 

E. Derived amino acids:

- **Derived amino acids found in proteins:** After the synthesis of proteins, some of the amino acids are modified, e.g. hydroxy proline and hydroxy lysine are important components of collagen
- **Derived amino acids not seen in proteins** (Non protein amino acids): Some derived amino acids are seen free in cells, e.g. Ornithine, Citrulline, Homocysteine. These are produced during the metabolism of amino acids.

II. Based on Nutritional Requirements

A. Essential or indispensable:

Their carbon skeleton cannot be synthesized by human beings and so preformed amino acids are to be taken in food for normal growth. Normal growth and optimal health will not occur, if one such amino acid is deficient in the diet.

They include: **I**soleucine, **L**eucine, **T**hreonine, **Ly**sine, **M**ethionine, **Ph**enylalanine, **T**ryptophan, and **V**aline are essential amino acids (VITTAL LYMPH).

B. Partially essential or Semiessential: Histidine and Arginine are semi indispensable amino acids. Growing children require them in food. But they are not essential for the adult individual.

C. Non-essential or Dispensable: The remaining 10 amino acids are non-essential, because their carbon skeleton can be synthesized by the body. So we need not have to ingest these amino acids as such. However, they are also required for normal protein synthesis. The non-essential amino acids are Alanine, Asparagine, Aspartic acid, Cysteine, Glutamine, Glutamic Acid, Glycine, Proline, Serine and Tyrosine.

III. Based on Metabolism

A. **Purely ketogenic:** Leucine is purely ketogenic because it is converted to ketone bodies.

B. **Ketogenic and glucogenic:** Lysine, Isoleucine, Phenylalanine, Tyrosine and Tryptophan are partially ketogenic and partially glucogenic. During metabolism, part of the carbon skeleton of these amino acids will enter the ketogenic pathway and the other part to glucogenic pathway.

C. **Purely glucogenic:** All the remaining 14 amino acids are purely glucogenic as they enter only into the glucogenic pathway.

PROPERTIES OF AMINO ACIDS

A) Physical properties:

1. All amino acids are soluble in water and alcohol (polar solvents); but insoluble in non-polar solvents (benzene).
2. All amino acids -except glycine- are optically active because they contain asymmetric carbon atom (=a-carbon). Thus they can rotate plane polarized

light (see carbohydrate chemistry). Glycinecontains no asymmetric carbon atom, so it is optically Inactive.

3. Amino acids are present in crystals with high ionic forces, stabilizing these crystals. So amino acids have high melting points above 200°C i.e. they are very stable molecules.
4. Glycine, alanine, valine, serine, tryptophan, histidine and proline are sweet in taste; leucine is tasteless; while isoleucine and arginine are bitter. Sodium glutamate is a flavoring agent. Aspartame, an artificial sweetener contains aspartic acid and phenylalanine.
5. **Zwitter ion and Isoelectric Point**

Monoamino-monocarboxylic acids present in aqueous solutions as **zwitter ion:** It is the amino acid that carries both positive and negative charges. It is electrically neutral and cannot migrate in electric field.

- Isoelectric pH (isoelectric point: Pi): it is the pH at which the zwitter ion is formed.

Each amino acid has certain pH at Which zwitter ion is formed.

B) Chemical properties

1. Amino acids give all the reactions expected for the carboxyl and amino groups e.g. salt formation with acids and alkalies, decarboxylation, deamination, esterification, etc.
2. Reaction with ninhydrin: Ninhydrin is a substance that reacts with amino acids to give C02, ammonia and aldehyde. Ninhydrin reacts with liberated ammonia to give blue colour. The intensity of the blue colour indicates the quantity of amino acids present.
3. Color reactions of amino: acids These depend on the nature of radical (R).
   - Millon's reaction: for tyrosine ~ Red color.
   - Rosenhelm's reaction: for tryptophan ~ Purple color.
   - Xanthoproteic reaction: for phenylalanine and tyrosine ~ Orange color.
4. Peptide bond formation: Amino acids can react together to form peptide bond

Peptides

Definition: Peptides are compounds, formed of less than 50 amino acids linked together by peptide bonds.

Types

1. Dipeptide (2 amino acids and 1 peptide bond).

2. Tripeptide (3 amino acids and 2 p.b).

3. Oligopeptide (3-10 amino acids).

4. Polypeptide (10-50 amino acids).

• **Peptide bond:**

- **Definition:** It is a covalent bond formed between the carboxyl group of one amino acid and the amino group of another with removal of water.

- Peptide formation needs energy getting from hydrolysis of ATP

• Primary structure of peptides:

- It's the arrangement of amino acids in a polypeptide chain.

- In a polypeptide chain the N-terminal amino acid (i.e. the only amino acid that contains free amino group) is always to the left side.

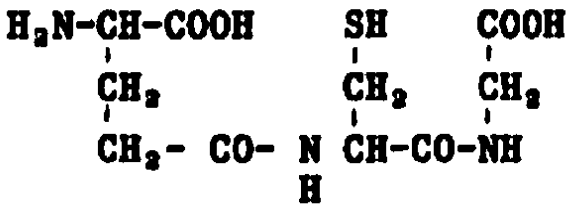
- The C-terminal amino acid (i.e. the only amino acid that contains free carboxyl group) is always to the right.

• Separation of peptides

A. By electrophoresis B. By exchange chromatography technique.

• **Biologically active peptide Peptides include many active compounds as:**

A. Glutathione:



Glutathione

- **Definition**: it is a Tripeptide formed of three amino acids: glutamate cysteine and glycine. It is also called "glutamyl-cysteinyl-glycine".

Glutathione is commonly abbreviated as G-SH where -SH indicates the sulfhydryl group of cysteine and it is the most active part of the molecule.

- **Functions of glutathione:**

1- Defense mechanism against certain toxic compounds (Detoxification).

2-Absorption of amino acid: glutathione has a role in transport of amino acids across intestinal cell membrane..

3- Protect against cell damage and hemolysis of RBCs: Glutathione breakdown the hydrogen peroxide (H2O2) which causes cell damage and hemolysis.

4- Activation of some enzymes. 5- Inactivation of insulin hormone.

B. Hormones:

1. Insulin and glucagon from pancreas.

2. Vasopressin and oxytocin from posterior pituitary gland.

3. ACTH from anterior pituitary gland.

Proteins

• Nature of proteins:

A. Composition:

1. Proteins are macromolecules formed of amino acids united together by peptide bonds.

2. The term protein is applied to describe molecules greater than 50 a.a.

3. Molecules contain less than 50 amino acids are termed peptides.

• Functions of proteins:

1. Enzymes: Enzymes are protein

2. Transport: Of small molecules and ions e.g.

a. Hemoglobin is a carrier for oxygen.

b. Lipids are transported as lipoproteins.

3. Structural elements: e.g.

a. Cell membrane contains proteins in the form of glycoproteins.

b. Skin and bone: e.g. contains proteins in the form of collagen.

4. Hormonal regulation:

a. Some hormones are protein in nature e.g. **growth hormone**.

b. Cellular **receptors** that recognize hormones are proteins

5. Defense mechanism:

a. Antibodies: (immunoglobulins) are protein in nature.

b. Keratin found in skin and other tissues is protein that protect against mechanical and chemical injury.

6. Blood clotting: Coagulation factors are proteins.

7. Storage: as **ferritin** which is a storage form of **iron**.

8. Control of genetic expression: many regulators of genes are protein in nature.

Bonds responsible for protein structure:

Protein structure is generally stabilized by 2 strong covalent bonds and 3 weak noncovalent bonds:

a. Covalent bonds (strong bonds):
   - peptide bond: the primary bond in protein structure
   - disulfide bond: secondary bond

b. Noncovalent bonds (week bonds): hydrogen, hydrophobic and electrostatic bonds. All are secondary bonds

copper sulphate giving violet complex.

Salting out:

When a neutral salt, such as ammonium sulfate or sodium sulfate is added to protein solution, the shell of hydration is removed and the protein is precipitated. This is called salting out. As a general rule, higher the molecular weight of a protein, the salt required for precipitation is lesser. Thus globulins are precipitated at half saturation of ammonium sulfate; but albumin will need full saturation of ammonium sulfate.

Precipitation of proteins: This can be done by:

1. Various concentrations of salt solutions: Salting out.

2. Various concentrations of alcohol.

3. By heavy metals e.g. mercury, silver. Heavy metals combine with proteins forming insoluble metalloproteins.

4. By alkaloidal reagents e.g. trichloroacetic acid and picric acid. Alkaloidal reagents form insoluble complex with proteins.

Denaturation of proteins:

Definition: unfolding and loss of secondary tertiary and quaternary structure of protein. It does not affect primary structure i.e. not accompanied by hydrolysis of peptide bond.

Effect of protein denaturation:

1. Loss of biological activity: e.g. insulin loses its activity after denaturation.

2. Denaturated protein are often insoluble.

3. Denaturated protein are easily precipitated.

Denaturating factors include:

1. Heat: causes coagulation and precipitation of certain proteins like albumin.

2. Organic solvents: They interfere with **hydrophobic bonds** of proteins.

3. Detergents: They contain both hydrophobic and hydrophilic groups i.e. amphipathic. They interfere with hydrophobic bonds of proteins.

4. Strong acids or bases: They lead to change in pH which affects the charges on polypeptide chains. As a result, hydrogen and electrostatic bonds will be disrupted.

5. Heavy metals: as lead and mercury salts:

a) They form ionic bonds with negatively charged ions in polypeptide chains. This leads to disruption of electrostatic bonds.

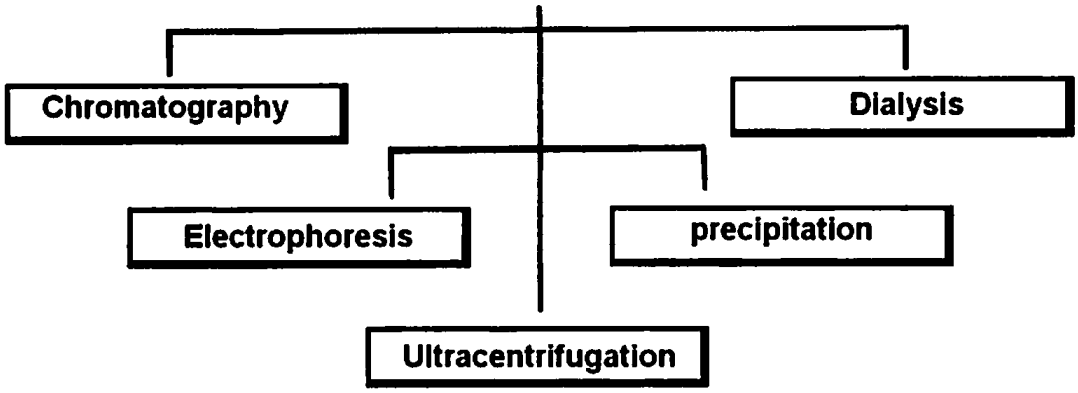
b) They unite with -SH (sulfhydryl) groups of proteins causing its denaturation (-S-Hg).

6. Enzymes: e.g. Digestive enzymes.

7. Urea, ammonium sulphate and sodium chloride: cause precipitation of proteins.

8. Repeated freezing and thawing: cause disruption of hydrogen and other bonds.

Techniques for Separation of proteins and amino acids



1. ***Chromatography:***

A. Definition:

1. Chromatography is a group of separation techniques, where a mixture of molecules is into its components

2. The separated molecules are divided between a stationary and mobile phase.

3. The separation process depends on the tendency of one type of molecules in the mixture to associate more strongly with one phase than the other.

2. ***Electrophoresis****:*

It is the movement of charged particles in an electric field towards the oppositely charged electrode.

Procedure:

1. Sample (e.g. serum containing a mixture of proteins) is applied to a strip of filter paper or cellulose acetate. Then both edges of the strip is dipped in alkaline buffer solution.

2. Because proteins are amphoteric, they will carry negative charges in alkaline medium.

3. When the current passes, proteins will migrate towards positive electrode (anode). The rate of migration depends on:

a) The amount of charges carried by each protein.

b) The molecular weight of proteins.

4. By this method, serum proteins can be separated into several bands, each band represents special type of protein. These types are: albumin, globulins α1, α2, β, and γ globulins.

5. The density of each band is directly proportional to its serum concentration. So albumin shows the densest band.

Diagnostic Importance:

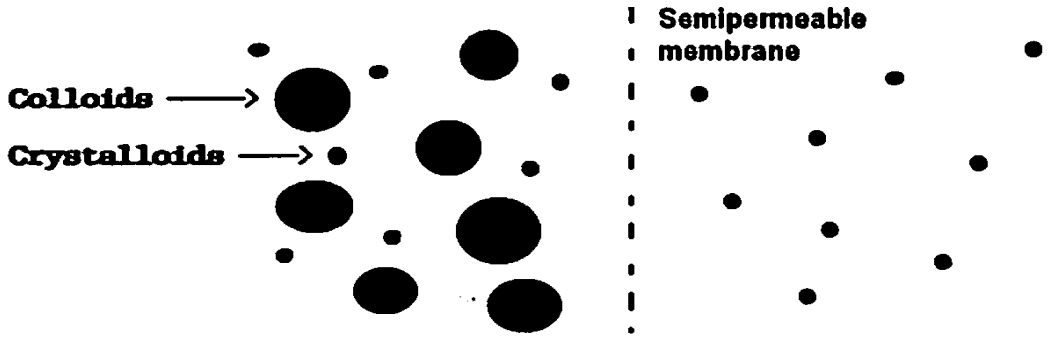
Serum electrophoresis may be used in diagnosis of certain diseases:

1. Hypoalbuminemia: (i.e. decreased serum albumin): The albumin band becomes less dense. This occurs for example in advanced liver disease as liver is the site of albumin synthesis.
2. Hypergammaglobulinemia: (i.e. increased y-globulins): Occurs in some malignant diseases called: multiple myeloma.

3.Dialysis:

- Dialysis means separation of colloids from crystalloids.
- Proteins have a high molecular weight. They form a colloidal solution
- If there is a mixture of proteins (colloids) and salts (crystalloids), they can be separated by dialysis i.e. by using a semi-permeable membrane. Crystalloids can pass through this membrane, while colloids cannot due to the large size of their particles.

Dialysis is used for renal failure patients. Blood passes through dialyzing machine to get rid of waste products and preserving the plasma proteins.



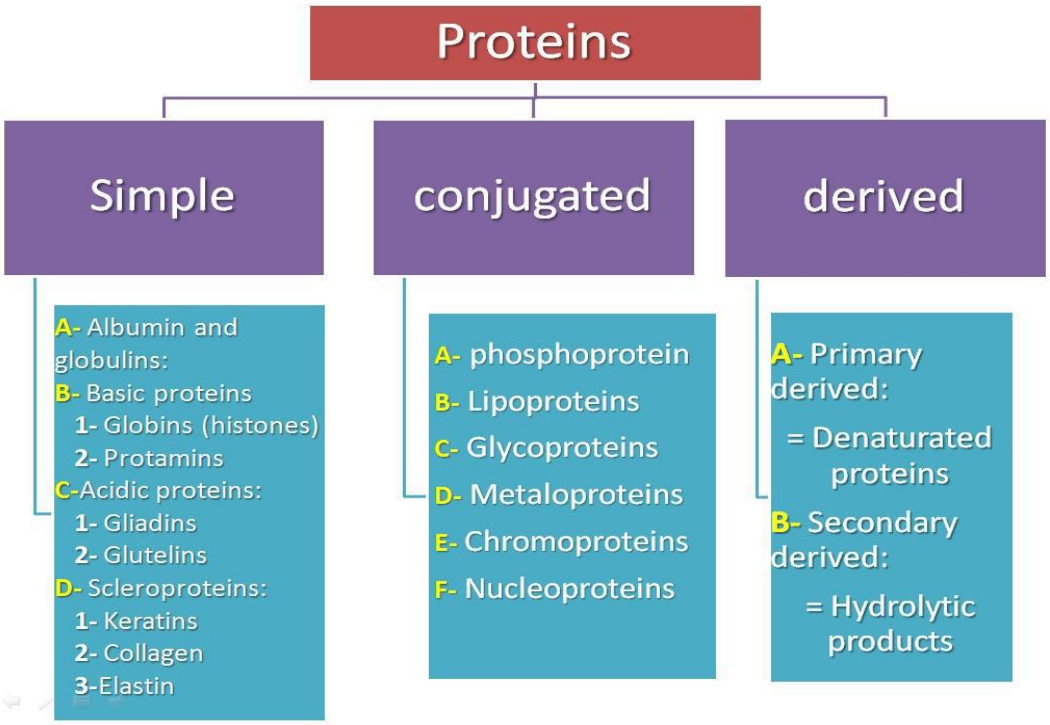
I. Ultracentrifugation*:*

By using a centrifuge of about 40000 rounds per minute (RPM).By this method, a mixture of proteins is separated into different fractions according to their densities.

II. Precipitation: previously discussed

Classification of proteins

III. Classification based on Composition and Solubility



Simple Proteins: they contain only amino acids.

A. Albumin and globulins:

	Albumin	globulins
coagulated by heat	coagulable	Same
biological value	high	Same
Solubility	Soluble in water	Soluble in salt solution
Molecular weight	68.000	150,000
Precipitation	By full saturated ammonium sulphate	By half saturated ammonium sulphate
Sources: **1)Blood** **2)Milk** **3)Egg**	 Serum albumin Lactalbumin Egg albumin	 Serum globulins Lactglobulin Egg globulin

B. basic proteins: Globins (=histones) and protamines:

Both are basic proteins i.e. rich in basic amino acids.

	Globins (=histones)	Protamins
Type of basic amino acid	Histidine	Lysine and Arginine
Solubility	In salt solution	1. In salt solution 2. In 70% ethanol
Sources: **1. Combined with DNA** **2. Combined with Heme**	 1. In plants & animals 2. To form hemoglobin	 In fish

C. Gliadins and Glutelins:

1. Both are acidic proteins i.e. rich in acidic amino acids: glutamic acid.
2. Both are present in cereals
3. Both are soluble in diluted acids and alkalies. Gliadins also soluble in 70% ethanol.

D. Scleroproteins:

They include: keratin, collagen, elastin and reticulin.

1. Keratins:

Location: They are found in hair, nail, enamel of teeth, and outer layer of skin.
Structure: They are **α-helical polypeptide** chains. They are rich in **cysteine** (which provides **disulfide bonds** between adjacent polypeptide chains).
Solubility: It is **insoluble** due to their high content of **hydrophobic A.A.**

2. **Collagen**

It is the protein of connective tissue present in skin, bones, tendons and blood vessels.

- Bones and teeth are made by adding mineral crystals to the collagen.
- Collagen may be present as a gel e.g. in extracellular matrix or in vitreous humour of the eye.

3. **Elastin:**

It is connective tissue protein. It is rubber like i.e. it can be stretched to several times as their normal length, but recoil to their original shape when the stretching force is relaxed.

It is present in lungs, the walls of large blood vessels and elastic ligaments.

Conjugated Proteins

They are combinations of protein with a non-protein part, called prosthetic Group.

Examples:

Conjugated Protein	*Protein part*	*Prosthetic group*
Hemoglobin	Globin	Heme
Nucleoprotein	Histones	DNA
Rhodopsin	Opsin	11-cis-retinal
Succinate dehydrogenase	Protein	Riboflavin as FAD
Ferritin	Apoferritin	Iron
Ceruloplasmin	Apocerulo-plasmin	Copper

IV. Classification Based on Shape

Globular Proteins

They are spherical or oval in shape. They are easily soluble, e.g. albumins, globulins and protamines.

Fibrous Proteins

The molecules are elongated or needle shaped. Their solubility is minimum. They resist digestion. Collagen, elastin and keratins are examples.

V. Classification Based on Nutritional Value

Nutritionally Rich Proteins

They are also called as complete proteins or first-class proteins. They contain all the essential amino acids in the required proportion. On supplying these proteins in the diet, children will grow satisfactorily. Good examples are casein of milk, albumin and globulin.

Incomplete Proteins

They lack one essential amino acid. They cannot promote body growth in children; but may be able to sustain the body weight in adults. Proteins from pulses are deficient in methionine, while proteins of cereals lack in lysine. If both of them are combined in the diet, adequate growth may be obtained.

Poor Proteins

They lack in many essential amino acids and a diet based on these proteins will not even sustain the original body weight. Zein from corn lacks tryptophan and lysine.

MUSCLE PROTEINS

Introduction:

The mass of a muscle is made up of 75% water and more than 20% protein.

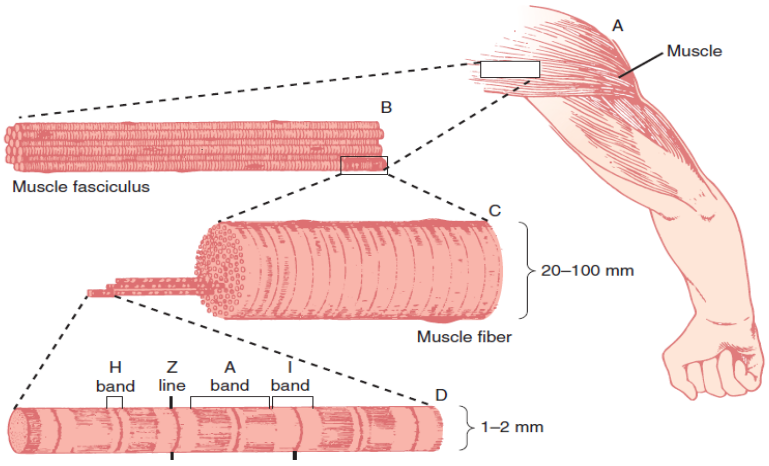


Figure (1) structure of skeletal muscles

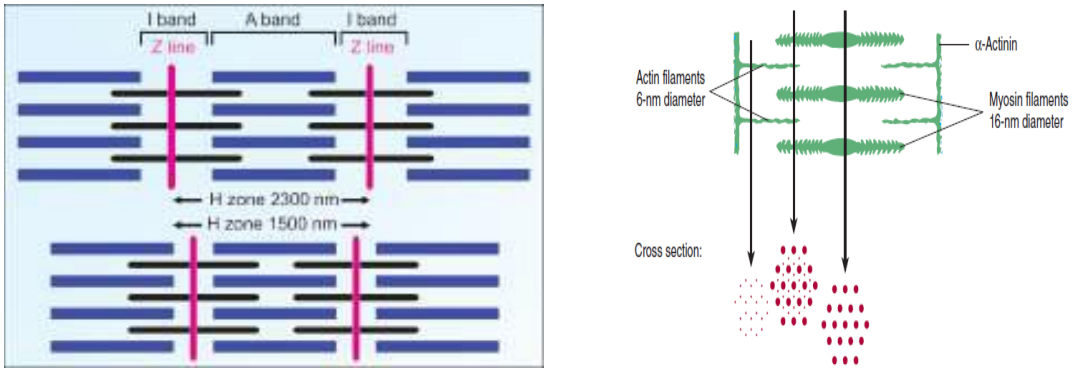


Figure (2) Sliding and shortening of actin and myosin is the basis ofmuscle contraction. Compare the distance between Z lines in the upper and lower pictures

Striated muscle is made up of multinucleated cells bound by plasma membrane called sarcolemma. Sarcomere is the functional unit of muscle. Each muscle cell contains myofibrils about 1 mm in diameter. The myofibrils are immersed in a cytosol that is rich in glycogen, ATP, creatin phosphate and glycolytic enzymes.

The functional unit of a myofibril is a sarcomere. The dark A bands and light I bands alternate regularly. The central H zone of A band is lighter, while the dark M line is

found in the middle of the H zone. The I band is bisected by a very dense narrow Z line.

These bands are formed by variable combination of thick and thin filaments. The thick filaments have a diameter about 150 Å whereas thin filaments have a diameter about 70 Å.

The thick filament is primarily myosin and thin filament contains actin, tropomyosin and troponin. The Z line contains 2 actin molecules and M protein is located in the M line (Fig. 1,2).

Thick and thin filaments slide past each other during the muscle contraction, so that the muscle shortens by as much as a third of its original length. However, the lengths of the thick and thin filaments do not change during muscle contraction (Fig. 2). During muscle contraction, myosin moves over actin filament (Fig. 3).

Figure (3): During muscle contraction, myosin moves over actin filament

Types of muscle proteins

1- Myosin

Myosin structure:

Myosin molecules are large (about 540 kD), each with 6 polypeptide chains; 2 identical heavy chains and 4 light chains. The myosin molecule has a double headed globular end. They are joined to a long double stranded alpha helical coil formed by the heavy chain. At the head portion of each heavy chain, 2 light chains are bound. The heavy chain is thus demarcated into an amino terminal globular head and C-terminal tail. Part of the amino acid sequence in the heavy chain is similar

to that at the active site of other ATPases.

Trypsin cleaves myosin into 2 parts; light meromyosin (LMM) and heavy meromyosin (HMM) types.

The LMM can formfilaments but has no enzymatic activity.

HMM has enzymatic activity and binds actin, but cannot form filaments.

HMM can further be split into:

- the S1 fragments having the ATPase site plus the actin binding site and
- the S2 subfragment

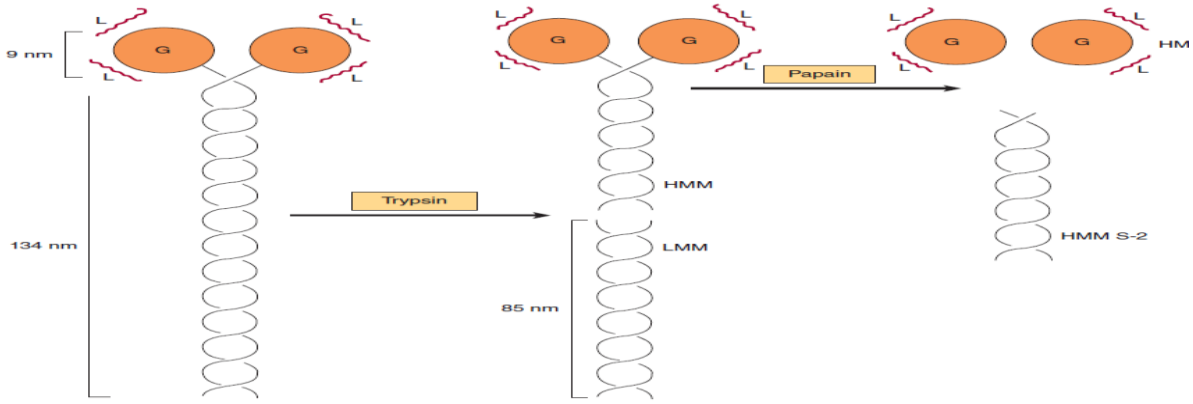


Figure (4): structure of myosin

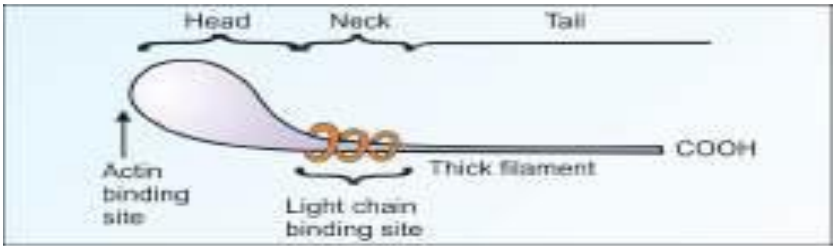


Figure (5): heavy chain of myosin

Functions of myosin:

a. Myosin molecules assemble into filaments (contributes 55% of muscle protein by weight and

forms the thick filaments).

b. Myosin has ATPase enzyme activity.

c. Myosin binds to actin polymer which is the major component of the thin filaments.

2- Actin

It is the major protein of the thin filaments. It is a monomeric protein often referred to as G-actin due to its globular shape. It can polymerize into a fibrous form, called F-actin, which is a helix of actin monomer.

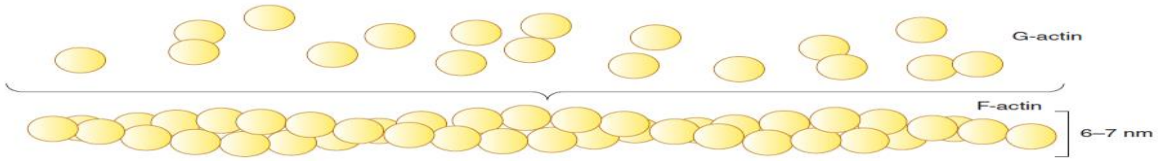


Figure (6): Actin structure

The muscle contraction results from interaction of actin and myosin, to form actomyosin, with energy provided by ATP. When the two thin filaments that bind the cross bridges of a thick filament are drawn towards each other, the distance between Z lines becomes shorter This could result in the process of contraction of muscle fibers. This needs energy from hydrolysis of ATP, effected by the ATPase activity of myosin.

The contractile force is generated by conformational changes, leading to dissociation of actin and S1 heads of myosin. There is a reversible attachment and detachment of myosin S1 head to actin.

3- Tropomyosin

It is a fibrous molecule that consists of two chains, alpha and beta, that attach to F-actin in the groove between its filaments. Tropomyosin is present in all muscular and muscle-like structures.

4- Troponin Complex:

The troponin complex is unique to striated muscle and consists of three polypeptides.

- **Troponin T (TpT):** binds to tropomyosin as well as to the other two troponin components.

Two isoforms of cardiac TnT, called TnT1 and TnT2 are present in adult human cardiac tissue. Serum levels of TnT2 increases within 4 hours of myocardial
infarction, and remains high for up to 14 days. The TnT2 is 100% sensitive index for myocardial infarction.

- **Troponin I (TpI):** also called "actomyosin-ATPase inhibitory element". It inhibits the F-actin-myosin interaction and also binds to the other components of troponin.
  Troponin I is a marker for myocardial infarction. Its level in serum is increased
  within 4 hours of myocardial infarction, and remains high for about 7 days. It is about 75% sensitive index for myocardial infarction. The cardiac form of TnI is 31 amino acids longer than the skeletal muscle form of TnI.
- **Troponin C** (TpC) is a calcium binding polypeptide that is structurally and functionally analogous to **calmodulin,** an important calcium-binding protein widely distributed in nature. Up to four calcium ions can bind per molecule of troponin C or calmodulin, both of which have a molecular mass of 17 kDa.

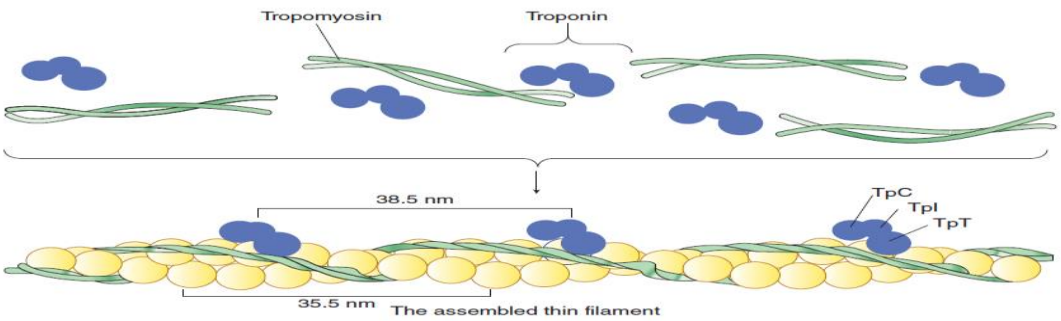


Figure (6): Components of thin filament

Inherited Diseases due to Abnormality of Muscle Proteins

Malignant hyperthermia

Following halothane and succinylcholine (used in anesthetic practice) high fever can be seen. Here calcium channels remain open, and so cytosolic calcium

concentration is remains high. The drug of choice in the treatment is dantrolene, which inhibits release of calcium from the sarcoplasmic reticulum, so that cytoplasmic calcium is kept at a reduced rate. In this condition, there are different mutations in the calcium release channel protein (*RYR1* gene) or in *DHPR* gene (dihydropyridine receptor, a voltage gated calcium channel).

Muscular dystrophies

Dystrophin is a structural protein, attached to muscle cell membrane. Dystrophin is part of a large complex, consisting of dystroglycan, laminin and sarcoglycans. Dystrophin links the actin of cytoskeleton of the cell into the extracellular matrix.

- Mutations in the dystrophin gene cause Duchenne muscular dystrophy or a milder form called Becker muscular dystrophy.
- Some forms of cardiomyopathy are also related to mutations in dystrophin. Mutations in sarcoglycans cause limb girdle dystrophy.
- Mutations in the genes for the glycosyl transferases (which add the sugar groups to proteins) are also responsible for some types of muscle dystrophies.
- Mutations in the cardiac myosin heavy chain cause familial hypertrophic cardiomyopathy.

Proteins of Extracellular Matrix (ECM)

Background:

Most mammalian cells are located in tissues where they are surrounded by a complex ECM often referred to as "connective tissue," which protects the organs and also provides elasticity
where required (eg, in blood vessels, lungs, and skin).

The ECM contains three major classes of biomolecules:

(1) Structural proteins, for example, collagen, elastin, and fibrillin,
(2) certain specialized proteins, such as fibronectin and laminin, which form a mesh of fibers
(3) proteoglycans: in which the protein fibers are impeded in.

The ECM has been found to be involved in many normal and pathologic processes

- It plays important roles in development, in inflammatory states, and in the spread of cancer cells.
- Involvement of certain components of the ECM has been documented in both rheumatoid

arthritis and osteoarthritis.

- Several diseases (eg, osteogenesis imperfecta and a number of types of the Ehlers-Danlos

syndrome) are due to genetic disturbances of the synthesis of collagen.

- Specific components of proteoglycans (the glycosaminoglycans; GAGs) are affected in the group of genetic disorders known as the mucopolysaccharidoses.
- Changes occur in the ECM during the aging process.

Extracellular Matrix (ECM) proteins:

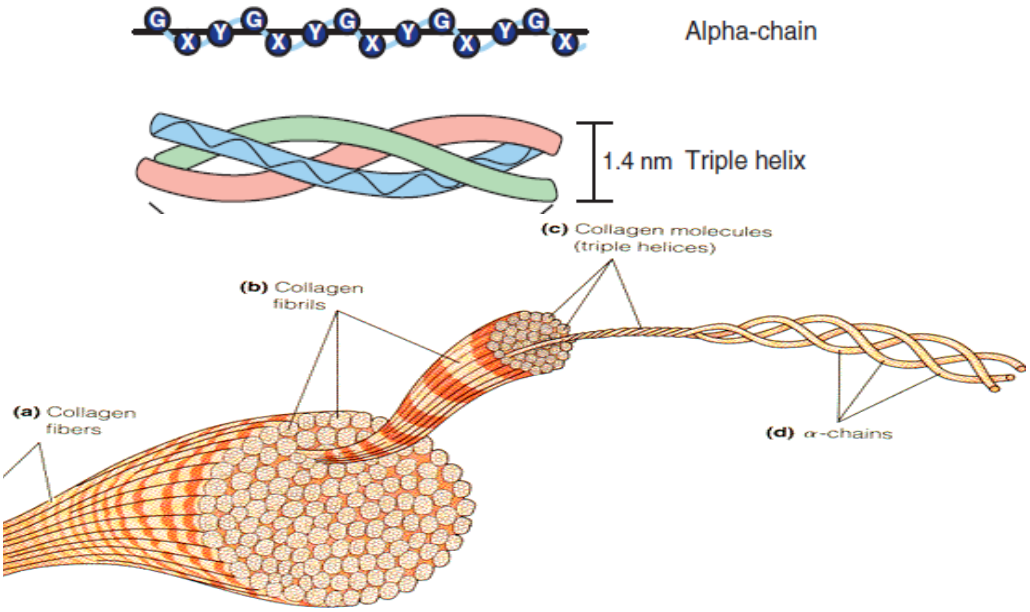
1. COLLAGEN

The major structural protein found in connective tissue is the collagen. It is the most abundant protein in the body. About 25–30% of the total weight of protein in the body is collagen. It serves to hold together the cells in the tissues. It is the major fibrous element of tissues like bone, teeth, tendons, cartilage and blood vessels.

Depending on the amino acid variations, there are 27 types of collagens described. Type I is the most abundant form, seen in connective tissues in almost all regions of the body. Type II is mainly seen in cartilage and vitreous humor, Type III is seen in skin, lung and vascular tissues and Type IV is seen in the basement membranes. Others are seen in minor quantities. About 30 genes are responsible for collagen synthesis, and the enzymes necessary for collagen synthesis

Collagen Structure

- All collagen types have a triple helical structure made up of three polypeptide chains called α-chains.
- Collagen contains **33%** glycine amino acid, **10%** proline, **10%** hydroxy proline and **1%** hydroxylysine.
- Every third amino acid in the α-chain is glycine.
- The repeating sequence is glycine-X-Y, where X is frequently proline and Y is often hydroxy-proline or hydroxylysine.



Synthesis of Collagen

The collagen is synthesized by fibroblasts in the ribosomes in a precursor form, **preprocollagen,** which contains a leader or signal sequence that directs the polypeptide chain into the lumen

of the endoplasmic reticulum.

Post-translational Modifications

1. **Cleavage of signal peptide**

2. **The hydroxylation of proline and lysine residues of collagen**.

Prolyl hydroxylase and lysyl hydroxylase are both di-oxygenases using molecular oxygen. The enzyme also contains ferrous iron at its active site and requires a reducing agent like ascorbic acid to preserve the iron in the reduced ferrous state. So, vitamin C deficiency leads to poor hydroxylation. It is the major biochemical defect in scurvy.

3. **Glycosylation of Procollagen**

The hydroxylated polypeptides are next glycosylated. The common carbohydrate residues added are galactose and glucose, which are added sequentially by galactosyl and glucosyl transferases. The glycosylation occurs only on the hydroxylysine residues.

1. **Extracellular Maturation of Collagen**

Inside the fibroblasts; polypeptides are synthesized, proline and lysine residues are hydroxylated and glycosylation of lysine takes place. Then the procollagen molecules are secreted. Outside the cell, procollagen is cleaved by fibroblast-specific peptidases. About 150 amino acids in N-terminal area and 300 amino acids in C-terminal area are cleaved off. Then tropocollagen molecules are assembled into collagen. Finally covalent crosslinks are formed.
Deficiency of the peptidase leads to dermatopraxis, where the skin is prone to be torn easily.

2. **Crosslinks in Collagen Fibers**

The collagen fibers are strengthened by covalent cross- links between lysine and hydroxylysine residues. The crosslinks are formed by lysyl oxidase which converts these amino acids into aldehydes. The older the collagen, the more the extent of crosslinkages. The process continues, especially in old age, so that the skin, blood

vessels and other tissues become less elastic and stiffer, contributing a great extent to the medical problems of the old people.

Functions of Collagen

1. To give support to organs.
2. To provide alignment of cells, so that cell anchoring is possible. This in turn, helps in proliferation and differentiation of cells.
3. It is the protein of connective tissue present in skin, bones, tendons and blood vessels.
4. Bones and teeth are made by adding mineral crystals to the collagen.
5. Collagen may be present as a gel e.g. in extracellular matrix or in vitreous humour of the eye.
6. In blood vessels, if collagen is exposed, platelets adhere and thrombus formation is initiated.

Degradation of Collagen

Collagenases are enzymes that can degrade collagen. Collagen is a protein resistant to attack by ordinary enzymes.

Adult human tissues do not have any appreciable amount of collagenase activity.

Degradation of collagen is seen when there is bone and cartilage resorption, osteoporosis, tumor metastasis, during postpartum involution of uterus, Paget's disease, rickets, osteoarthritis, rheumatoid arthritis, and scurvy.

Solubility and denaturation:

1) Solubility: Collagen is insoluble in all solvents. It is protein of low biological value and not
digestible.

2) Denaturation:

When collagen is heated, it loses all of its structure. The triple helix unwinds and the chains
are separated. Then when this Denaturated mass cools down, it soaks up all of the surrounding water like sponge, forming Gelatin.

- Gelatin is soluble in water and digestible.
- Gelatin is given for patients during convalescence (in the form of jelly).

2. ELASTIN

Elastin is a protein found in connective tissue and is the major component of elastic fibers. The elastic fibers can stretch and then resume their original length. They have high tensile strength. They are found in the ligaments, lungs as well as in the walls of the blood vessels, especially large vessels like aorta. One-third of the residues are glycine. Proline is present in large amounts, so also alanine. Hydroxyproline is present in small amounts while hydroxylysine and glycosylated hydroxylysine are absent. Triple helix structure is also absent. When elastin matures, desmosine cross links are formed from 4 lysine residues. (Collagen has aldol cross links, while elastin has desmosine crosslinks). Once mature, elastin is very stable; the turn over rate is very low.

Major Differences Between Collagen and Elastin

Collagen	Elastin
1. Many different genetic types	One genetic type
2. Triple helix	No triple helix; random coil conformations permitting stretching
3. $(Gly\text{-}X\text{-}Y)_n$ repeating structure	No $(Gly\text{-}X\text{-}Y)_n$ repeating structure
4. Presence of hydroxylysine	No hydroxylysine
5. Carbohydrate-containing	No carbohydrate
6. Intramolecular aldol crosslinks	Intramolecular desmosine cross-links
7. Presence of extension peptides during biosynthesis	No extension peptides present during biosynthesis

3. Fibronectin

Fibronectin is a cell surface protein that is involved in the interaction of cells with the extracellular matrix. It has been found to play key roles in cell adhesion, cell migration, blood clotting and wound healing.

Deficiency of fibronectin in tumor cells account for their lack of adhesive properties and chances of metastasis.

Plasma fibronectin is produced by hepatocytes and secreted into bloodstream. Cellular fibronectin is produced by fibroblasts. Fibronectin has binding sites for collagen, integrin, heparin, fibrin, DNA and cell surface.

4. Laminin

It is a basement membrane protein with adhesive properties that enable epithelial cells to fix to underlying connective tissue. It is the first extracellular matrix protein manifested during embryogenesis. It has a vital role to play in neuronal outgrowth and nerve regeneration. It is a glycoprotein with three polypeptide chains. High levels of laminin have been reported in patients suffering from Alzheimer's disease. Increased expression of laminin is associated with senile plaques and amyloid proteins.

Biochemistry of immunoglobulins

Background

Immunology is one of the rapidly advancing branches of medical science.

Three major features of immunological reactions are:

Recognition of self from nonself or foreign substances; specificity of the reactions, and memory of the response.

When injected with 100 different proteins, the animal will produce 100 different antibodies; this is called specificity.

If the same antigen is introduced for a second time, body will react immediately; this memory is the basis of vaccination.

Immune Response

The lymphocytes generated from the bone marrow, passed through and processed by the thymus gland, are then called **T-lymphocytes**. They can directly kill the target cells and are the effector cells for the **cell-mediated immunity** (CMI. In peripheral blood 80% lymphocytes are T-cells, and 15% are B-cells. Immunoglobulins are secreted by **Plasma cells** belonging to the B-lymphocytes. The B-cells govern the **humoral immunity**

Cell Mediated Immunity

The following are the major activities of T-lymphocytes.

A. **Immunity against infections:** T-cells mediate effective immunity against bacteria, such as mycobacteria, many viruses and almost all parasites.

B. **Rejection of allograft:** When an organ (heart, kidney) is transplanted from one person to another, it is called allograft. Body tries to reject such transplanted organs, mainly by T-cell mediated mechanism.

C. **Tumor cell destruction:** Although other mechanisms are also involved in killing tumor cells, T-cell activity is the predominant one.

D. **Helper function:** T helper (TH) cells are a sub-group of cells which carry CD4 determinants on the cell surface (CD = cluster determinant). They are necessary for optimal antibody production by plasma cells and for generation of cytotoxic T-cells. They are selectively destroyed in AIDS.

E. **Suppressor function:** T suppressor (TS) cells are CD8 positive cells. They downregulate the activities of both T-and B-cells.

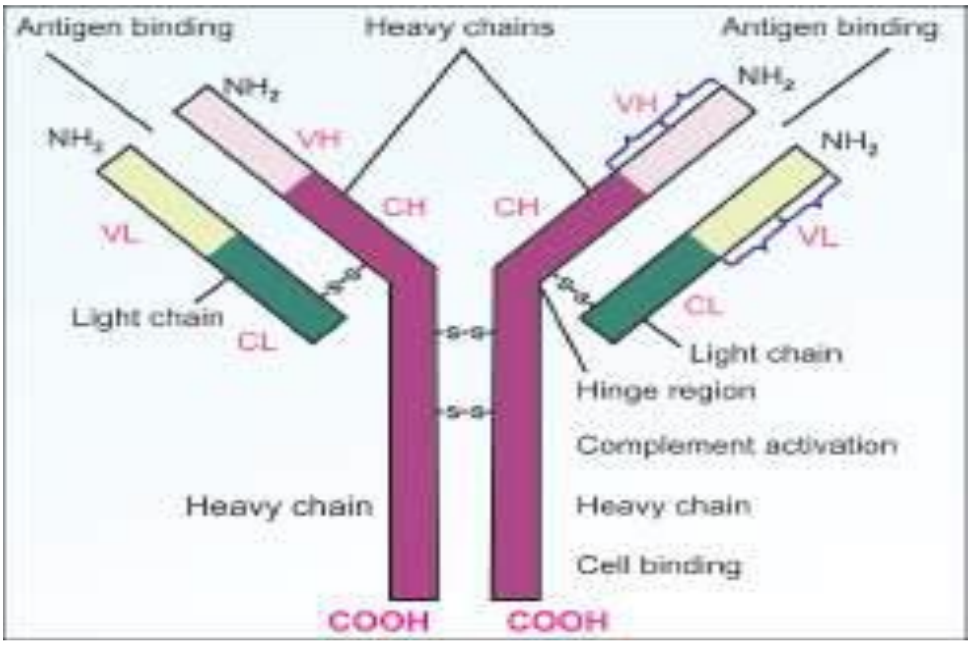
Humoral Immunity

Antibodies are produced by plasma cells. These are immunoglobulins, described in detail below. The antigen antibody reaction leads to activation of complement system, which destroys the foreign cells. The antibodies can destroy
the target cells by the following mechanisms:
(1) Classical complement pathway, (2) Antibody dependent cell mediated cytotoxicity (described below), (3) Agglutination,
(4) Opsonization of target cells, thereby making them more susceptible to phagocytosis.

Immunoglobulins (Gamma globulins):

Definition: These are a group of proteins (gamma globulins) produced by
the body (from B lymphocytes and plasma cells) in response to the presence of foreign substances.
The antibody reacts with antigen very specifically. This property is widely used
in purification of proteins. This affinity is based on the complementary nature of the three-dimensional structure of antigen and antibody.

Structure of Immunoglobulins



Structure of lgG molecule is shown in the figure above. The basic unit of all immunoglobulin molecules consists of 4 polypeptide chains 2 heavy (H) chains and 2 light (L) chains, combined through disulfide bridges.

Depending on the heavy chain make up, the immunoglobulins are differentiated into 5 major classes.

1. **Immunoglobulin-G (lgG)** is made up of heavy chain γ (gamma)

2. **lgM** has μ (mu) heavy chain

3. **lgA** has α (alpha) heavy chain

4. **lgD** contains δ (delta)

5. **lgE** heavy chain is called ε (epsilon).

The **light chains** are either κ (kappa) or λ (lambda) in all the classes. For example, lgG may consist of either γ2 κ2 or γ2 λ2. In human beings, 60% light chains are of κ variety and 40% are of λ type.

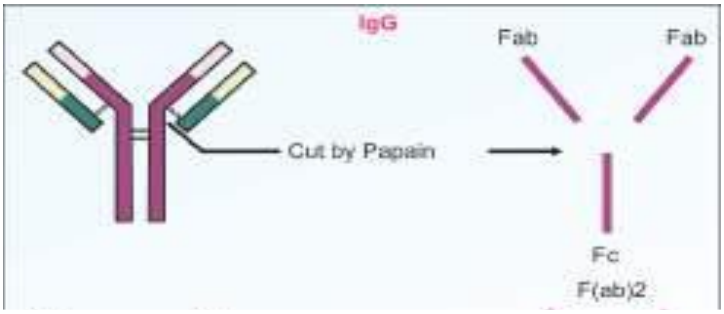
Variable and Constant Regions

Both the heavy and light chains contain relatively variable (V) and constant (C) regions with regard to their amino acid composition. VL and CL are the general terms for these regions on the light chain; while VH and CH specify variable and constant regions on the heavy chain. The first 108 amino acids in light chains and first 118 amino acids in g heavy chains constitute the variable region. Here the amino acid sequence can vary in H and L chains, so that the body could synthesize enormous varieties of different antibodies.

Fab and Fc Portions

- **Papain** (a proteolytic enzyme) cleaves the lg, so that two Fab (fraction antibody) portions and one Fc (fraction crystallizable) portion are produced.
- The **antigen binding part** of the antibody is in the Fab fragment.
- The cleavage takes place in the **hinge region**, where lg molecule can have mobility in 3-dimensional space,/so as to adjust for tight grip on the antigen. Carbohydrate groups of the Ig molecule are also situated in the hinge region.

- The area capable of **complement binding** lies in the Fc portion.



Different Classes of Immunoglobulins

Immunoglobulin G (IgG)

- IgG is formed of one unit only contains two heavy chains and two light chains; heavy chains being of gamma type.
- IgG is the major antibody; it constitutes about 75–80% of total immunoglobulins in circulation. It is the antibody seen in **secondary** immune response.
- It can pass from vascular compartment to interstitial space. It can cross placental barrier, and protects the newborn child from infections. These maternal antibodies are seen in neonatal circulation up to 2–4 months.

Immunoglobulin M (IgM)

- IgM is formed of 5 monomers, each having 4 peptide chains (total 10 heavy chains and 10 light chains) are joined together by a J-chain polypeptide.
- It can combine with 5 antigens simultaneously, and so IgM is very effective for agglutinating bacteria.
- Being a large molecule, it cannot come out of vascular space (can not cross the placenta).
- lgM is the major immunoglobulin during the primary immune response i.e. it is the first of the antibodies which act on introducing of a foreign antigen into the plasma. Its presence indicates recent infection.

- Natural antibodies are IgM in nature. Thus, a person having blood group A antigen will have anti-B antibodies in his circulation (isohemagglutinins). These are produced without any known antigenic.

Immunoglobulin A (IgA)

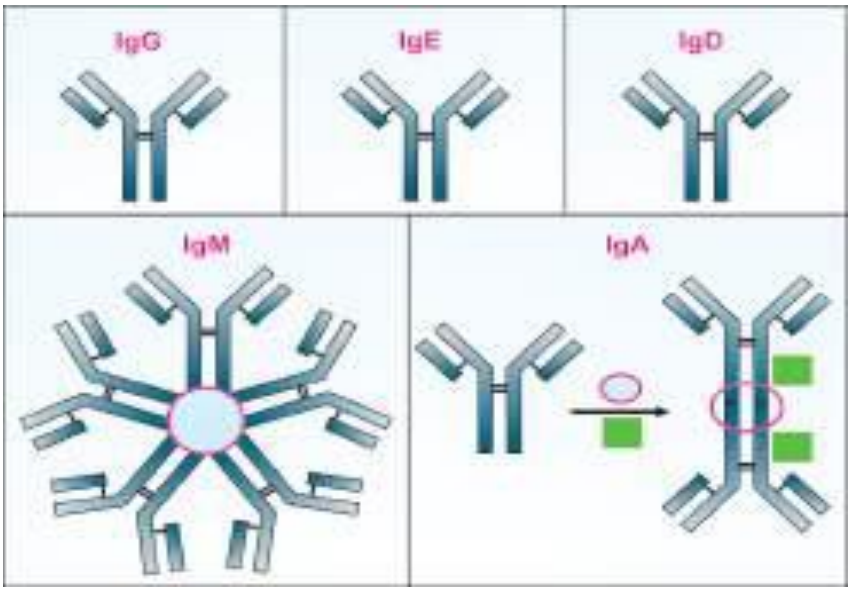
- IgA usually is dimer (total 4 heavy chains and 4 light chains). The J chain connects the dimers
- They are the secretory antibodies seen in seromucous secretions of gastrointestinal tract, nasopharyngeal tract, urogenital tract, tears, saliva, sweat, etc.
- The dimers are stabilized against proteolytic enzymes by the secretory piece. The secretory piece is produced in liver, reaches to the intestinal mucosal cells, where it combines with IgA dimer to form the secretory IgA which is then released.

Immunoglobulin E (IgE)

- They are cytophilic antibodies. They mediate allergy hypersensitivity and anaphylaxis.
- They have the property to fix on mast cells and basophils. When certain antigens such as penicillin are injected a few times, IgE class antibodies are produced which anchor on mast cells. When the same chemical is injected next time, the antigens fix on such antibodies, causing mast cell degranulation, and release of **histamine** and slow reacting substance. This leads to vasodilatation, hypotension and bronchiolar constriction. This is the basis of penicillin anaphylaxis, asthma by pollen and urticaria by absorbed food elements. The peak of this reaction will be at about 30 minutes; hence called **immediate type** hypersensitivity.
- IgE level in serum is markedly increased in helminthic infections.

Immunoglobulin D (lgD):

- It is present in a very low concentration in serum.
- It cannot cross the placenta.
- lgD is a cell membrane immunoglobulin found on the surface of B-lymphocytes. It serves in this location as a specific antigen receptor.
- IgD has activity against thyroid tissue, insulin, and diphtheria toxoid.



	IgG	*IgA*	*IgM*	*IgD*	*IgE*
Nomenclature of heavy chain	γ	α	μ	δ	ε
Heavy chain domains	4	4	5	4	5
No. of basic 4-peptide units (2L + 2H)	1	2	5	1	1
Additional unit	—	S and J	J piece	—	—
Molecular weight (Daltons)	1,46,000	3,85,000	9,70,000	1,85,000	1,90,000
Sedimentation coefficient	7 S	11 S	19 S	7 S	8S
Carbohydrate content (%)	2–3	8–10	12	10–13	11–12
Concentration in normal serum / 100 mL	800–1200 mg	150–300 mg	50–200 mg	1–10 mg	1.5–4.5 μg
Half-life in days	20	6	10	3	2
Distribution (% intravascular)	45	5	95	75	50

VITAMINS

Definition: vitamins are organic compounds occurring in small quantities in different natural foods and necessary for growth and maintenance of good health. They are essential for the proper utilization of carbohydrates, lipids and proteins. Many of them act as **coenzymes**

Provitamins:

These are **precursors** of vitamins that converted into vitamins inside the body e.g. **carotenes** are **Provitamin A.**

Classification:

1. Fat-soluble vitamins are A, D, E and K

2. Water soluble vitamins are named as B complex and vitamin C.

	Fat soluble vitamins	*Water soluble vitamins*
Solubility in fat	Soluble	Not soluble
Water solubility	Not soluble	Soluble
Absorption	Along with lipids Requires bile salts	*Absorption simple
Carrier proteins	Present	*No carrier proteins
Storage	Stored in liver	*No storage
Excretion	Not excreted	Excreted
Deficiency	Manifests only when stores are depleted	*Manifests rapidly as there is no storage
Toxicity	Hypervitaminosis may result	Unlikely, since excess is excreted
Treatment of deficiency	Single large doses may prevent deficiency	Regular dietary supply is required
Major vitamins	A, D, E and K	B and C
*Vitamin B_{12} is an exception.		

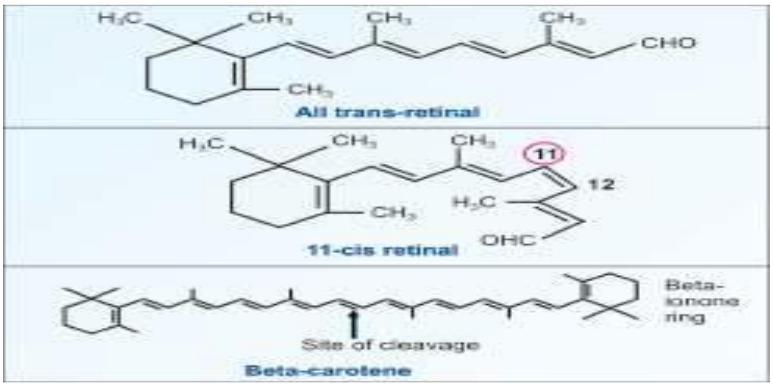
I. Fat soluble vitamins:

VITAMIN A

Chemistry

- Vitamin A is fat soluble. The active form is present only in animal tissues.
- The **pro-vitamin**, beta-carotene is present in plant tissues.
- All the compounds with vitamin A activity are referred to as **retinoids**.

- Three different compounds with vitamin A activity are **retinol** (vitamin A alcohol), **retinal** (vitamin A aldehyde) and **retinoic acid** (vitamin A acid).
- The vitamin A from liver is transported to peripheral tissues as trans-retinol by the retinol binding protein or RBP



Functions of vitamin A:

1. Vision: Retinal is essential for vision.

2. Reproduction: Retinol is essential for reproduction. It supports sperm formation **(**spermatogenesis) in males and maintains fetal life in females.

3. Growth: Retinol is essential for normal growth and bone & teeth formation.

4. Maintenance of epithelial cells: Retinol and retinoic acid are essential for normal differentiation of epithelial cells. This is important for smoothness of skin and mucus membranes. Retinol is also essential for intact cornea.

5. Retinoic acid: is important for

a) **Glycoprotein** synthesis.

b) **Phospholipids** synthesis in the lungs (lung surfactant).

6. Antioxidant (anticancer) action:

a) Retinoids and Carotenoids **(carotenes) act as antioxidants** and protect tissues from toxic effect of some oxidants that may lead to epithelial tissue cancer.

Role of vitamin A in vision:

a) The human retina contains two types of receptor cells for vision; **cones** and **rods**:

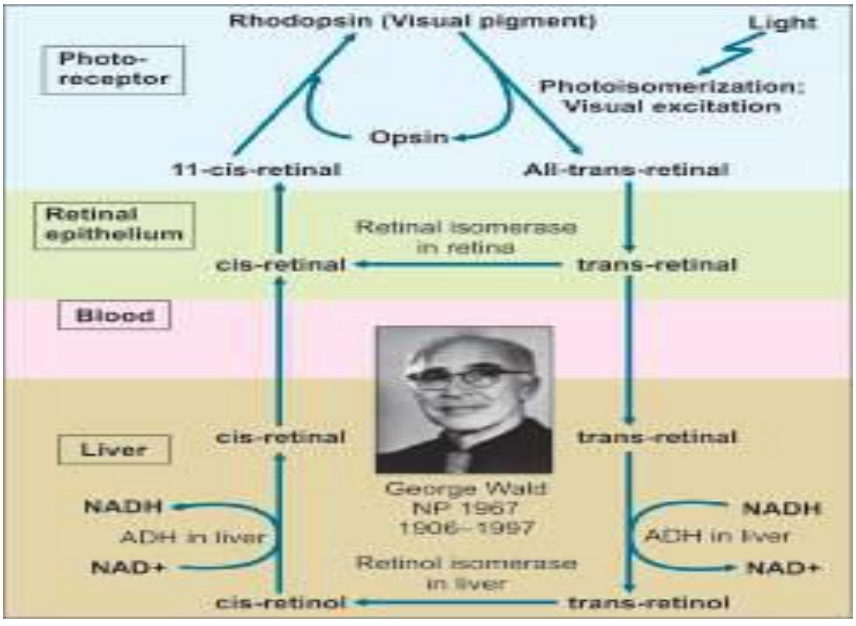
1) Cone cells are responsible for day vision and color.

2) Rod cells are responsible for vision in poor light e.g. at night.

b) Vitamin A is a component of a **visual pigment** (rhodopsin) present in **rods** and conopsin of cons.

Visual cycle:

- Rhodopsin consists of protein called opsin bound to 11-cis retinal (double bond at position 11 is in Cis form, while other double bonds are in Trans form).
- When rhodopsin is exposed to dark light, 11 Cis retinal is converted into all Trans retinal (all double bonds are in Trans form).
- All Trans retinal changes the permeability of cell membrane of rod cells. This allows the calcium ions to pass out of the cell membrane. This stimulates the nerve impulse in optic nerve. Thus the brain perceives light.
- Rhodopsin must regenerate for vision. All Trans retinal are, converted back to 11-cis retinal.
- The all-trans-retinal is isomerized to 11-cis-retinal in the retina itself in the dark by the enzyme **retinal isomerase.** This reaction is taking place in retinal pigment epithelium. The 11-cis retinal can recombine with opsin to regenerate rhodopsin.
- Alternatively, all-trans-retinal is transported to liver and then reduced to all-trans-retinol by **alcohol dehydrogenase** (ADH), an NADH dependent enzyme. ADH contains zinc, and therefore, **zinc** is important in retinol metabolism. The all-trans-retinol is isomerized to 11-cis-retinol and then oxidized to 11-cis-retinal in liver. This is then transported to retina.



Causes for Vitamin A Deficiency

1. Decreased intake.Obstructive jaundice causing defective absorption.
2. Cirrhosis of liver leading to reduced synthesis of **retinol binding protein** (RBP)
3. Severe malnutrition, where amino acids are not available for RBP synthesis.
4. Chronic nephrosis, where RBP is excreted through urine.

Manifestations of vitamin A deficiency:

1. Eye:
- Night blindness (Nyctalopia): impaired dark adaptation.
- Xero-ophthalmia (Bitot spots): dryness and roughness of cornea.
- Keratomalacia: degradation of corneal epithelium.
- Blindness.

2. Growth retardation.

3. Skin and- mucus membranes: Roughness of skin and mucus membranes of different body systems e.g. urinary system. This leads to infection.

4. Alopecia: loss of hair from the head or body.

Requirements of vitamin A:

Children = 400–600 mg/day, Men = 750–1000 mg/day.

Women = 750 mg/day, Pregnancy = 1000 mg/day

Excess vitamin A (overdose):

- It occurs when excessive vitamin A intake exceeds the capacity of RBP.
- Free retinol will release in blood with the following toxic effects:
- Headache - Nausea - Bone pain - Loss of hair.

Vitamin D (Calciferol),1, 25 dihydroxycholecalciferol ,Calcitriol

Dietary sourses of vitamin D: Ergocalciferol (vitamin D2), found in plants, and chole calciferol (vitamin D3), found in animal tissues, are sources of preformed vitamin D activity.

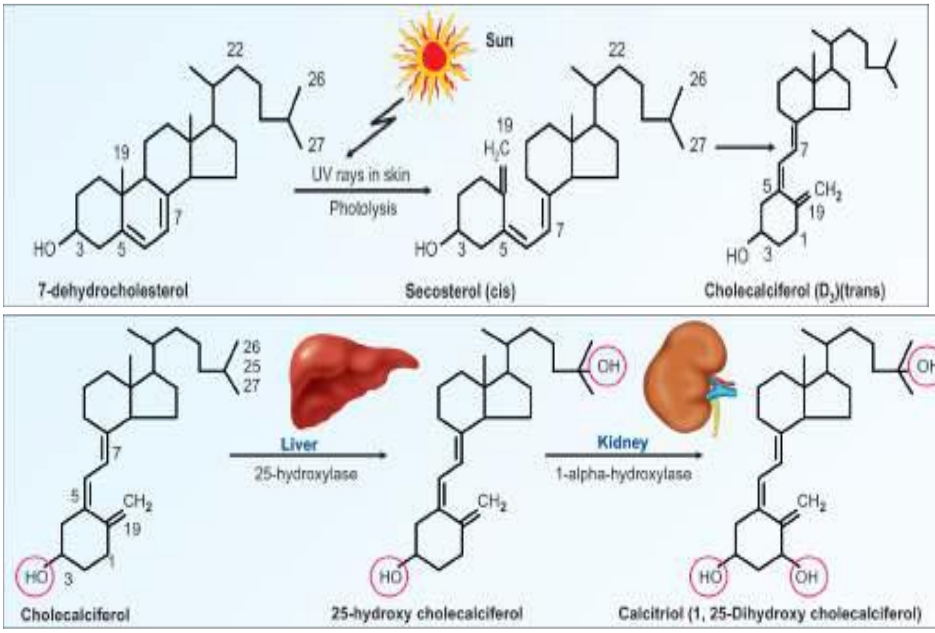
Synthesis of Vitamin D in the body

1. Cholesterol is converted into 7-dehydrocholesterol and transported to skin.
2. UV sunlight (290-320nm) penetrates the skin to break provitamine (7-dehydrocholesterol) to previtamine and it is then converted to Cholecalciferol by the process of isomerisation.
3. In the liver, cholecalciferol undergoes 25-hydroxylation to yield 25(OH) Vit-D (calcidiol).
4. In the kidney, calcidiol undergoes further 1α-hydroxylation to produce 1,25 – dihydroxy Vit-D (Calcitriol). Its production in the kidney is catalyzed by 1α -hydroxylase.

   1α -hydroxylase activity is increased by:

   Decreased serum Ca2+ Increased PTH level Decreased serum phosphate

   Estrogen, prolactin and growth hormone also stimulate 1α -hydroxylase thus increasing Ca absorption during pregnancy, lactation and growth



Functions of 1,25-dihydroxycholecalcififerol (Calcitriol)

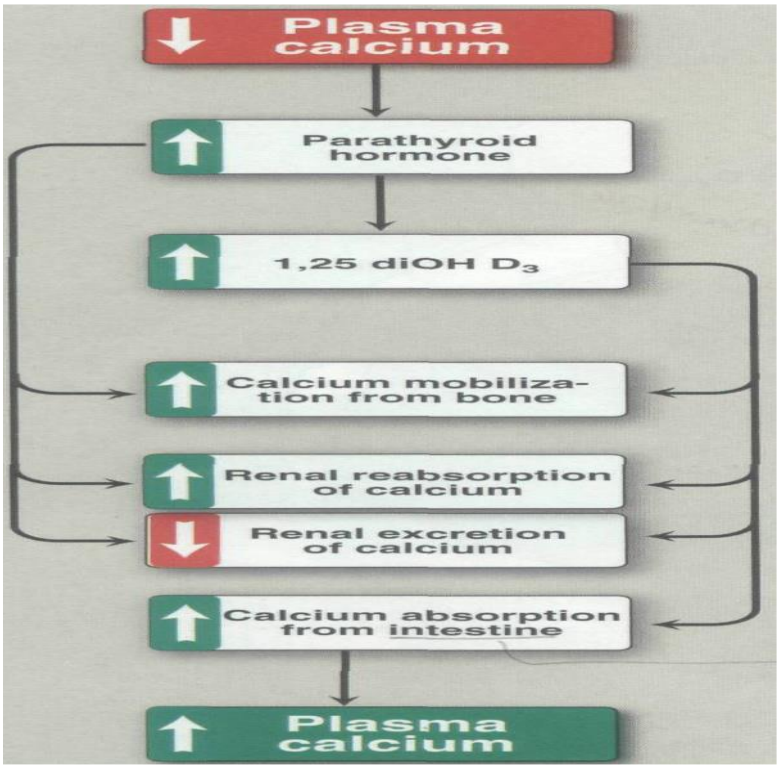
1. **Normalization of serum calcium**
2. **Vitamin D and absorption of calcium:** Calcitriol promotes the absorption of calcium and phosphorus from the intestine. Calcitriol enters the intestinal cell and binds to vitamin D receptor (VDR). The hormone-receptor complex interacts with DNA and causes consequent transcription of specific genes that

code for Calbindin. Due to the increased availability of calcium binding protein, the absorption of calcium is increased. Hence, blood calcium level tends to be elevated.

3. **Vitamin D and bone:** Vitamin D is acting independently on bone. Vitamin D increases the number and activity of osteoblasts, the bone forming cells. It also has a role in osteoclastogenesis. Calcitriol stimulates osteoblasts to secrete alkaline phosphatase. Due to this enzyme, the local concentration of phosphate is increased. The ionic product of calcium and phosphorus increases, leading to **mineralization and remodeling of** bone.

But in case of hypocalcemia, vitamin D stimulates osteoclastic bone resorption.

4. **Vitamin D and renal tubules:** Calcitriol increases the reabsorption of calcium and phosphorus by renal tubules, therefore both minerals are conserved. (PTH conserves only calcium).



Causes for Vitamin D Deficiency

1. Deficiency of vitamin D can occur in people who are not exposed to sunlight properly.
2. Nutritional deficiency

3. Malabsorption of vitamin (obstructive jaundice and steatorrhea). High phytate content in diet may also reduce the absorption of vitamin.
4. Abnormality of vitamin D activation. Liver and renal diseases may retard the hydroxylation reactions.

Manifestations of Vitamin D deficiency in Children & Adults

- In the vitamin D deficiency disease **rickets,** the bones of children are undermineralized as a result of poor absorption of calcium. Similar problems occur as a result of deficiency during the adolescent growth spurt.
- **Osteomalacia** in adults results from the demineralization of bone, especially in women who have little exposure to sunlight, especially after several pregnancies. Although vitamin D is essential for prevention and treatment of osteomalacia in the elderly, there is less evidence that it is beneficial in treating **osteoporosis.**

Requirements:

Children = 10 mg (400 IU)/day, Adults = 5 to 10 mg (200 IU)/day
Pregnancy, lactation = 10 mg/day, Above the age of 60 = 600 IU per day.

Excess vitamin D: (overdose or hypervitaminosis D):
This leads to abnormal calcification of tissues and deposition of calcium and phosphate in different systems e.g. renal stones.

Vitamin E (Tocopherols)

Sources: Vegetables and seed oils. It is present also in fish liver oils.

• **Functions of vitamin E:**

1. Vitamin E is the most powerful natural antioxidant: Vitamin E prevents non-enzymatic oxidation of cell components (e.g. polyunsaturated fatty acids, DNA and cell membranes) by molecular oxygen or free radicals.
2. Vitamin E removes peroxide formation in polyunsaturated fatty acids.
3. Vitamin E protects RBC from hemolysis. By preventing the peroxidation, it keeps the structural and functional integrity of all cells.
4. Protection against heart disease. Vitamin E acts as antioxidant. It prevents oxidation of LDL. Oxidized LDL causes heart disease.

Vitamin E deficiency

- No major disease states have been found to be associated with vitamin E deficiency due to adequate levels in the average diet.

- Vitamin E deficiency is seen in persons (a) who cannot absorb dietary fat, (b) in premature infants (birthweight less than 1500 grams), (c) in abetalipoproteinemia and (d) in mutations in the gene for the tocopherol transfer protein.
- Vitamin E deficiency causes:

1. neurological problems due to poor nerve conduction. These include neuromuscular problems such as spinocerebellar ataxia, retinopathy, peripheral neuropathy and myopathies.
2. Hemolysis of RBCs and anemia: due to lack of protection against peroxides

Vitamin K (anti hemorrhagic Vitamin)

Chemistry:

- There are three forms (Vitamers) of vitamin K: K1, K2 and K3.
- Vitamin K1 has 20C side chain. Vitamin K2 has a 30C side chain.
- Vitamin K3 is synthetic. It is water soluble and more potent than K1 & K2.

Sources of Vitamin K

- **Green leafy vegetables** are good dietary sources.
- Even if the diet does not contain the vitamin, **intestinal bacterial synthesis** will meet the daily requirements, as long as absorption is normal.

Biochemical Role of Vitamin K

- Vitamin K is necessary for coagulation. Factors dependent on vitamin K are Factor II (**prothrombin**); Factor VII Factor IX; Factor X.
- Vitamin K is also necessary for the functional activity of osteocalcin (calcium binding protein) in bones, as well as structural proteins of kidney, lung and spleen.

Causes for Deficiency of Vitamin K

In normal adults dietary deficiency seldom occurs since the intestinal bacterial synthesis is sufficient to meet the needs of the body.

However, deficiency can occur in conditions of:

- malabsorption of lipids. This can result from obstructive jaundice, chronic pancreatitis, sprue, etc.
- Prolonged antibiotic therapy and gastrointestinal infections with

diarrhea will destroy the bacterial flora and can also lead to vitamin K deficiency.

Clinical Manifestations of Deficiency

- Hemorrhagic disease of the newborn is attributed to vitamin K deficiency. The newborns, especially the premature infants have relative vitamin K deficiency. This is due to lack of hepatic stores, limited oral intake (breast milk has very low levels, 15 mg/liter) and absence of intestinal bacterial flora. It is often advised that preterm infants be given prophylactic doses of vitamin K (1 mg Menadione).
- In children and adults, Vitamin K deficiency may be manifested as bruising tendency, echymotic patches, mucous membrane hemorrhage, post-traumatic bleeding and internal bleeding.
- Prolongation of prothrombin time and delayed clotting time are characteristic of vitamin K deficiency.
- Measurement of prothrombin time (PT) is taken as an index of liver function. When liver function is considerably lowered, prolongation of PT occurs due to deficient synthesis of the coagulation factors. In such cases, administration of vitamin fails to restore PT to normal levels. Hence before undertaking any surgery on jaundiced patients, PT before and after
  administration of vitamin K should be done.
- Warfarin and dicoumarol will competitively inhibit the gamma carboxylation system due to structural similarity with vitamin K. Hence they are widely used as anticoagulants for therapeutic purposes.
- Treatment of pregnant women with warfarin can lead to fetal bone abnormalities (fetal warfarin syndrome).

Daily Requirement of Vitamin K

Recommended daily allowance is 50–100 mg/day. This is usually available in a normal diet.

Hypervitaminosis K

Hemolysis, hyperbilirubinemia, kernicterus and brain damage are the manifestations of toxicity.

II. Water soluble vitamins:

Vitamin C (L-Ascorbic acid)

Sources:

- Fruits especially citrus fruits (lemon, orange), melon and strawberry.
- Vegetables especially green leafy vegetables as lettuce, tomatoes, potatoes, raw cabbage and green peppers.
- Guava is very rich in vitamin C.

Chemistry

- It is water soluble and is easily destroyed by heat, alkali and storage. In the process of cooking, 70% of vitamin C is lost.
- Only L-ascorbic acid and dehydroascorbic acid have antiscorbutic activity. D-ascorbic acid has no activity.

Functions of vitamin C:

1. Formation of collagen protein:

a) Ascorbic acid is essential for the conversion of the Procollagen (immature collagen) into collagen. Procollagen is a protein containing proline and lysine. Hydroxylation of both amino acids is catalyzed by hydroxylase enzymes and by vitamin C as a **coenzyme**. This converts Procollagen into collagen.

b) Collagen is essential for the synthesis of connective tissue, bone, cartilage and teeth.

2. Absorption and mobilization of iron: Ascorbic acid is a potent reducing agent, keeping iron in ferrous state:

3. Acts as co-enzyme for many hydroxylase enzymes in the pathway of:

a) Bile acids synthesis: by 7 α hydroxylase.

b) Osteocalcin synthesis: osteocalcin is calcium binding protein in bones.

c) Carnitine synthesis: carnitine is a substance formed in the muscle. It stimulates fatty acid oxidation in mitochondria.

d) Epinephrine synthesis: by hydroxylase required for conversion of tyrosine into epinephrine.

3. **Antioxidant action:** Vitamin C acts as antioxidant and protect tissues from toxic effect of some oxidants that may lead to cancer.

Deficiency: → (**scurvy**): It is characterized by

1. Manifestations due to decreased collagen formation:

a) Bleeding into gum, muscles, joints, kidneys, GIT and pericardium.

b) Defective formation of bone and teeth.

c) Defective healing of wounds.

d) Necrosis of gums and loss of teeth.

2. Anemia: due to decreased absorption of iron and bleeding.

3. Manifestations due to decreased neurotransmitters (epinephrine):

a) Behavioral changes.

b) Severe emotional disturbances.

4. Manifestations due to decrease carnitine and fatty acids oxidation:

a) General weakness.

Excessive vitamin C:

- Intake of high doses of vitamin C produces hyperoxaluria (increased oxalate in urine) and may lead to stone formation.

Requirement of Vitamin C

Recommended daily allowance is 75 mg/day (equal to 50 mL orange juice). During pregnancy, lactation, and in aged people requirement may be 100 mg/day. Smokers and those on oral contraceptives have lower vitamin C levels. Aspirin has been found to block the uptake of vitamin by white blood cells. Hence these people require around 100 mg/day.

Vitamin B complex:

These are a group of vitamins of different chemical molecules. They were put together in one group because:

1. All are soluble in water.

2. All are present in the same sources. B vitamins are particularly abundant in whole grain cereals, liver and yeast.

3. Due to their presence in the same foods: deficiencies of B vitamins are often multiple rather than singular.

Functions of vitamin B complex:

1. All B-complex vitamins serve as coenzymes in enzymatic reactions.

2. Folic acid and B**12** act as coenzymes in hematopoiesis (formation of red blood cells).

Absorption of vitamin B complex:

1. The B vitamins are absorbed in the intestine and transported in the portal circulation.

2. The tissue stores of most B vitamins are minimal. The depletion occurs over several weeks in response to dietary restriction or increased the requirements as in pregnancy. Body stores of folic acid and vitamin B**12** are more extensive than other B vitamins.

Toxicity of vitamin B complex:

Toxic effects are relatively uncommon, since excessive ingestion of water soluble vitamins is followed by saturation of body stores and rapid loss of excess vitamins in the urine.

The important members of vitamin B complex group are:

- Thiamine (vitamin B1, antiberiberi factor)
- Riboflavin (vitamin B2)
- Niacin (nicotinic acid, B3)
- Pyridoxine (vitamin B6)
- Pantothenic acid
- Biotin
- Folic acid
- Vitamin B12

Vitamin B12 = Cobalamins

Sources:

1. Meat, egg, milk and milk products.
2. Vitamin B12 is not present in plant sources. This means that strict vegetarians (vegans) are at risk of developing vitamin B12 deficiency.
3. Intestinal microorganisms synthesize in human colon, but it is not absorbed through the mucosa in this region of the gastrointestinal tract.

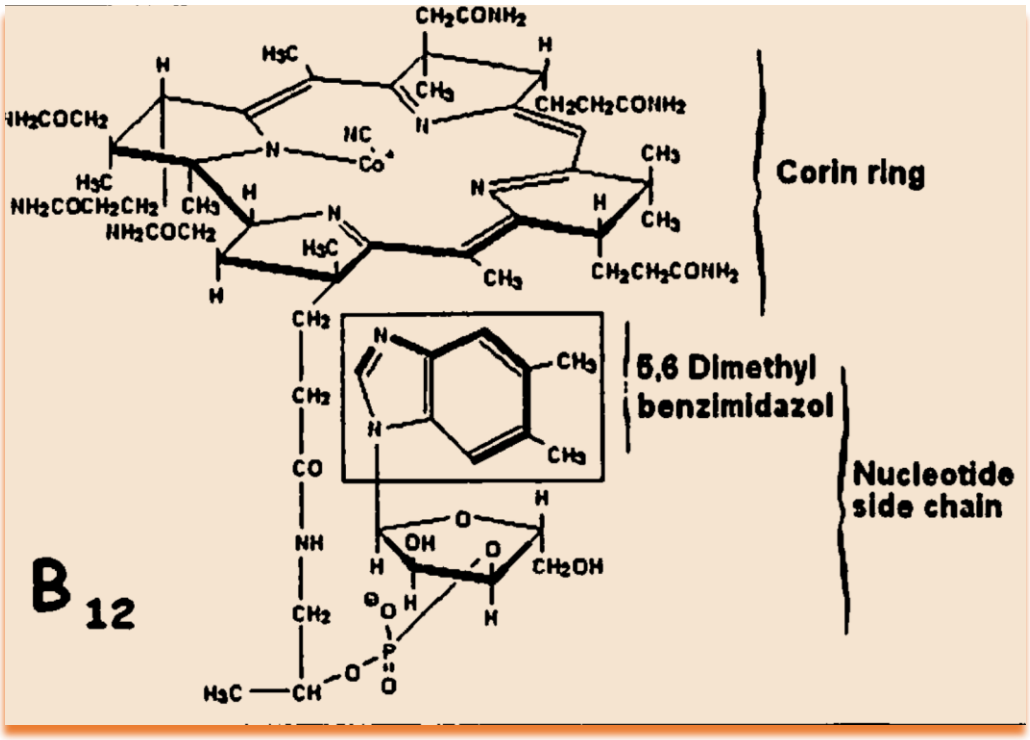
Chemistry:

- Vitamin B12 or cobalamin consists of:

1. A corin ring [formed of 4 pyrrole rings connected by methenyl groups (=CH-)].

2. Cobalt ion at in the center of corin ring. The cobalt is red in color. This is the cause of the red color of vitamin B12.
3. A nucleotide side chain.
4. 4. A cyano group (CN) attached to cobalt ion forming cyanocobalamin.

- Vitamin B12 is water soluble, heat stable.



Absorption of Vitamin B12

- Absorption of vitamin B12 requires two binding proteins:

1. First is the intrinsic factor (IF) secreted by the gastric parietal cells. It is a glycoprotein in nature.
2. The second factor is cobalophilin, secreted in the saliva. Gastric pepsin releases the vitamin from proteins of the food, and then B12 binds with cobalophilin. In duodenum, cobalophilin is hydrolyzed by trypsin of pancreatic juice; vitamin is released, and then vitamin binds to intrinsic factor. In pancreatic insufficiency (absence of trypsin), the vitamin may not be released. Then vitamin cobalophilin complex is excreted, resulting in vitamin deficiency.

- One molecule of IF can combine with 2 molecules of B12. This IF-B12 complex is attached with specific receptors on mucosal cells. The whole IF-B12 complex is internalized.

- B12 is absorbed from ileum
- **Transport and Storage**
- In the blood, **Transcobalamin**, a glycoprotein, is the specific carrier.
- It is stored in the liver cells. B complex vitamins are not stored in the body, B12 is an exception. Whole liver contains about 2 mg of B12, which is sufficient for the requirement for 2–3 years. So, B12 deficiency is seen only years after gastrectomy.

Physiological functions:

Vitamin Bu is required in humans for synthesis of

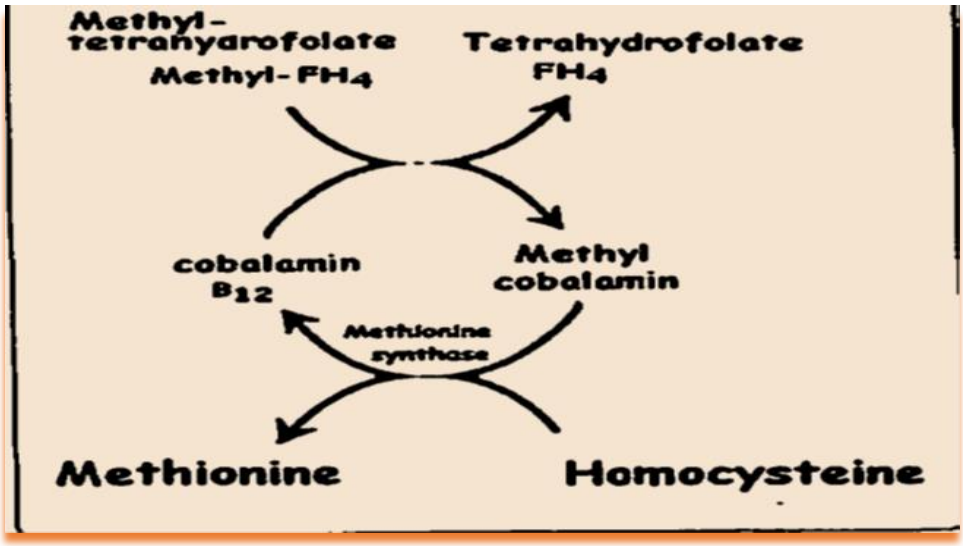
1. methionine
2. Tetrahydrofolate,
3. myelin sheath

Methionine:

a) **Synthesis:**

1) Vitamin 8 12 (in the form of methylcobalamin) acts as coenzyme for methionine synthase enzyme.

2) This enzyme catalyzes the conversion of homocysteine into methionine.



***b)* Functions:**

It is Important for:-

1) Synthesis of phospholipids which enters in the structure of myelin sheath.

2) Prevention of fatty liver.

Tetrahydrofolate:

a) **Synthesis**:

1) Methyl-tetrahydrofolate (methyl-FH4) is converted into Tetrahydrofolate (FH4) by transferring methyl group (-CH3) to cobalamin. Then methylcobalamin transfers the (-CH3) to homocysteine to form methionine.

b) **Functions:**

(FH4) is required for cell division e.g., hemopoiesis.

Deficiency:

Causes:

- Decrease vitamin B 12 intake. This may occur among vegetarians.
- Drug induced vitamin B12 deficiency as neomycin antibiotic and alcohol.
- Atrophy of gastric mucosa leading to lack of intrinsic factor (pernicious anemia).

Manifestations

I. Megaloblastic anemia:

It is a macrocytic hyperchromic anemia. It is due to abnormal replication of DNA in hematopoietic tissue. It is due to direct insufficiency of folate or indirectly to a cobalamin insufficiency.

II. Neuro logical manifestations:

1) They are due to the lack of myelin sheath formation due to the deficiency of methionine and disturbance in the metabolism of odd number fatty acids.

2) They include: -

- Subacute combined degeneration of the spinal cord where both motor and sensory tracts are affected.
- Peripheral neuritis leads to numbness. tingling and weakness of extremities.

III. **Folate trap:** Vitamin B12 deficiency causes simultaneous folate deficiency due to the folate trap. Therefore all the manifestations of folate deficiency are also seen.

Treatment

If megaloblastic anemia is treated with folic acid alone, the anemia may improve, but associated neurological symptoms are aggravated. Hence all macrocytic anemias are generally treated with folate and vitamin B12.

Requirement of Vitamin B12

Normal daily requirement is 1–2 mg/day. During pregnancy and lactation, this is increased to 2 mg/day. Those who take folic acid, should also take vitamin B12. Elderly people are advised to take B12 supplementation.

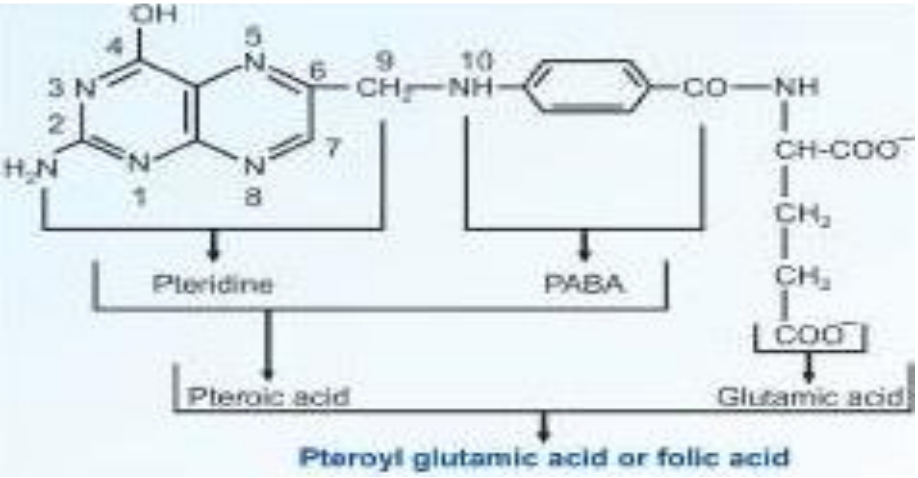
Folic Acid

Sources:

1. The major source is leafy vegetables.

2. Liver, beans and whole grain cereals.

Chemistry:



It is composed of three constituents: The pteridine group linked with para-amino benzoic acid (PABA) is called pteroic acid. It is then attached to glutamic acid to form pteroyl glutamic acid or folic acid.
Folic acid is soluble in water. When exposed to light, it is rapidly destroyed.

Absorption of Folic Acid

Folic acid is readily absorbed by the upper part of jejunum. Folic acid is not stored in tissues.

Functions of folic acid:

1. The active form is tetrahydrofolic acid (H4 folate) which functions as a carrier for one-carbon groups.
2. The one carbon groups are utilized for the synthesis of:

a) Synthesis of DNA and RNA through synthesis of purines (adenine and guanine) and methylation of uracil into thymine. Thus, folate is required for cell formation including blood cells.

b) Synthesis of nonessential amino acids e.g. serine and glycine.

c) Conversion of homocysteine into methionine.

Deficiency:

Causes:

Folic acid deficiency is very common.

- **Pregnancy:** Folate deficiency is commonly seen in pregnancy, where requirement is increased.
- **Defective absorption:** In sprue, celiac disease, gluten induced enteropathy, resection of jejunum.
- **Drugs:** Anticonvulsant drugs (hydantoin, dilantin, phenytoin, phenobarbitone) reduce folate absorption
- **Dietary deficiency:** Absence of vegetables in food for prolonged periods may lead to deficiency.
- **Folate trap:** The only way for generation of free THFA. When B12 is deficient, this reaction cannot take place, leading to folate deficiency

The manifestations

- They are due to defective synthesis of DNA and RNA leading to defective cell formation including blood cells.
- The manifestations of folate deficiency include:

a) Pancytopenia: i.e. all blood cells are affected.

1) Megaloblastic anemia: (macrocytic anemia).
2) Leucopenia: decreased W.B.Cs.
3) Thrombocytopenia: decreased Platelets.

b) Impaired growth and neural tube defects in fetus.

Minerals

Classification:

According to the body needs, minerals may be divided into 2 groups:

A. Macrominerals:

1. They are required in amounts greater than 100 mgfday.
2. They include 6 elements: calcium, phosphorus, magnesium, sodium, potassium and chloride.

B. Microminerals (trace elements):

1. They are required in amounts less than 100 mg/day.
2. They include 10 elements: chromium, cobalt, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, zinc and silicon.

Calcium

Calcium distribution:

- Most of the calcium in the body exists as the mineral hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

Dietary sources:

Milk and dairy products, Green leafy vegetables, seafood, almonds, blackstrap molasses, broccoli, enriched soy and rice milk products, figs, soybeans and tofu.

Absorption of Ca:

- Absorption is taking place from the first and second part of duodenum against concentration gradients. Absorption required a carrier protein, helped by Ca-dependent ATPase.

  Causes of increased calcium absorption-

  1- calcitriol , active form of Vit-D
  2- 2- PTH
  3- 3-acidic pH
  4- Lysine and Arginine amino acids

Causes of inhibited Ca absorption:

1- **Phytic acid:** Hexaphosphate of inositol is present in cereals. Fermentation and cooking reduce phytate content.
2- **Oxalates:** They are present in some leafy vegetables, which cause formation of insoluble calcium oxalates.
3- **Malabsorption syndromes:** Fatty acid is not absorbed, causing formation of insoluble calcium salt of fatty acid.
4- **Phosphate:** High phosphate content will cause precipitation as calcium phosphate. The optimum ratio of calcium to phosphorus which allows maximum absorption is 1:2 to 2:1 as present in milk.

Biological functions of Calcium

1- Bone and teeth mineralization
2- Regulate neuromuscular excitability: Calcium mediates excitation and contraction of muscle fibers.
3- Myocardium: Ca++ prolongs systole. In hypercalcemia, cardiac arrest is seen in systole. This fact should be kept in mind when calcium is administered intravenously. It should be given very slowly.
4- Blood coagulation: Calcium is known as factor IV in blood coagulation cascade
5- Vascular Permeability: Calcium decreases the passage of serum through capillaries. Thus, calcium is clinically used to reduce allergic exudates.
6- Enzyme reactions:
   Calmodulin is a calcium binding regulatory protein, with a molecular weight of 17,000 Daltons. Calmodulin can bind with 4 calcium ions. Calcium binding leads to activation of enzymes e.g. Glycerol-3-phosphate dehydrogenase, Glycogen synthase. Calmodulin is part of various regulatory kinases. Some other enzymes are activated directly by Ca++ without the intervention of calmodulin; examples are
   pancreatic lipase; enzymes of coagulation pathway; and rennin (milk clotting enzyme in stomach).
7- Release of hormones and neurotransmitters: Calcium is necessary for transmission of nerve impulses from pre-synaptic to post-synaptic

region, and mediates secretion of Insulin, parathyroid hormone, calcitonin, vasopressin, etc. from the cells.

8- Intracellular second messenger: Calcium and cyclic AMP are second messengers of
different hormones e.g., glucagon.

Calcium in Blood

- Normal blood level: Normal calcium level is 9–11 mg/dL. (10 mg/dL of Ca++ = 5 mEq/L).
- Ionized calcium: About 5 mg/dL of calcium is in ionized form and is metabolically active (Fig. 39.4). Another 1 mg/dL is complexed with phosphate, bicarbonate and citrate. These two forms are diffusible from blood to tissues.
- Protein bound calcium: About 4 mg/dL of calcium is bound to proteins in blood and is non diffusible.

Factors Regulating Blood Calcium Level

I. Vitamin D

Action of 1,25-dihydroxycholecalcififerol (Calcitriol)

1. **Normalization of serum calcium**

a. Vitamin D and absorption of calcium: Calcitriol promotes the absorption of calcium and phosphorus from the intestine. Calcitriol enters the intestinal cell and binds to vitamin D receptor (VDR). The hormone-receptor complex interacts with DNA and causes consequent transcription of specific genes that code for Calbindin. Due to the increased availability of calcium binding protein, the absorption of calcium is increased. Hence, blood calcium level tends to be elevated.

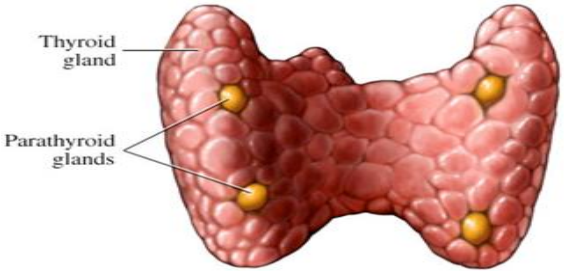
b. in case of hypocalcemia, vitamin D stimulates osteoclastic bone resorption.

c. Vitamin D and renal tubules: Calcitriol increases the reabsorption of calcium and phosphorus by renal tubules, therefore both minerals are conserved. (PTH conserves only calcium).

2. **Mineralization of bone**: Vitamin D stimulates mineralization and remodeling of bone.

II. Parathyroid hormone (PTH)

It is a polypeptide synthesized as pre-pro-PTH(115aa) and is cleaved to pro-PTH(90aa) with cleavage before secretion of PTH (84aa). It is secreted from the chief cells of the parathyroid glands.



Functions:

1. Increase renal phosphate excretion and decrease plasma phosphate levels.
2. Increases plasma calcium by:

   Increasing osteoclastic resorption of bone (occurring rapidly).

   Increasing intestinal absorption of calcium (a slower response).

   Increasing synthesis of 1,25-(OH)2D3 (stimulating GIT absorption).

   Increasing renal tubular reabsorption of calcium

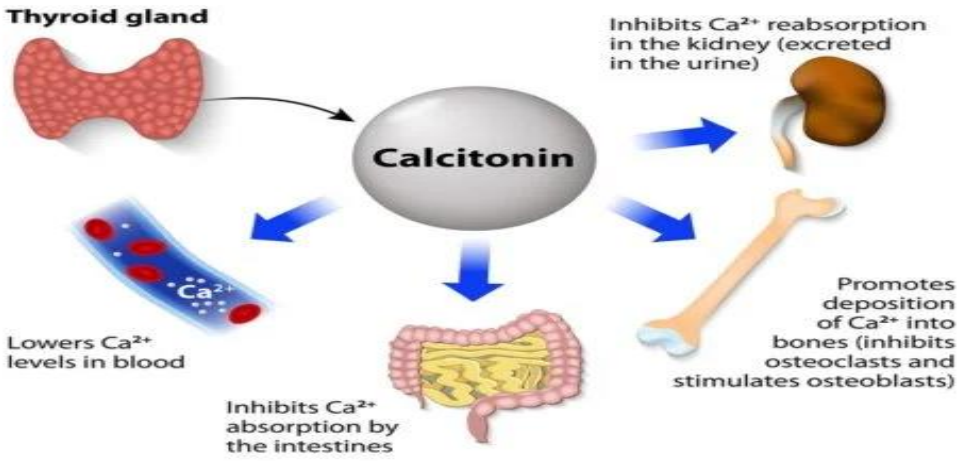
III. Calcitonin

This is produced from the C-cells of the thyroid. It is a Ploypeptide (32 aa) , MW 35KD.

The major stimulus of calcitonin secretion is a rise in plasma Ca++ levelsCalcitonin is a physiological antagonist to PTH with regard to Ca++ homeostasis.

Calcitonin acts via increased cAMP concentrations to inhibit osteoclast motility and cell shape and inactivates them. It inhibits Ca reabsorption in the kidney.

The major effect of calcitonin administration is a rapid fall in Ca2+ caused by inhibition of bone resorption.



Alterations of plasma calcium:

1. Hypercalcemia:

Causes

- Primary hyperparathyroidism: usually due to adenoma (benign tumor).
- Ectopic cells as in some malignancy that secrete PTH
- Excess intake of vitamin D: or calcium or both. Usually it is due to over dosage or self-medication with vitamin D.
- Milk-alkali syndrome: This is hypercalcemia present in patients who received, for long periods, excessive absorbable alkalies and milk (source of calcium), for the treatment of peptic ulcer.
- Bone diseases: (increased bone resorption) as in malignancy, leukemia, multiple myeloma and Paget's disease.
- Drugs: As thiazide diuretics.
- Other causes: As thyrotoxicosis, Cushing's syndrome.

Effects:

Stone formation: e.g renal stones. Calcification in different tissues.

2. Hypocalcemia:

Causes:

1) Hypoparathyroidism.
2) Alkalosis.
3) Kidney diseases where activation of vitamin D is inhibited.

Effects:

1) Acute deficiency: if ionized calcium is much decreased, tetany with carpopedal spasm results.
2) Chronic deficiency: In children, Rickets and in adults, Osteomalcia.

Daily Requirement of Calcium

An adult needs **500 mg** per day and a child about 1200 mg/day. Requirement may be increased to 1500 mg/day during pregnancy and lactation. After the age of 50, there is a general tendency for osteoporosis, which may be prevented by increased calcium (1500 mg/day) plus vitamin D (20 mg/day).

IRON (Fe)

Distribution of Iron

Total body iron content is 3 to 5 g; 75% of which is in blood, the rest is in liver, bone marrow and muscles. Iron is present in almost all cells. Blood contains 14.5 g of Hb per 100 mL. About 75% of total iron is in hemoglobin, and 5% is in myoglobin and 15% in ferritin.

Sources of Iron

1. Liver, heart, kidney, spleen and fish.
2. Sugar cane syrup (molasses).
3. Dates and egg yolk.
4. Leafy vegetables, spinach -unlike popular- is a poor source of iron because it is bound to phytate, which is difficult to absorb

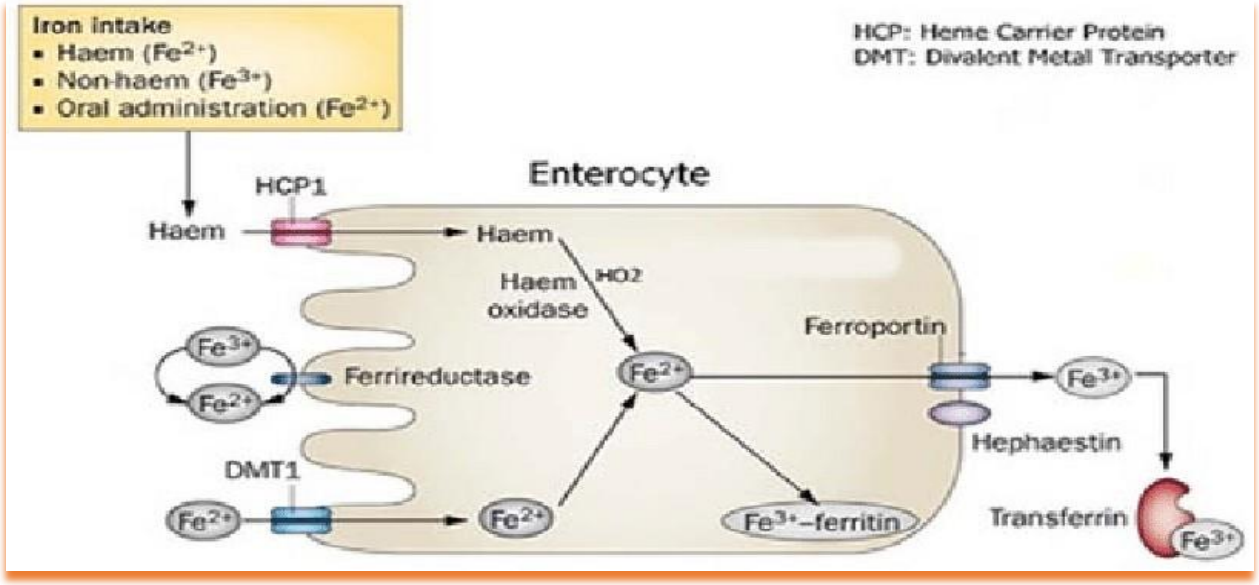
Iron Absorption

- Absorption of iron occurs in the duodenum and the proximal part of the jejunum.
- Only ferrous (and not ferric) form of iron is absorbed
- Free iron in the intestine is reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state on the luminal surface of intestinal enterocytes and transported into the cells through the action of the **divalent metal transporter** (DMT1).
- Intestinal uptake of heme iron occurs through the help of **heme carrier protein** (HCP1). The iron is then released within the enterocytes by the

enzyme **heme oxygenase**. The iron can be temporarily stored within intestinal enterocytes bound to **ferritin**.

- Iron is transported across the basolateral membrane of intestinal enterocytes into the circulation, through the action of the transport protein **ferroportin**
- The enzyme hephaestin (a copper-containing ferroxidase oxidizes the ferrous form back to the ferric form.
- Once in the circulation, ferric form is bound to **transferrin** and passes to liver.

.



Factors affecting iron absorption: most of dietary iron is present in the ferric state (Fe+++) as ferric organic compounds.

***a)* Factors promoting iron absorption:**

1) Cooking of food and gastric HCI facilitates the liberation of ferric ions (Fe+++) from organic compounds.

2) Reducing substances: vitamin C and cysteine (-SH) of dietary protein help the reduction of ferric ions (Fe•••) into the absorbable ferrous (Fe••) state.

3) Body needs: absorption occurs only if the body is in need to iron. More iron is absorbed when there is iron deficiency or when erythropoiesis is increased.

***b)* Factors inhibiting iron absorption:**

1) High dietary phosphate and phytate: They form insoluble, non-absorbable organic iron complexes.

2) Steatorrhea: Where fatty acids form non-absorbable iron soaps.
3) Alkalies and tea.

Storage of Iron

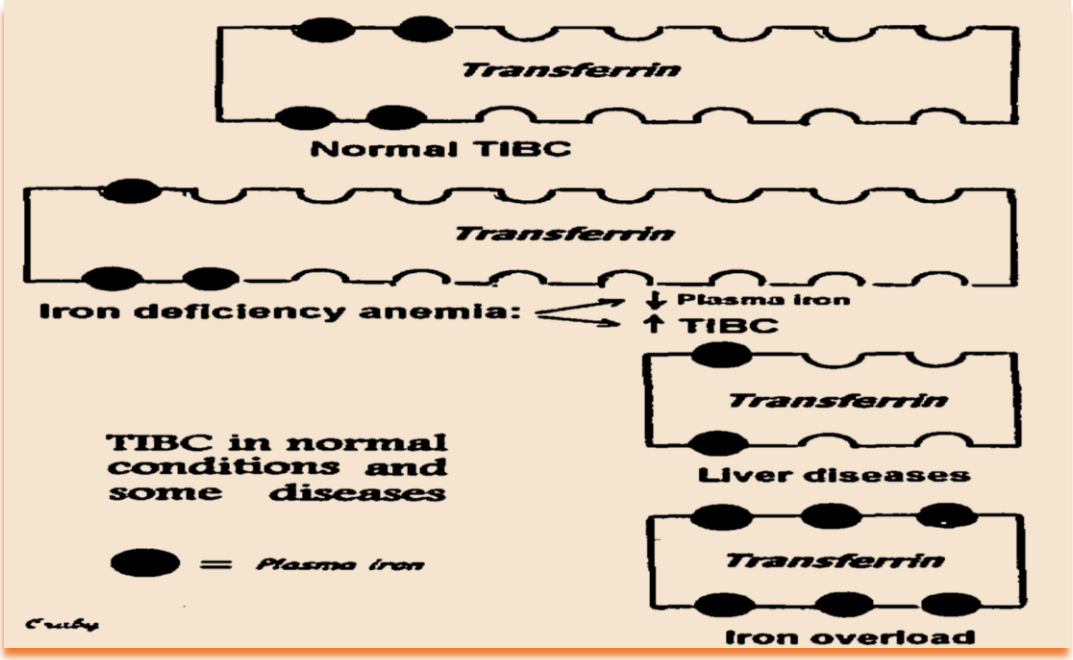
- The storage form is **ferritin**. It is seen in intestinal mucosal cells, liver, spleen and bone marrow.
- The **apoferritin** has a molecular weight of about 440 kilo Daltons. It has 24 subunits. It can take up to 4,000 iron atoms per molecule. Ferritin contains about 23% iron.
- Normal plasma contains very little ferritin. Ferritin in plasma is elevated in iron overload. Thus ferritin level in blood is an index of body iron stores.
- Ferritin is an acute phase reactant protein that is elevated in inflammatory diseases. Estimation of ferritin is also indicated in chronic kidney disease to assess the extent of anemia.

Plasma iron:

- Plasma iron: Ranges from 6o- 160 ug/dl.
- Plasma transferrin:
  This is a plasma glycoprotein that acts as carrier for iron. It is synthesized in the liver. Each molecule can carry 2 atoms of iron in ferric state.
- Transferrin may carry up to 180-450 ug iron/dl. This is known as total iron binding capacity of transferrin (TIBC). As the plasma iron is 6o-t6o ug/dl, thus only 30% of the TIBC of transferrin is saturated.
- TIBC is therefore defined as maximum amount of iron that can be carried by transferrin per deciliter.

Abnormalities of plasma TIBC concentration:

1. In iron deficiency anemia: Plasma iron is decreased. Liver synthesizes more transferrin with subsequent increase of TIBC.
2. In liver diseases: Both plasma iron and transferrin synthesis tend to decrease (↓plasma iron and ↓TIBC).
3. In iron overload: transferrin synthesis is inhibited. This leads to increased plasma iron and decreased Total iron binding capacity.



- **Plasma ferritin:**

A low plasma ferritin indicates the presence of depleted iron stores e.g. in iron deficiency anemia. A raised plasma ferritin is found in iron overload and also in many patients with liver disease and cancer.

Tissue iron: it includes:

1. **Ferritin**
2. **Hemosedrin:**

   When body contains very high content of iron more than the capacity of apoferritin, some of iron is found in grauules called hemosedrin that deposited in tissues. These granules are composed of iron, protein, and polysaccharides.

3. **Non-available iron forms:** cannot be used even if there are body needs. All these forms arc hemoproteins. They include:

- Myoglobin: It is hemoprotein formed of a single heme ring attachted to one long polypeptide chain. It is present in muscles and heart. It acts as oxygen reservoir for quick utilization by contracting muscles.
- Respiratory cytochromes (b c., c, a, a 3): These are components of respiratory chain in mitochondria. They act as electron carriers.
- Catalase and peroxidase: These are two enzymes that act on the toxic
- hydrogen peroxide (H2O2) converting it into H2O.

- Tryptophan oxygenase: This enzyme is important for tryptophan metabolism.
- Cytochrome P 450: These are a specific group of enzymes that present in liver, lung, kidney, gut, adrenal cortex, heart, and brain. They are used in xenohiotics metabolism.

Alterations of plasma iron:

1. Iron deficiency anemia

Causes:

Deficient intake.

Impaired absorption: e.g. steatorrhea, abdominal surgery.

Excessive loss e.g. menstrual loss, gastrointestinal bleeding due to some parasites (anchylostoma).

Biochemical changes:

Plasma iron is decreased.

Plasma TIBC is increased.

Plasma ferritin is decreased.

RBCs show: hypochromic, microcytic cells.

2. **Iron overload:**

Causes:

Repeated blood transfusion.

Intravenous administration of iron.

Hemochromatosis (hemosiderosis, bronze diabetes): This is a rare hereditary disease characterized by abnormal increase of iron absorption.

Iron is deposited in the form of hemosedrin in:

- Liver: causing liver cirrhosis.
- Pancreas: causing fibrosis and diabetes mellitus.
- Skin: causing bronze discoloration of skin.

Biochemical changes:

Plasma iron is increased. Plasma TIBC is decreased. Plasma ferritin is increased.

Enzyme Chemistry

Definition: These are specific protein catalysts that accelerate the rate of chemical reactions.

- Enzyme structure is not changed by entering the reactions.

- Enzyme does not affect the equilibrium constant (i.e. end products) of the reactions.

Characteristics of Enzymes

- Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.
- They are heat labile.
- They are water-soluble.
- They can be precipitated by protein precipitating re
- agents (ammonium sulfate or trichloroacetic acid)
- **Ribozymes**: it is stated that all enzymes are proteins. Invariably, all rules will have exceptions. Ribozymes are RNA molecules with enzymatic activity, which catalyze cutting of nascent mRNA or primary transcript

Cellular distribution of enzymes:

A. Intracellular enzymes: Produced and act inside the cells e.g. metabolic enzymes.

B. Extracellular enzymes: Produced inside the cells and act outside the cells e.g. digestive

enzymes.

Properties of ENZYMES

- The general properties of enzymes are those of proteins:

1. They are globular proteins.

2. They can be denatured by physical and /or chemical agents and they loose their biological function as the denaturation change their conformation.

3. Enzymes are usually specific in action and the specificity varies in degree (see later).

4. Some enzymes are simple proteins, others are conjugated proteins.

5. Each enzyme has a characteristic tertiary structure and undergoes a conformational change suitable to the specific substrate

6. Some enzymes are secreted as proenzymes (zymogens) then they are activated at the time of action.

Zymogens

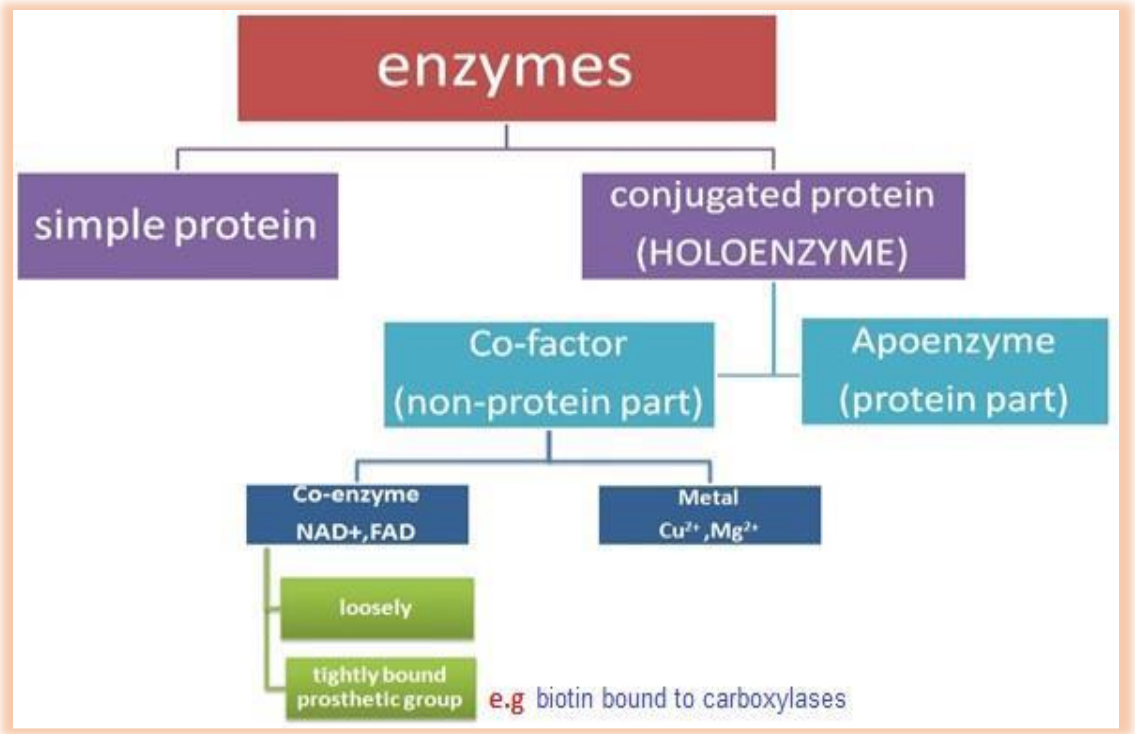
- They are inactive enzymes.
- Zymogens are inactive because their catalytic sites are masked by a polypeptide chain.
- Activation of zymogen, into active enzyme is done by removal of the polypeptide chain to open the catalytic site for its substrate.
- Examples of zymogens: are pepsinogen and trypsinogen.

ENZYME STRUCTURE

- Enzymes may be simple proteins, or complex enzymes, containing a non-protein part, called the prosthetic group. The prosthetic group is called the co-enzyme. It is heat stable.
- The protein part of the enzyme is then named the apoenzyme. It is heat labile. These two portions combined together are called the holo-enzyme.
- Co-enzymes may be divided into two groups:

  a. Those taking part in reactions catalyzed by oxidoreductases by donating or accepting hydrogen atoms or electrons.

  b. Those co-enzymes taking part in reactions transfer ring groups other than hydrogen.



ENZYMES SPECIFICITY

1. **Bond specificity (Relative specificity):** Most of the proteolytic enzymes are showing group (bond) specificity. For example, trypsin can hydrolyze peptide bonds formed by carboxyl groups of arginine or lysine residues in any protein. lipase enzymes act on different TAG

2. Group Specificity: One enzyme can catalyze the same reaction on a group

of structurally similar compounds, e.g. hexokinase can catalyze phosphorylation of glucose, galactose and mannose.

3. **Optical specificity (Stereospecificity):**

- Enzymes act on D or L isomers e.g.

D - Amino acid oxidase acts only on D-amino acids

L- Amino acid oxidase acts only on L-amino acids

- Enzymes act on specific type of linkages according to the type of linkage (α or β) of the compounds attached to it e.g.

α Amylase hydrolyses α-1-4 glycosidic linkage of starch.

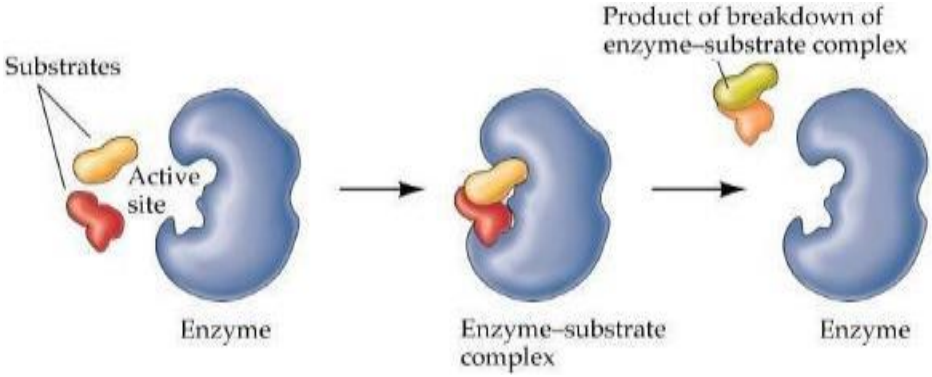
4. **Absolute specificity:** One enzyme acts only on one substrate e.g. urease enzyme acts only on urea.

MECHANISM OF ENZYME ACTION

- Enzymes lower the energy of activation.
- Activation energy is defined as the energy required to convert all molecules of a reacting substance from the ground state to the transition state.
- Substrates are remaining in an energy trough, and are to be placed at a higher energy level, whereupon spontaneous degradation can occur.
- During enzyme substrate binding, weak interactions between enzyme and substrate are optimized.

  During the enzyme action, there is a temporary combination between the enzyme and its substrate forming enzyme-substrate complex. This occurs at active site of enzyme.

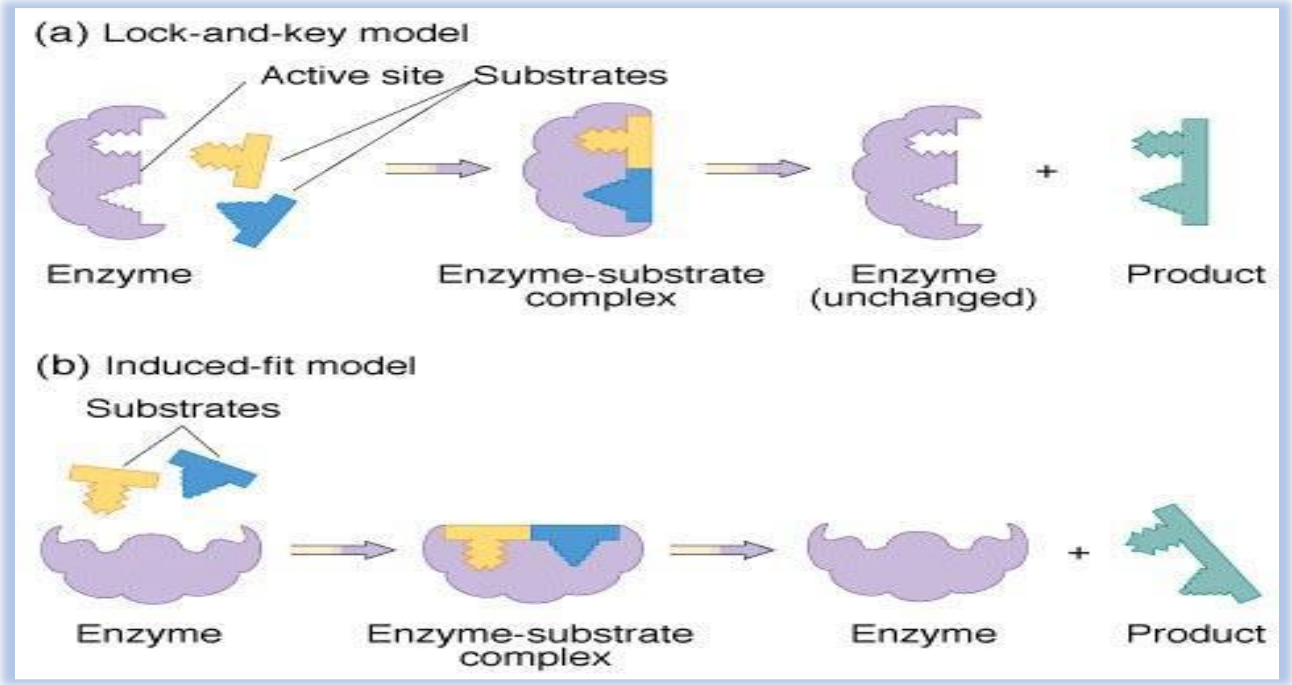
  2) This is followed by dissociation of this complex into enzyme again and products



Theories of enzyme action: Two theories have been proposed to explain the specificity of
enzyme action:

a) The lock and key theory: The active site of the enzyme is complementary in conformation to the substrate so that enzyme and substrate "recognize" one another.

b) The induced fit theory: The enzyme changes shape upon binding the substrate, so that the conformation of substrate and enzyme protein are only complementary after the binding reaction.

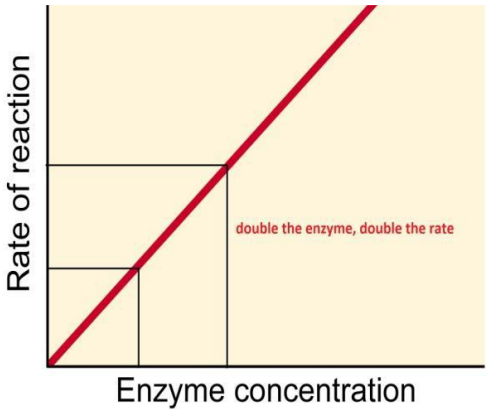


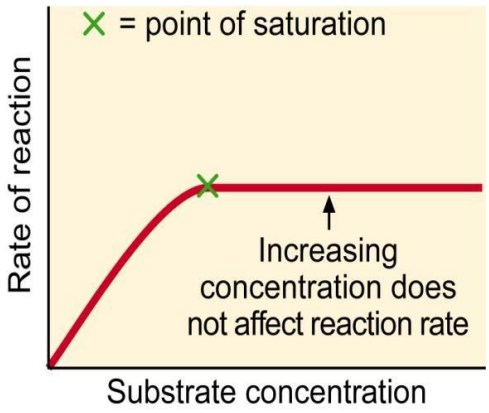
FACTORS AFFECTING ENZYME ACTIVITY

1. Enzyme concentration
2. Substrate concentration
3. Product concentration
4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitors
8. Allosteric regulation)
9. Covalent modification.

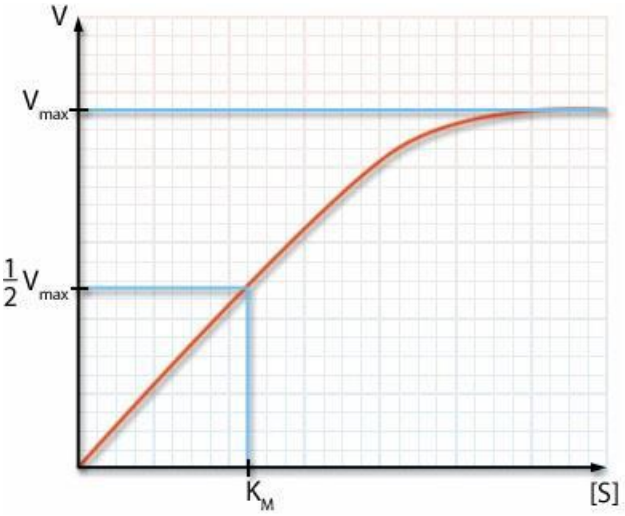
1. **Concentration of enzyme:** The initial velocity of a reaction is directly proportional to the amount of the enzyme present, provided that all other conditions remain constant.
2. **Concentration of substrate:** The initial velocity of a reaction is directly proportional to the amount of substrate present till it reaches a maximum point

known as maximum velocity (Vmax), where any further increase in the amount of substrate causes no increase in the velocity of the reaction. This is true if all other conditions especially enzyme concentration remain constant.





- **Definition of maximum velocity (V max):** It is the maximum point in substrate velocity curve where any further increase in the amount of substrate causes no increase in the velocity of the reaction due to enzyme saturation.
- **Michaelis constant Km:** It is substrate concentration that produces half maximum velocity.

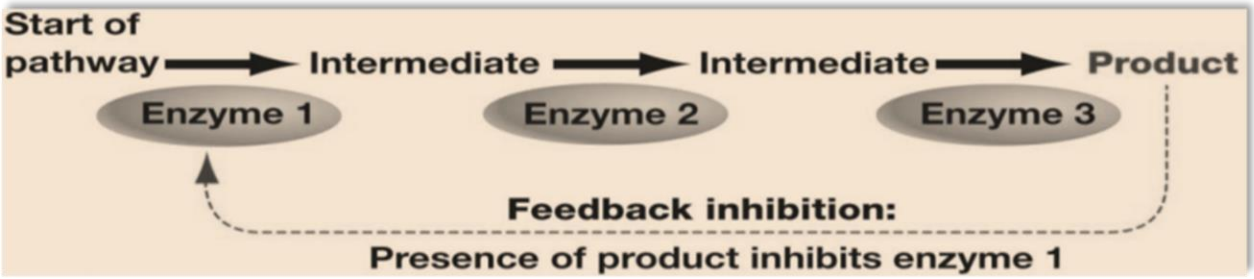


- K**m** is a constant, characteristic of an enzyme and a particular substrate. K**m** reflects the affinity of the enzyme for the substrate.
- The smaller the Km value → the more active the enzyme.

3. Effect of product concentration:

Increased product concentration decreases enzyme activity, this may be due to:

- Change in the pH of the medium.
- The product is more or less similar to the substrate, so it may compete it to catalytic site of the enzyme.
- The product may bind to the enzyme at the allosteric Site (in case of allosteric enzyme).



4. Effect of temperature:

- The optimal temperature for enzymatic activity in human body is **37 °C** i.e. the temperature of the cells.
- At zero temperature, the enzyme is inactive. The reaction velocity increases with increase of temperature until a maximum velocity is reached.
- Further elevation of the temperature results in a decrease in reaction velocity. At 55°C - 60°C, most enzymes are denaturated and become permanently inactive.

5. Effect of pH:

- The optimal pH for enzyme activity is that pH at which the enzyme acts maximally.
- Above or below this pH, the ionic state of both enzyme and substrate will be changed, and the rate of reaction will therefore decrease.
- Each enzyme has its own optimal pH e.g. Salivary amylase 6.8. - Pepsin 2. Trypsin 8 - Alkaline phosphatase 8.4
- Extremes of pH can also lead to denaturation of the enzyme.

6.Enzyme activators:

- Activators increase the rate of enzyme catalyzed reactions.
- Velocity of the reaction depends on activator concentration.
- Some enzymes are activated by different ways:

- Removal of peptide converts inactive forms of the enzyme (zymogen) to active. e.g. pepsinogen Pepsin or HCI pepsin.
- Some enzymes containing SH groups e.g. glyceraldehyde 3-P dehydrogenase require reducing agents (vitamin C) to be activated.
- Some enzymes require minerals, they are called metal activated enzymes eg. Cl- for amylases - Mg++ for kinases
- Allosteric activators (Allosteric modifiers): The binding of allosteric activator produces conformational changes in the protein structure of the enzyme resulting in increased velocity of the reaction e.g. AMP is an allosteric activator of phosphofructokinase enzyme.

7.Enzyme inhibitors:

Definition: These are substances that can diminish the velocity of enzymatic reactions.

Types

A) Reversible inhibitors

- Bind to enzymes through non covalent bonds.
- Dilution of the enzyme-inhibitor complex dissociates the reversibly bound inhibitor and recovery of enzyme activity.

a. Competitive inhibitors:

- Similar to substrate.
- compete with substrate for active site of the enzyme.
- Both substrate (S) and inhibitor (I) can bind with the catalytic site of the enzyme to form either Enz-S-complex or Enz-l-complex.
- Effect of competitive inhibitor on Vmax and Km:

a) Effect on Vmax: A competitive inhibition does not affect Vmax.

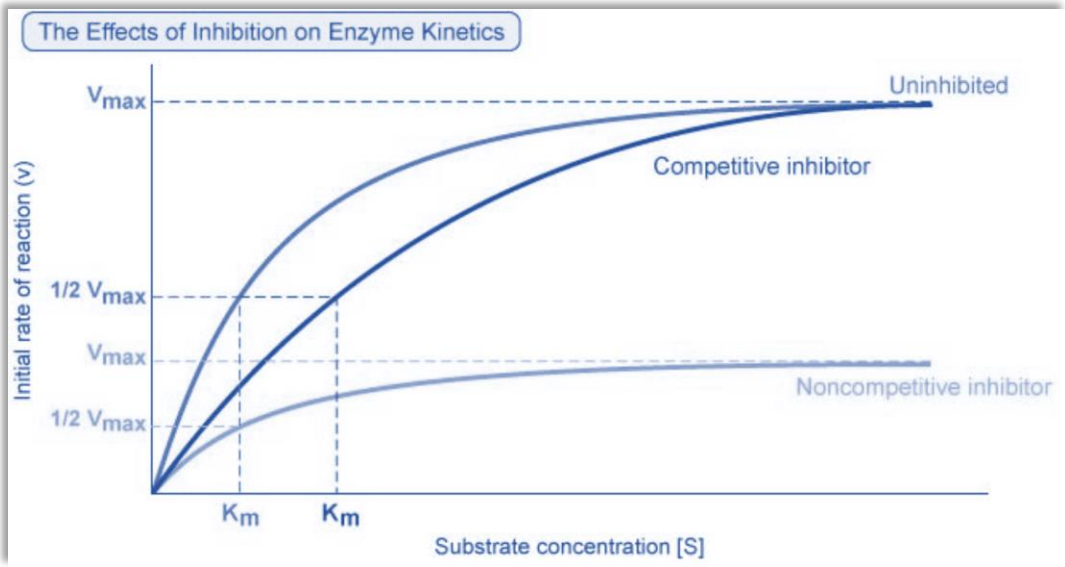
b) Effect on K: A competitive inhibition increases the Km of substrate.

b. Non-competitive inhibitors:

- Inhibitor and substrate bind to different sites on the enzyme.
- The inhibitor does not alter the catalytic site.
- There is no structural similarity between substrate and inhibitor.
- The inhibitor can bind either free enzyme or the enzyme-substrate complex.

- Both enzyme inhibitor complex and enzyme substrate inhibitor complex are inactive.
- Effect of noncompetitive inhibitor on Vmax and Km:

a) Vmax is decreased.
b) Km is unchanged.



B. **Irreversible inhibitors**

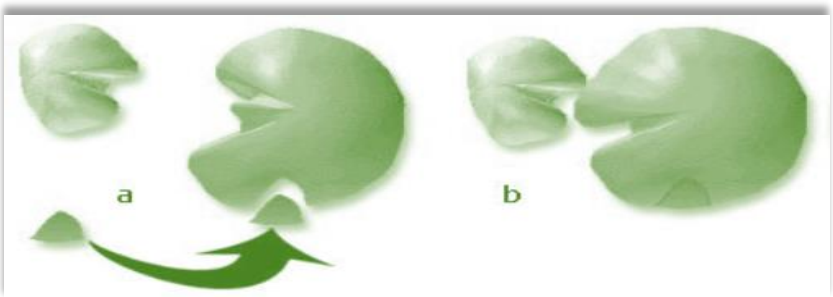
- This type of inhibition cannot be reversed by adding more substrate
- The inhibitor alters the catalytic site.
- Irreversible inhibitors include the following:

1. All compounds that produce Denaturation of proteins.
2. Inhibitors of sulfhydryl group
3. Antienzymes: e.g. Antithrombin III
4. Removal of catalytic ions: by addition of EDTA.
5. Inhibition by phosphorylation and dephosphorylation
6. Cyanide and carbon monoxide inhibit cytochrome oxidase.

8. Allosteric Regulation

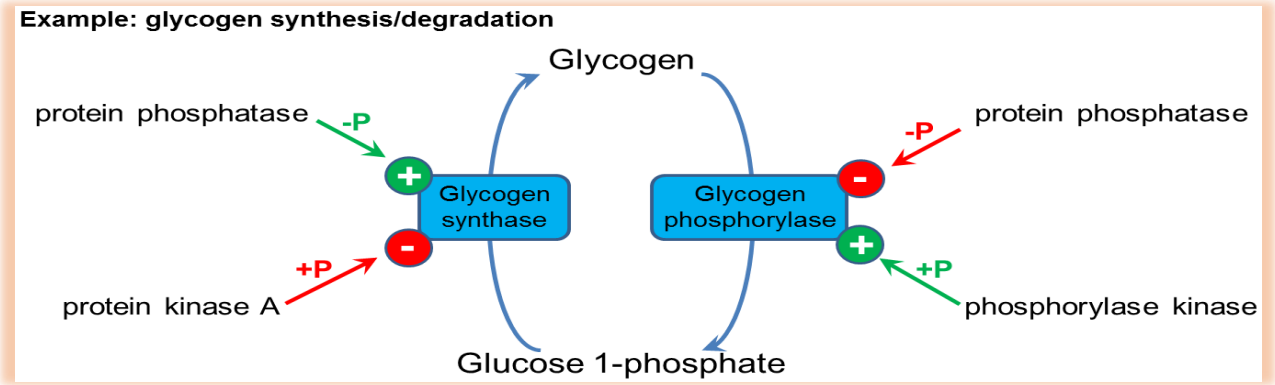
- Allosteric enzyme has one catalytic site where the substrate binds and another separate allosteric site where the modifier binds (*allo* = other).

- Allosteric and substrate binding sites may or may not be physically adjacent.
- The binding of the regulatory molecule can either enhance the activity of the enzyme (allosteric activation), or inhibit the activity of the enzyme (allosteric inhibition. It is partially reversible, when excess substrate is added. Km is usually increased. Vmax is reduced.
- Allosteric enzymes generally catalyze the irreversible steps in metabolic pathways.
- Body uses allosteric enzymes for regulating metabolic pathways. Such a regulatory enzyme in a particular pathway is called the key enzyme or rate limiting enzyme.



9. Covalent modification: phosphorylation / dephosphorylation:

- Some enzymes may be regulated by covalent modification, most frequently by the addition or removal of phosphate groups from the enzymes.
- Phosphorylation reactions are catalyzed by a family of enzymes, called: protein kinase. It utilizes ATP as a phosphate donor.
- Phosphate groups are removed from Phosphorylated enzymes by the action of phosphoprotein phosphatase



Isoenzymes

- Definition: Isoenzymes are different molecular forms of the enzyme that activate the same
reaction, use the same coenzyme and same substrate but they are different in chemical protein
structure. This leads to:

1. Different immunological reactions.
2. Different Km and Vmax.
3. Different physical properties.

Examples:

1. **Lactate dehydrogenase enzyme (LD)**

   The LDH catalyzes reversible conversion of pyruvate to lactate.
   LDH enzyme is a tetramer with 4 subunits. But the subunit may be either H (heart) or M (muscle) polypeptide chains. Although both of them have the same molecular weight (32 kD), there are minor amino acid variations.

- So, 5 combinations of H and M chains are possible; H4, H3M,H2M2, M3H and M4 varieties, forming 5 iso-enzymes. All these 5 forms are seen in all persons.

LDI_1	HHHH	Heart	Serum LDI_1 increases in certain heart diseases (myocardial infarction).
LDI_2	HHHM	Red cells	Acute leukemia
LDI_3	HHMM	Lungs	Acute leukemia
LDI_4	HMMM	Other tissues	
LDI_5	MMMM	Liver	Serum LDI_5 increases in certain liver diseases (infective hepatitis)

2. Creatine kinase (CK) (CPK):

- It catalyzes the reaction:

CPK

Creatine phosphate + ADP --------→ ATP + Creatine

- CK is a dimer; each subunit has a molecular weight of 40 kD. The subunits are called B for brain and M for muscle. Therefore, three iso-enzymes are circulation.

- **CK-BB:** in brain. Its level increases in cerebral stroke
- **CK-MB:** in Skeletal muscle. Its level increases in cases of myopathies and muscle destruction.
- **CK-MM:** in myocardium. Its level increases in myocardial infarction(MI).

Iso-enzyme	*Electrophoretic mobility*	*Tissue of origin*	*Mean percentage in blood*
MM(CK3)	Least	Skeletal muscle	80%
MB(CK2)	Intermediate	Heart	5%
BB(CK1)	Maximum	Brain	1%