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Table of Contents

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INTRODUCTION TO BIOCHEMISTRY

Medical biochemistry is an essential component of curriculum for all categories of health professionals. Contemporary Biochemistry plays a crucial role in the Medical field, be it metabolic pathways, storage diseases, mechanism action of varied biomolecules or inter and intra cellular communications.

A lecture note on Medical biochemistry integrates and summarizes the essentials of the core subject. Topics are carefully selected to cover the essential areas of the subject for graduate level of Health sciences. The chapters are organized around the following major themes:

1. Conformation of biomolecules, structure and their relationship to biological activity
2. synthesis and degradation of major metabolites
3. Production and storage of energy
4. Biocatalysts and their application
5. Intercellular communication by hormones
6. Molecular events in gene expression and regulation

Enzymes:

Body proteins perform a large number of functions. One such unique function is, they act as biological catalysts (Enzymes) .They are responsible for highly complex reactions. They direct the metabolic events and exhibit specificity toward substrates, regulate the entire metabolism. Thus, they play key role in the degradation and synthesis of nutrients, biomolecules etc. The most important diagnostic procedures invol7 Tw[(most impeatio)*.0011ionshi9D.0006 n(i)5.5(then 0606c

the protein. The structural organization of proteins could be primary, secondary, tertiary and quaternary. The three dimensional structure is the most biologically active one.

The unfolding and disorganization of the proteins results in denaturation, the process is mostly irreversible. Such a protein may lose its biological function. Many amino acid derived peptides are of biological importance and special products formed from them are of critical importance to the body.

Carbohydrates

They are biomolecules, found abundantly in living organisms. They contain more than one hydroxyl group (polyhydric) In addition to aldehyde or ketone group. Thus, they form in to polyhydroxy aldoses or polyhydroxy ketoses. Carbohydrates can be classified in to Monosaccharide, disaccharide, and polysaccharides. Mono is the smallest sugar unit, disaccharide is made up of two monosaccharides joined by glycosidic linkages .The linkage can be or β. A polymer with more than 10 monosaccharide units is called polysaccharide.

Carbohydrates have a wide range of functions. They provide energy; act as storage molecules of energy. Serve as cell membrane components and mediate some forms of communication between cells.

Absence of a single enzyme like lactase causes discomfort and diarrhea. The failure of Galactose and fructose metabolism due to deficient enzymes leads to turbidity of lens proteins (Cataract). Blood glucose is controlled by different hormones and metabolic processes. People suffer from Diabetes if the insulin hormone is less or not functioning well, such people are prone to atherosclerosis, vascular diseases, and renal failure.

Integrative Metabolism and Bioenergetics

Oxygen is utilized for the conversion of glucose to pyruvate. The same metabolite also forms from amino acid and protein metabolism. Other precursors like Glycerol, propionate can give rise to pyruvate. The main breakdown product of pyruvate is acetyl CoA, which is the common intermediate in the energy metabolism of carbohydrates, lipid and amino acids. It enters central metabolic pathway, the Citric acid cycle in t

Lipids

The bulk of the living matter is made up of Lipids, carbohydrates and proteins.

Lipids are water insoluble, but can be extracted with non-polar solvents like Benzene, methanol,

Hormones

Hormones are chemical messengers secreted by endocrine glands and specific tissues. They reach distant organs and stimulate or inhibit the function. They play important role in carrying

UNIT ONE

ENZYMES

General Properties

Enzymes are protein catalysts for chemical reaction in biological systems. They increase the rate of chemical reactions taking place within living cells with out changing themselves.

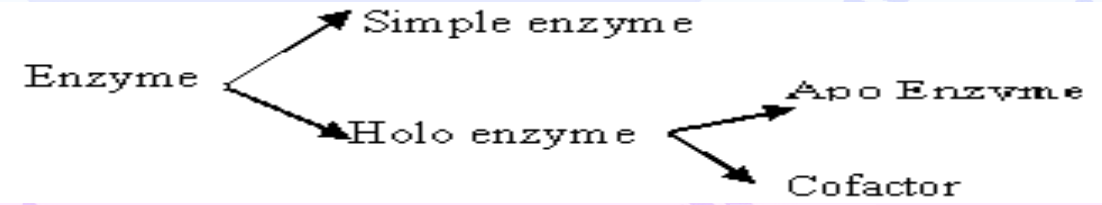
Nature of Enzymes

Most enzymes are protein in nature. Depending on the presence and absence of a non-protein component with the enzyme enzymes can exist as, simple enzyme or holoenzyme

1. Simple enzyme: It is made up of only protein molecules not bound to any non-proteins. Example: Pancreatic Ribonuclease.
2. Holo enzyme is made up o protein groups and non-protein component.
   - The protein component of this holo enzymes is called **apoenzyme**
   - The non-protein component of the holo enzyme is called a **cofactor**. I

If this cofactor is an organic compound it is called a **coenzyme** and if it is an inorganic groups it is called **activator**. (Fe $^{2+}$, Mn $^{2+}$, or Zn $^{2+}$ ions).

If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a **prosthetic group**



COENZYMES-

Coenzymes are derivatives of vitamins without which the enzyme cannot exhibit any reaction. One molecule of coenzyme is able to convert a large number of substrate molecules with the help of enzyme.

- Coenzyme accepts a particular group removed from the substrate or donates a particular group to the substrate
- Coenzymes are called co substrate because the changes that take place in substrates are complimentary to the changes in coenzymes.

- Â The coenzyme may participate in forming an intermediate enzyme-substrate complex

  Example: **NAD, FAD, Coenzyme A**

Metal ions in enzymes

Many enzymes require metal ions like **ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+}** for their activity.

Metal-activated enzymes-form only loose and easily dissociable complexes with the metal and can easily release the metal without denaturation. Metalloenzymes hold the metal tightly on the molecule and do not release it even during extensive purification.

Metal ions promote enzyme action by

a. Maintaining or producing the active structural conformation of the enzyme (e.g. glutamine synthase)
b. Promoting the formation of the enzyme-substrate complex (Example: Enolase and carboxypeptidase A.)
c. Acting as electron donors or acceptors (Example: Fe-S proteins and cytochromes)
d. Causing distortions in the substrate or the enzyme Example: phosphotransferases).

Properties of Enzyme

A**. Active site**

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate.

The active site binds the substrate, forming an enzyme-substrate (ES) complex. ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product.

For the combination with substrate, each enzyme is said to possess one or more active sites where the substrate can be taken up.

The active site of the enzyme may contain free hydroxyl group of serine, phenolic (hydroxyl) group of tyrosine, SH-thiol (Sulfhydryl) group of cysteine or imindazolle group of histidine to interact with there is substrates.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E. **Zymogens** (- inactive form of enzyme)

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called Zymogens (Proenzymes).

Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group

Examples: Pepsinogen - This zymogen is from gastric juice.When required Pepsinogen converts to Pepsin

Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

* The activation is brought about by specific ions or by other enzymes that are proteolytic.

Pepsinogen + H^+ ⟶ Pepsin

Trypsinogen $\xrightarrow{\text{Enteropeptidase}}$ Trypsin

Zymogen forms of enzymes a protective mechanism to prevent auto digestion of tissue producing the digestive enzymes and to prevent intravascular coagulation of blood.

F. Isoenzymes (Isozymes)

These are enzymes having similar catalytic activity, act on the same substrate and produces the same product but originated at different site and exhibiting different physical and chemical characteristics such as electrophoretic mobilities, amino acid composition and immunological behavior.

Example: LDH (Lactate dehydrogenase) exists in five different forms each having four polypeptide chains. H= Heart and M=Muscle.

Type	Polypeptide chain
LDH-1	H H H H
LDH-2	H H H M
LDH-3	H H M M
LDH-4	H M M M
LDH-5	M M M M

Example. CPK (Creatine phospho kinase) exists in three different forms each having two polypeptide chains. Characteristic sub units are B=Brain and M= Muscle.

Type	Polypeptide chain
CPK-1	BB
CPK-2	MB
CPK-3	M

Classification of Enzymes

Enzymes are classified on the basis of th


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Example: Enzymes catalyzing formation of C-N-bonds

L- Glutamine: ammonia ligase (ADP) [Glutamine Synthetase]

ATP + L-Glutamate + NH_4 = ADP + orthophosphate + L-Glutamine

Example: Enzymes catalyzing formation of C-C bonds

Acetyl-CoA: CO_2 ligase (ADP) [acetyl-CoA carboxylase] ATP+ Acetyl-COA-CO_2 → Malonyl-CoA+ADP+pi.

MECHANISM OF ACTION OF ENZYMES

Emil Fischer's model Lock and Key model 1890.

Lock: Key model of enzyme action implies that the active site of the enzyme is complementary in shape to that of its substrate, i.e. the shape of the enzyme molecule and the substrate molecule should fit each other like a lock and Key

In 1958, Daniel Koshland, postulated another model; which implies that the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.

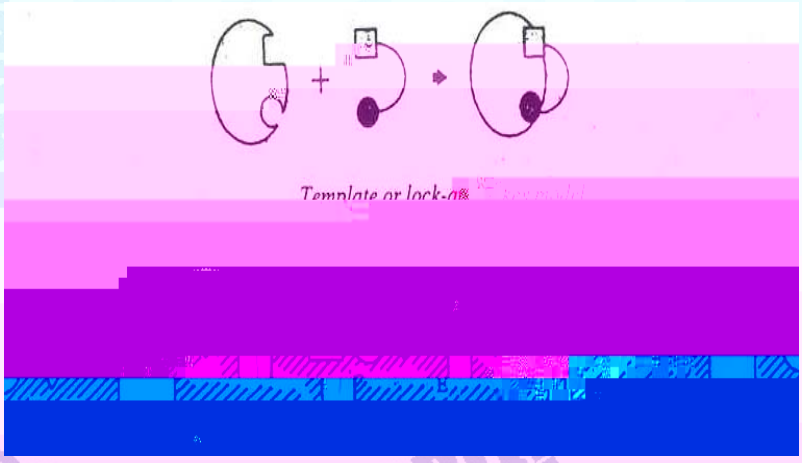

Figure: **Models of enzyme- substrate interactions**

Mechanism of Enzyme Action (1913)

Michaels and Menten have proposed a hypothesis for enzyme action, which is most acceptable. According to their hypothesis, the enzyme molecule (E) first combines with a substrate molecule (S) to form an enzyme substrate (ES) complex which further

dissociates to form product (P) and enzyme (E) back. Enzyme once dissociated from the complex is free to combine with another molecule of substrate and form product in a similar way.

ENZYMES ENHANCE THE RATE OF REACTION BY LOWERING FREE ENERGY OF ACTIVAION

A chemical reaction S P (where S is the substrate and P is the product or products) will take place when a certain number of S molecules at any given instant posses enough energy to attain an activated condition called the "**transition state**", in which the probability of making or breaking a chemical bond to form the product is very high.

The transition state is the top of the energy barrier separating the reactants and products. The rate of a given reaction will vary directly as the number of reactant molecules in the transition state. The "energy of activation is the amount of energy required to bring all the molecules in 1 gram-mole of a substrate at a given temperate to the transition state

A rise in temperature, by increasing thermal motion and energy, causes an increase in the number of molecules on the transition state and thus accelerates a chemical reaction. Addition of an enzyme or any catalyst can also bring about such acceleration.

The enzyme combines transiently with the substrate to produce a transient state having c lower energy of activation than that of substrate alone. This results in acceleration of the reaction. Once the products are formed, the enzyme (or catalyst) is free or regenerated to combine with another molecule of the substrate and repeat the process.

Activation energy is defined as the energy required to convert all molecules in one mole of reacting substance from the ground state to the transition state.

Enzyme are said to reduce the magnitude of this activation energy.

* During the formation of an ES complex, the substrate attaches itself to the specific active sites on the enzyme molecule by Reversible interactions formed by Electrostatic bonds, Hydrogen bonds, Vanderwaals forces, Hydrophobic interactions.

Factors Affecting Enzyme Activity

Physical and chemical factors are affecting the enzyme activity. These include

1. Temperature
2. pH
3. Substrate/enzyme concentration etc.

Temperature

Starting from low temperature as the temperature increases to certain degree the activity of the enzyme increases because the temperature increase the total energy of the chemical system .

There is an optimal temperature at which the reaction is most rapid (maximum). Above this the reaction rate decreases sharply, mainly due to denaturation of the enzyme by heat.

The temperature at which an enzyme shows maximum activity is known as the optimum temperature for the enzyme. For most body enzymes the optimum temperature is around 37^0c, which is body temperature.

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For example, Catalytic activity may require that an amino-group of the enzyme be in the protonated form ($-NH_3^+$) At alkaline pH this group is deprotonated and the rate of reaction therefore declines.

Extreme pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid chains.

The pH at which maximum enzyme activity is achieved is different for different enzymes, and after reflects the pH^+] at which the enzyme functions in the body.

For example, pepsin, a digestive enzyme in the stomach, has maximum action at pH 2, where as other enzymes, designed to work at neutral pH, are denatured by such an acidic environment.

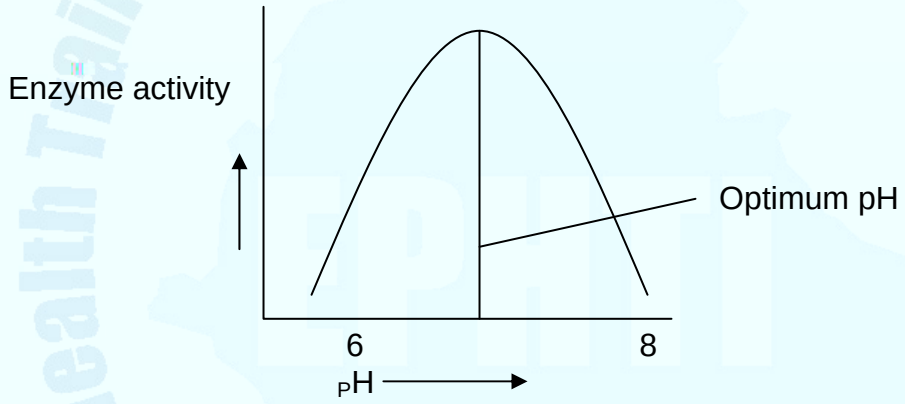


Figure. **Effect of pH on enzymatic reaction**

3. **Concentration of substrate**

At fixed enzyme concentration pH and temperature the activity of enzymes is influenced by increase in substrate concentration.

An increase in the substrate concentration increases the enzyme activity till a maximum is reached. Further increase in substrate concentration does not increase rate of reaction.

This condition shows that as concentration of substrate is increased, the substrate molecule combine with all available enzyme molecules at their active site till not more active sites are available (The active Sites become saturated). At this state the enzyme is obtained it maximum rate (V max).

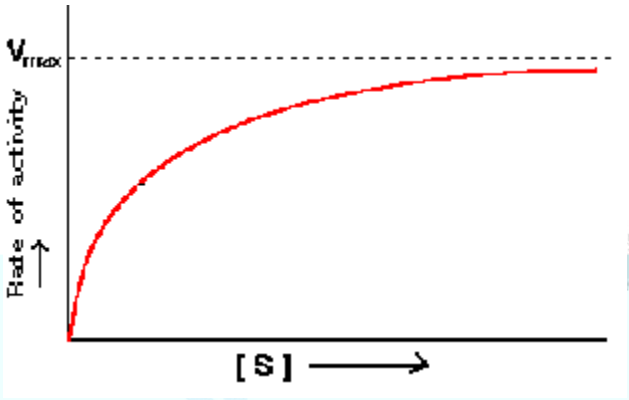


Figure. **Effect of Concentration of substrate on enzyme activity**

The characteristic shape of the substrate saturation curve for an enzyme can be expressed mathematically by the Michaelis Menten equation:

$$E + S \underset{K-1}{\overset{K1}{\rightleftharpoons}} ES \xrightarrow{K2} E + P \qquad Km = K3 + K2 / K1$$

$$V \quad \frac{V\max[S]}{Km \quad [S]}$$

Where: V= Velocity at a given concentration of substrate (initial reaction velocity)

Vmax = Maximal velocity possible with excess of substrate

[S] = concentration of the substrate at velocity V

Km = michaelis-constant of the enzyme for particular substrate.

Relationship between [S] and Km

Km shows the relationship between the substrate concentration and the velocity of the enzyme catalyzed reaction.

Take the point in which 50% of the active site of the enzyme will be saturated by substrate, Assume that at ½ Vmax-50% of the active site of enzyme becomes saturated. Therefore:

Vo = ½ Vmax, at 50% saturation

½ Vmax = $\frac{Vmax[S]}{Km + [S]}$

2[S] = Km + [S]

Km= [S]

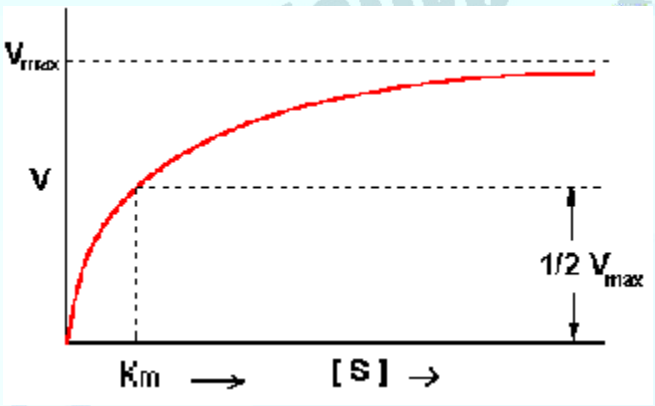


Figure:

High Km Value f an enzyme means the catalysis of that enzyme is slow compared to low Km.

Km does not vary with the concentration of enzyme.

4. Relationship of Velocity to Enzyme Concentration

The rate of the reaction is directly proportional to enzyme concentration at all substrate concentration. For example, if the enzyme concentration halved, the initial rate of the reaction (Vo) is reduced to one half that of the original.

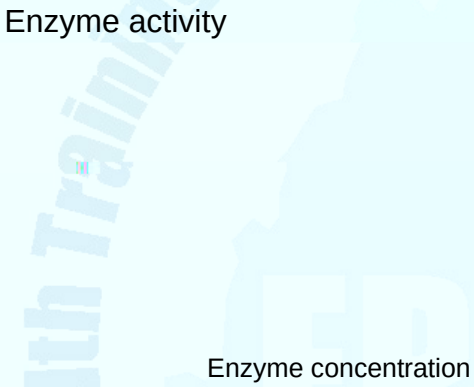


Figure. **Effect of Enzyme concentration on enzymatic reaction**

Order of Reaction

When [S] is much less than Km, the velocity of the reaction is roughly proportional to the substrate concentration. The rate of reaction is then said to be first order configuration with respect to substrate. When [S] is much greater than Km, the velocity is constant and equal to V max. The rate of reaction is then independent of substrate concentration and said to be 08 1.007[(zTcInhibi1.3(me Co)-4.1(n)-4.1tion

Irreversible Inhibition

The type of inhibition that can not be reversed by increasing substrate concentration or removing the remaining free inhibitor is called Irreversible inhibition

Eg. Diisopropyl & luorophosphate (DFP) Inhibits the enzyme acetyl cholinesterase, important in the transmission of nerve impulses. Acetyl cholinesterase catalyzes the hydrolysis of Acetylcholin (to acetic acid and choline) a neurotransmitter substance functioning in certain portions of the nervous system

- DEP inhibits also trypsin, chymotry

Effect of Competitive inhibitors

1. Effect on Vmax: The effect of a competitive inhibitor is reversed by increasing [s]. at a sufficiently high substrate concentration, the reaction velocity reaches the Vmax. observed in the absence of inhibitor.
2. Effect on Km: A competitive inhibitor increases the apparent Km for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve ½ Vmax.

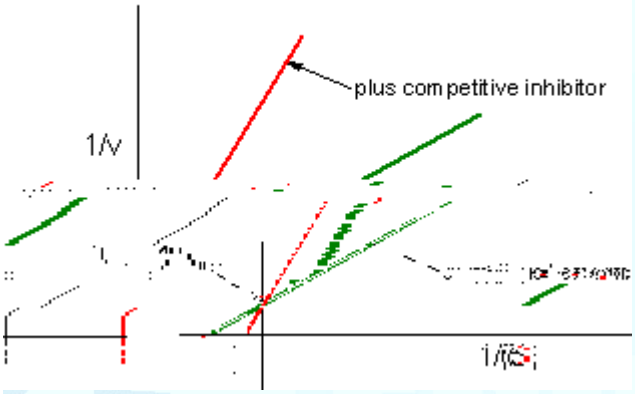


Figure: **Competitive inhibition**

Non-Competitive Inhibition

In non-competitive inhibition the inhibitor binds at different site rather than the substrate-binding site. When the inhibitor binds at this site there will be a change in conformation of the enzyme molecules, which leads to the reversible inactivation of the catalytic site. Non-competitive inhibitors bind reversibly either to the free-enzyme or the ES complex to form the inactive complexes EI and ESI (Enzyme substrate Inhibition)

The most important non-competitive inhibitors are naturally occurring metabolic intermediates that can combine reversibly with specific sites on certain regulatory enzymes, that changes the activity of their catalytic sites.

An Example: is the inhibition of L-threonine dehydratase by L-isoleucine.

*Such type of Enzyme is called Allosteric Enzyme, which has a specific sites or allosteric site other than the substrate-binding site.

1. Effect on Vmax.

Non-Competitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, non-competitive inhibitors decrease the Vmas of the reaction.

2. Effect on Km:

Non-competitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same Km in the presence or absence of the non-competitive inhibitor.

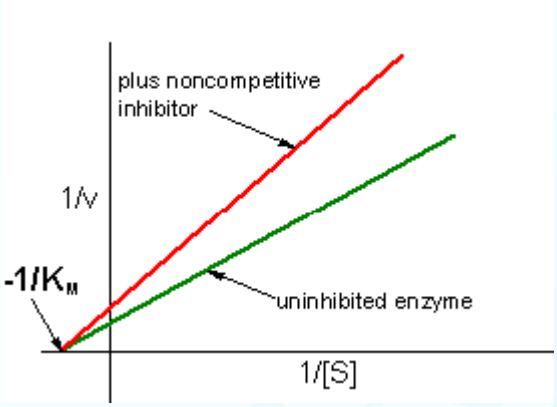


Figure: **Noncompetitive inhibition**

Uncompetitive Inhibition

Uncompetitive Inhibitor binds only to ES complex at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor-binding site available. Inhibition cannot be reversed by substrate.

In this case apparent Vmax. and Km decreased.

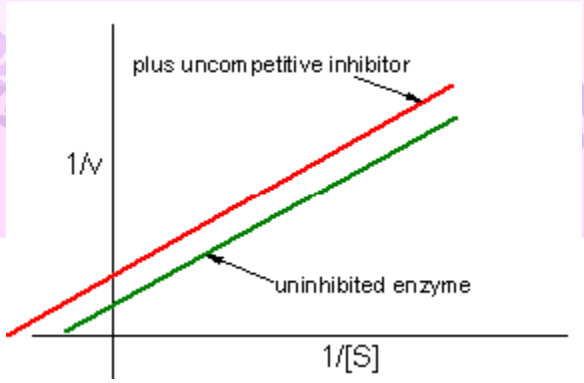


Figure: **Uncompetitive inhibition**

Regulation of enzyme activity

There are several means by which the activity of a particular enzyme is specifically regulated.

1**. Irreversible covalent Activation / Zymogen activation**

Some enzymes are secreted in an inactive form called Proenzymes or zymogens. At the site of action specific peptide bonds are hydrolysed either enzymatically or by PH changes to convert it into active form, e.g. Pepsinogen Ą pepsin, Trypsinogen Ą trypsin, plasminogen Ą plasmin. After hydrolysis when it is activated, it cannot be reconverted into proenzyme form.

2. Reversible Covalent Modification

By addition of or removal of phosphate or adenylate, certain enzymes are reversibly activated and inactivated as per the requirement. Protein kinase of muscle phosphorylate phosphorylase kinase, glycogen synthetase by making use of ATP.

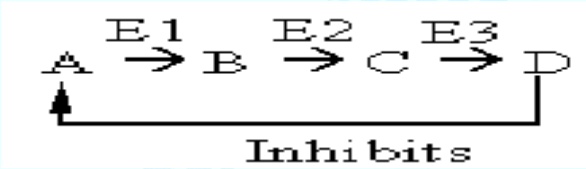
3. **Allosteric Modulation**

In addition to simple enzymes that interact only with substrates and inhibitors, there is a class of enzymes that bind small, physiologically important molecules and modulate activity in ways other than those described above. These are known as **allosteric enzymes**; the small regulatory molecules to which they bind are known as **effectors**. Allosteric effectors br

There are two ways that enzymatic activity can be altered by effectors: the V_{max} can be increased or decreased, or the K_m can be raised or lowered.

4. Feedback inhibition

In allosteric regulation in which end products inhibit the activity of the enzyme is called" feedback inhibition".



A high conc. D typically inhibits conversion of A Ą B.

This involves not simple backing up of intermediates but the activity of D to bind to and inhibit E1. D thus acts as negative allosteric affector or feedback inhibitor of E1.

The kinetics of feedback inhibition cay be competitive, mixed, etc. It is the commonest way of regulation of a biosynthetic pathway. Feedback regulation generally occurs at the earliest functionally irreversible step unique in the biosynthetic pathway.

ENZYMES IN CLINICAL DIAGNOSIS

Plasma enzymes can be classified into two major groups

1. Those, relatively, small group of enzymes secreted into the plasma by certain organs (i.e. Enzymes those have function in plasma) For example: - the liver secretes zymogens of the enzymes involved in blood coagulation.
2. Those large enzyme species released from cells during normal cell turnover. These enzymes are normally intracellular and have no physiologic function in the plasma. In healthy individuals the levels of these enzymes are fairly constant and represent steady state in which the rate of release from cells into the plasma is balanced by an equal rate or removal from the plasma.

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely

determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the diagnosis and prognosis for the patient.

Measurement of enzymes concentration of mostly the latter type in plasma gives valuable informatio0n about disease involving tissues of their origin.

1. Lipase:

It is an enzyme catalyzing the hydrolysis of fats. It is secreted by pancreas and Liver. The plasma lipase level may be low in liver disease, Vitamin A deficiency, some malignancies, and diabetes mellitus. It may be elevated in acute pancreatitis and pancreatic carcinoma.

2. - Amylase

- amylase is the enzyme concerned with the break down of dietary starch and glycogen to maltose. It is present in pancreatic juice and saliva as well as in liver fallopian tubes and muscles. The enzyme is excreted in the Urine. The main use of amylase estimations is in the diagnosis of acute pancreatitis. The plasma amylase level may be low in liver disease and increased in high intestinal obstruction, mumps, acute pancreatitis and diabetes.

3. Trypsin

Trypsin is secreted by pancreas. Elevated levels of trypsin in plasma occur during acute pancreatic disease.

4. Alkaline phosphates (ALP)

The alkaline phosphates are a group of enzymes, which hydrolyze phosphate esters at an alkaline pH. They are found in bone, liver, kidney, intestinal wall, lactating mammary gland and placenta. In bone the enzyme is found in osteoblasts and is probably

important for normal bone function. The level of these enzymes may be increased in rickets and osteomalacia, hyperparathyroidism, paget's disease of bone, obstructive jaundice, and metastatic carcinoma. Serum alkaline phosphatase levels may be

7. Lactate Dehydrogenase (LDH)

It catalyzes the reversible interconversion of lactate and pyruvate. It is widely distributed with high concentrations in the heart, skeletal muscle, liver, kidney, brain and erythrocytes.

The enzyme is increased in plasma in myocardial infarction, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of it isoenzymes is more useful in clinical diagnosis to differentiate hepatic disease and myocardial infarction.

8. Creatine kinase (CK) or ceratin phosphokinase (CPK)

CK (CPK) is found in heart muscle brain and skeletal muscle. Measurement of serum creatine phosphokinase activity is of value in the diagnosis of disorders affectin

UNIT TWO
CARBOHYDRATS

Objectives

Define carbohydrates in chemical terms

Classify carbohydrates in to three major groups with examples of each group

List the monosaccharides of biological importance and learn their properties

List the disaccharides of biological importance and learn their properties

List the polysaccharides of biological importance and learn their properties

Study the chemistry and functions of glycoproteins

Introduction

Carbohydrates are the most abundant macromolecules in nature. They are the main source and storage of energy in the body. They serve also as structural component of cell membrane. The general molecular formula of carbohydrate is $C_nH_{2n}O_n$ or $(CH_2O)_n$, where $n \geq 3$. Chemically, they contain the elements Carbon, hydrogen and oxygen. Thus they are Carbon compounds that contain large quantities of Hydroxyl groups.

Carbohydrates in general are polyhydroxy aldehydes or ketones or compounds which give these substances on hydrolysis.

Chemistry of Carbohydrates

Classification and Structure

Classification

There are three major classes of carbohydrates

Monosaccharides (Greek, mono = one)

Oligosaccharides (Greek, oligo= few) 2-10 monosaccharide units.

Polysaccharides (Greek, Poly = many) >10 monosaccharide units.

Monosaccharides

Monosaccharides also called simple sugars. They consist of a single polyhydroxy aldehyde or ketone units. The most abundant monosaccharides in nature are the 6-carbon sugars like D-glucose and fructose.

Structure

Monosaccharide has a backbone, which is un- branched, single bonded carbon chain. One of the carbon atoms is double bonded to an oxygen atom to form carbonyl group.

Physical properties

Physical properties of Monosaccharides

They are colorless, crystalline compounds, readily soluble in water. Their solutions are optically

and L isomers are present, the resulting mixture has no optical activity, since the activities of each isomer cancel one another. Such a mixture is called racemic or DL mixture.

Epimers

When sugars are different from one another, only in configuration with regard to a single carbon atom (around one carbon atom) they are called **epimers** of each other. For example glucose and mannose are epimers. They differ only in configuration around C_2. Mannose and Galactose are epimers of Glucose

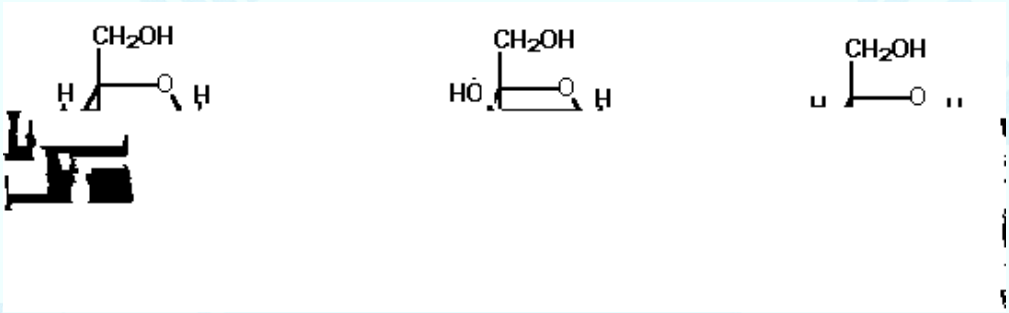


Fig 2.3**: Structure of D - glucose, D-mannose and D- Galactose**.

Anomers

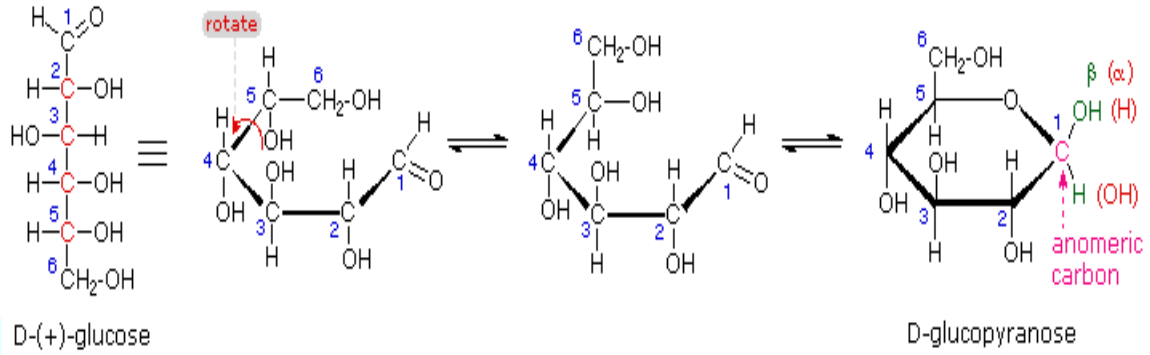
The two stereoisomers at the hemiacetal (anomeric) carbon are:

- The alpha anomer: Where- OH group is down (Haworth)
- The beta anomer:Where- OH group is up (Haworth)

Anomers are diastereomers (having different physical properties)

Cyclization of monosaccharides

Monosaccharides with five or more carbon atoms in the backbone usually occur in solution as cyclic or ring structure, in which the carbonyl group is not free as written on the open chain structure but has formed a covalent bond with one of the hydroxyl group along the chain to form a hemiacetal or hemiketal ring. In general, an aldehyde can react with an alcohol to form a hemiacetal or acetal.



The C-1 aldehyde in the open-chain form of glucose reacts with the -5th carbon atom containing hydroxyl group to form an intramolecular hemiacetal. The resulting six membered ring is called pyranose because of its similarity to organic molecule Pyran.

Two different forms of glucose are formed when the OH group extends to right it is -D-Glucose and when it extends to left, it is -D-Glucose commonly called as Anomers.

Similarly, a ketone can react with an alcohol to form a hemiketal or ketal.

The C-2 keto group in the open chain form of fructose can react with the 5th carbon atom containing hydroxyl group to form an intramolecular hemiketal. This five membered ring is called furanose because of its similarity to organic molecule furan

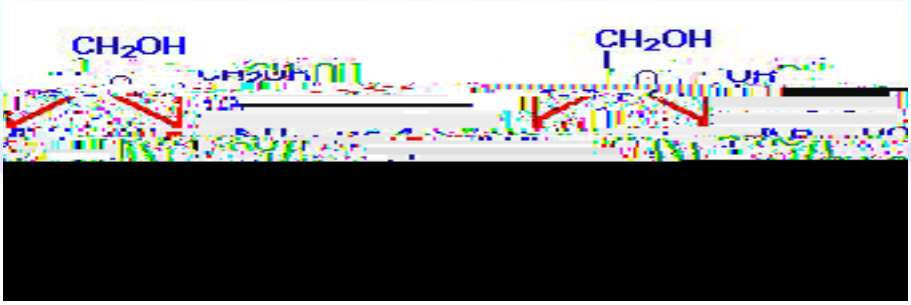


Fig 2.4. and β forms of Fructose

Oligosaccharides

Oligosaccharides contain 2 to 10 monosaccharide units. The most abundant oligosaccharides found in nature are the Disaccharides.

Disaccharides

When two monosaccharides are covalently bonded together by glycosidic linkages a disaccharide is formed. Glycosidic bond is formed when the hydroxyl group on one of the sugars reacts witr1 Do.8(s)-1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The inner part of glucose units in amylopectin are joined by -(1,4) glycosidic linkage as in amylose , but the branch points of amylopectin are - (1,6) linkages. The branch points repeat about every 20 to 30 (1-4) linkages

Glycogen

- Glycogen is the main storage polysaccharide of animal cells (Animal starch).
- It is present in liver and in skeletal muscle.
- Like amylopectin glycogen is a branched polysaccharide of D-glucose units in - (1, 4) linkages, but it is highly branched.
- The branches are formed by -(1,6) glycosidic linkage that occurs after every 8 -12 residues. Therefore liver cell can store glycogen within a small space. Multiple terminals of branch points release many glucose units in short time.

Cellulose

Cellulose is the most abundant structural polysaccharide in plants. It is fibrous, tough, water insoluble. Cellulose is a linear unbranched homopolysaccharide of 10,000 or more D- glucose units connected by -(1, 4) glycosidic bonds. Humans cannot use cellulose because they lack of enzyme (cellulase) to hydrolyze the -(1-4) linkages.

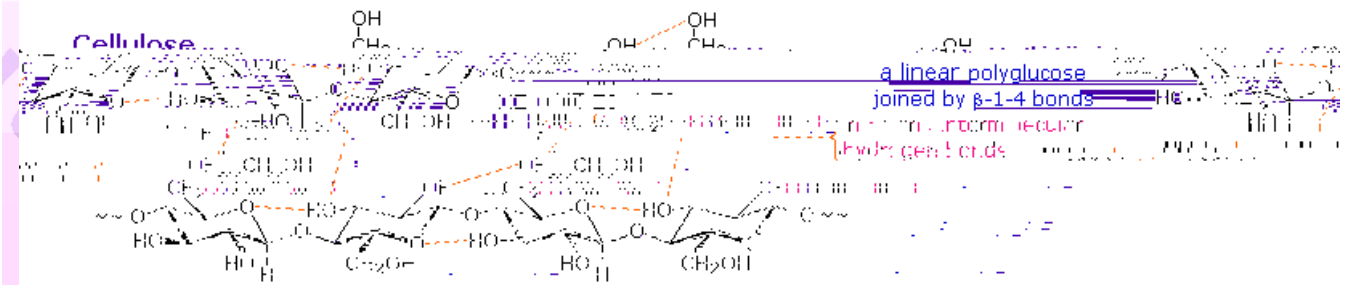

Figure: **Structure of Cellulose**

Dextrins

These are highly branched homopolymers of glucose units with -(1, 6), -(1, 4) and -(1, 3) linkages. Since they do not easily go out of vascular compartment they are used for intravenous infusion as plasma volume expander in the treatment of hypovolumic shock.

Hetero polysaccharides

These are polysaccharides containing more than one type of sugar residues

1. Glycosaminoglycans, (GAGs or mucopolysaccharides)

They are long, usually unbranched, composed of a repeating disaccharide units

* They are negatively charged heteroplolysaccharid chains (polyanions)

The amino sugar is either D-glucosamine or D-galactosamine in which the amino group is usually acetylated, thus eliminating its positive charges.

The amino sugar may also be sulfated on carbon 4, 6, or on a monoacetylated nitrogen.

The acidic sugar is either D-glucuronic acid or its carbon 6 epimer, L-uronic acid. For example Hayluronic acid, Heparin and chondatin sulphate.

Function of Glycosammoglycans. (GAGS)

1. They have the special ability to bind large amounts of water, there by producing the gel-like matrix that forms the basis of the body's ground substance.
2. Since they are negatively charged, for example, in bone, glycosaminoglycans attract and tightly bind cattions like ca^{++}, they also take-up $Na^{+}$and K^{+}
3. GAGs stabilize and support cellular and fibrous components of tissue while helping maintain the water and salt balance of the body.
4. Its essential components of the extra cellular matrix, GAGs' play an important role in mediating cell-cell interactions

   Ground substance is a part of connective tissue, which is a gel like substance containing water, salt, proteins and polysaccharides.

An example of specialized ground substance is the synovial fluid, which serves as a lubricant in joints, and tendon sheaths.

3. Heparin:

contains a repeating unit of D-glucuronic and D-gluconsamine, with sulfate groups on some of the hydroxyl and aminx-groups

It is an important anticoagualtn, prevents the clotting of blood by inhiginting the conversion of prothrombin to throbin. Thrombin is an enzyme that acts on the conversion of plasma fibrinogen into the fibrin.

It is found in mast cells in lung, liver skin and intestinal mucosa.

Glycoproteins (Mucoproteins)

Glycoprotiens are proteins to which oligosaccharides are covalently attached. They differ from the glycosaminoglycans in that the length of the glycoproteins carbohydrate chain is relatively short (usually two to ten sugar residues in length, although they can be longer), whereas it can be very long in the glycosaminoglycans.


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Action of pancreatic Amylase

It is also an - amylase, optimum pH 7.1. Like ptyalin it also requires Cl^- for activity. The enzyme hydrolyzes -(1,4) glycosidic linkage situated well inside polysaccharide molecule. Other criteria and end products of action are similar of ptyalin.

1. Digestion in Small Intestine

Action of Intestinal Juice

a. pancreatic amylase:

It hydrolyzes terminal -(1,4), glycosidic linkage in polysaccharides and Oligosaccharide molecules liberating free glucose molecules.

b. Lactase

It is a - glycosidase, its pH range is 5.4 to 6.0. Lactose is hydrolyzed to glucose and galactose.

Lactose —(*lactase*)→ *Glucose + Galactose*

Lactose Intolerance

Lactose is hydrolyzed to galactose and glucose by lactase in humans (by - Galactosidase in Bacteria).Some adults do not have lactase.Such adults cannot digest the sugar.It remains in the intestines and gets fermented by the bacteria. The condition is called as Lactose intolerance. Such patients suffer from watery diarrhea, abnormal intestinal flow and chloeic pain. They are advised to avoid the consumption of Lactose containing foods like Milk.

C. Maltase

The enzyme hydrolyzes the -(1,4) glycosidic linkage between glucose units in maltose molecule liberating two glucose molecules. Its pH range is 5.8 to 6.2.

Maltose —(***Maltase***)→ ***Glucose + Glucose***

D. Sucrase

PH ranges 5.0 to 7.0. It hydrolyzes sucrose molecule to form glucose and fructose.

Sucrose —(***Sucrase***)→ ***Glucose + fructose***


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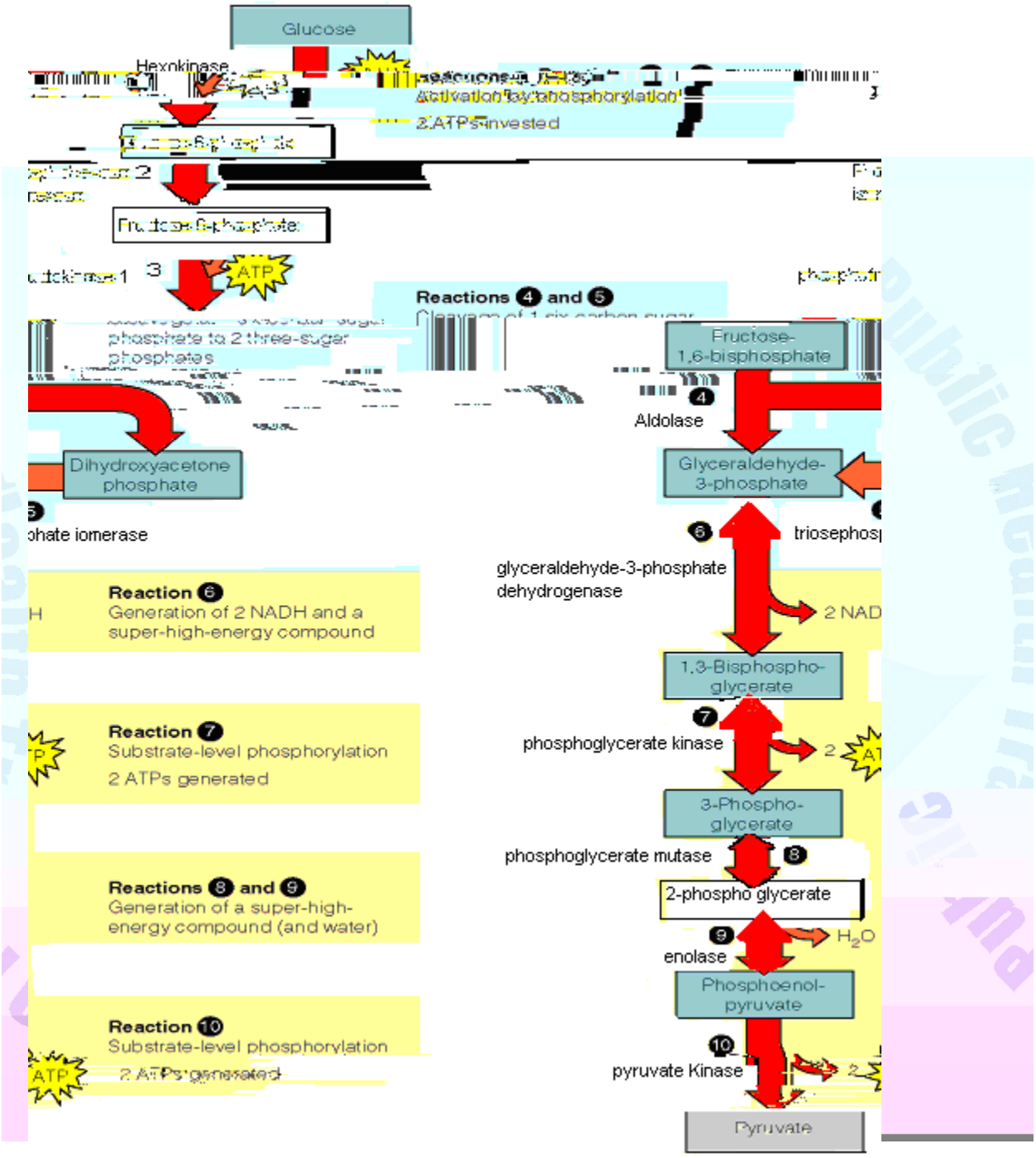

Fig 2.8: **Glycolysis reactions**

ATP acts as PO_4 donor in the presence of Mg .One high energy PO_4 bond is utilized and ADP is produced. The reaction is accompanied by considerable loss of free energy as heat, and hence under physiological conditions is regarded as irreversible.

Glucose 6 phosphate formed is an important compound at the junction of several metabolic pathways like glycolysis, glycogenesis, glycogenolysis, glyconeogenesis, Hexosemonophosphate Shunt, uronic acid partway. Thus is a "committed step" in metabolic pathways.

2. Conversion of G- 6- phosphate to Fructose6-phosphate

Glucose6 phosphate after formation is converted to fructose 6-p by phospho- hexose isomerase, which involves an aldose- ketose isomerization. The enzyme can act only on - anomer of Glucose 6 phosphate.

Phospho- hexose **isomerase**

Glucose 6 phosphate ⟶ Fructose 6 phosphate

3. Conversion of Fructose 6phosphate to Fructose 1, 6 bisphosphate

The above reaction is followed by another phosphorylation. Fructose-6-p is phosphorylated with ATP at 1- position catalyzed by the enzyme phospho- fructokinase-1 to produce the symmetrical molecule fructose –1, 6 bis phosphate.

Note:

reaction one is irreversible

One ATP is utilized for phosphorylation of glucose at position 6

Phosphofruvctokinase I is the key enzyme in glycolysis that regulates the pathway. The enzyme is inducible, as well as allosterically modified

Phosphofructokinase II is an is enzyme which catalyzes the reaction to form fructose-2 6-bis phosphate.

Fructose-6-phosphate + ATP ⟶ Fructose-2, 6-bisphosphate + ADP

Energetics

Note that in this stage glucose oxidation does not yield any useful energy rather there is expenditure of 2 ATP molecules for two phosphorylations (-2 ATP).

Stage II

Here,Fructose, 1, 6- bisphosphate is split by the enzyme aldolase into two molecules of triose-phosphates, an Aldotriose, glyceraldehyde3 phosphate and a Ketotriose, Dihydroxy acetone phosphate.

Note

The reaction is reversible

There is neither expenditure of energy nor formation ATP

Aldolases are tetramers, containing 4 subunits. Two isoenzymes.A,B

Aldolase B: occurs in liver and kidney

The fructose- 6-p exists in the cells in "furanose" form but they react with isomerase, phosphofructokinase-1 and aldolase in the open-chain configuration.

Both triose phosphates are interconvertable

D- glyceradehyde-3 –p

Stage III

This is the energy- yielding reaction. Reactions of this type in which an aldehyde group is oxidized to an acid are accompanied by liberation of large amounts of potentially useful energy.

This stage consists of the following two reactions:

1. Oxidation of Glyceraldehyde 3phosphate to 1,3 bis phosphoglycerate

   Glycolysis proceeds by the oxidation of glyceraldehde-3-phosphate,to form1,3-bis phosphoglycerate.

Dihydroxyacetone phosphate also forms 1, 3 - bisphosphoglycerate via glyceraldehydes-3-phosphate shuttle.The enzyme responsible is Glyceraldehyde 3 phosphate dehydrogenase, which is NAD+ dependant.

Energetics

1. In first reaction of this stage- NADH produced will be oxidized in electron transport chain to produce 3 ATP in presence of O_2. Since two molecules of triose phosphate are formed per molecule of glucose oxidtes,


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Significance of lactate formation:

Under anaerobic conditions NADH is re oxidized via lactate formation. This allows glycolysis to proceed in the absence of oxygen. The process generates enough NAD for another cycle of glycolysis.

B. Clinical Importance

Tissues that function under hypoxic conditions will produce lactic acid from glucose oxidation. Produces local acidosis. If lactate production is more it can produce metabolic acidosis

Vigorously contracting skeletal muscle will produce lactic acid.

Whether O_2 is present or not, glycolysis in erythrocytes always terminated in to pyruvate and lactate.

Entry of fructose in to glycolysis:

Liver contains specific enzymes fructokinase. It converts fructose to fructose 1 phosphate in the presence of ATP. In liver fructose1-phosphate is split to glyceraldehyde and dihydroxy acetone phosophate by AldolaseB.

Glyceraldehyde enters glycolysis, when it is phosphorylated to glyceraldehyde-3-P by triose kinase.

Dihydroxy aceton phosphate and glyceraldehyde-3-P may be degraded via glycolysis or may be condensed to form glucose by aldolase.

Lack of fructose kinase leads to fructosuria. Absence of aldolaseB leads to hereditary fructose intolerance. If fructose 1, 6 bisphosphatase is absent, causes fructose induced hypoglycemia. The reason being high concentration of Fructose 1 phosphate and fructose 1, 6 bis phosphate inhibit Liver phosphorylase by allosteric modulation.

As in case of Galactose, fructose intolerance can also lead to cataract formation.

Galactose:

Milk sugar contains galactose. Galactokinase converts galatose to galactose-1-P.It reacts with

Galactosemia:

Some people cannot metabolize galactose. It is an inherited disorder that the defect may be in the galactokinase, uridlyl transferase or 4-epimerase.Most common is uridyl transferase. Such patients have high concentration of Galactose in blood (Galactosemia).In lense, Galactose is reduced to galactitol by aldose reductase.The product accumulates in lense and leads to accumulation of water by osmotic pull. This leads to turbidity of lense proteins (Cataract).

If uridyl transferase was absent galctose 1-phosphate accumulates.Liver is depleted of inorganic phosphate. This ultimately causes failure of liver function and mental retardation.

If 4-epimerase is absent, since the patient can form UDP-galactose from glucose the patient remains symptom free.

Glycogen metabolism

Introduction

Glycogen is the major storage form of carbohydrate in animals .It is mainly stored in liver and muscles and is mobilized as glucose whenever body tissues require.

Degradation of Glycogen (glycogenolysis)

A. Shortening of chains

Golycogen phosphorylase cleaves the

B. Removal of Branches

A debranching enzyme also called Glucantransferase which contains two activities, Glucantransferase and Glucosidase. The transfer activity removes the terminal 3 glucose residues of one branch and attaches them to a free C_4 end of the second branch.The glucose in -(1,6) linkage at the branch is removed by the action of Glucosidase as free glucose.

Fig.2.9 Summary of Glycogenolysis

Synthesis of Glygogen (Glycogenesis)

Synthesis of glycogen from Glucose is carried out by the enzyme Glycogen Synthase.The

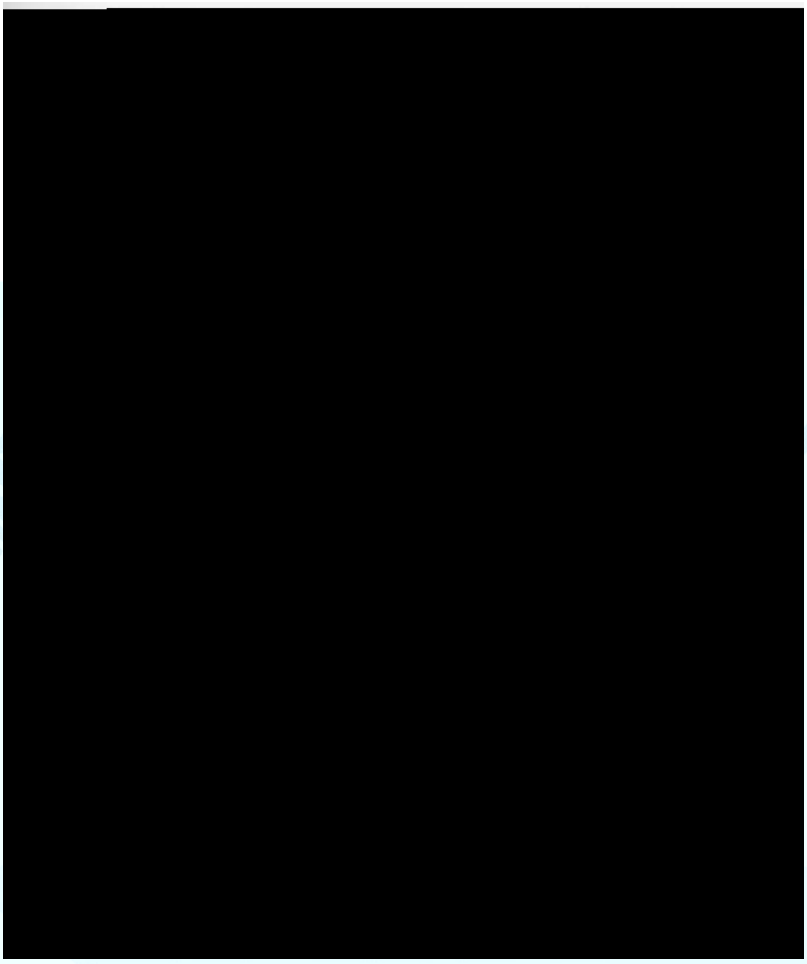

Fig 2.10. Glycogenesis

Glycogen storage diseases

These are a group of genetic diseases that result from a defect in an enzyme required for either glycogen synthesis or degradation.They result in either formation of glycogen that has an abnormal structure or the accumulation of excessive amounts of normal glycogen in specific tissues,

A particular enzyme may be defective in a single tissue such as the liver or the defect may be more generalized, affecting muscle, kidney, intestine and myocardium. The severity of the diseases may range from fatal in infancy to mild disorders that are not life threatening some of the more prevalent glycogen storage diseases are the following.


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requires NADPH as the electron source, therefore, any rapidly proliferating cell needs large quantities of NADPH.

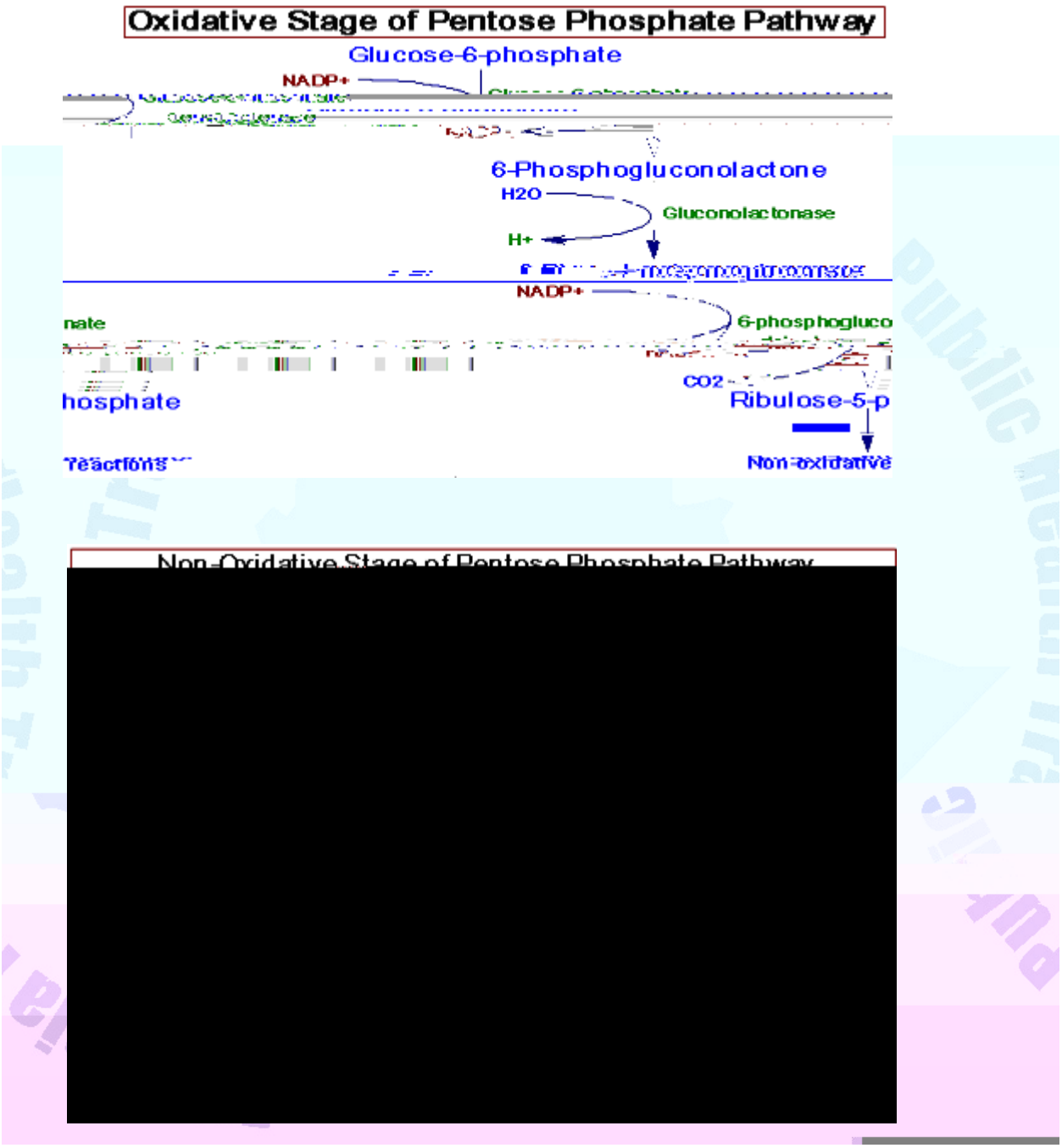

Fig 2.11 Oxidative and non –oxidative phases of HMP shunt

Significance of HMP shunt

The net result of the PPP, if not used solely for R5P production, is the oxidation of G6P, a 6 carbon sugar, into a 5 carbon sugar. In turn, 3 moles of 5 carbon sugar are converted, via the

enzymes of the PPP, back into two moles of 6 carbon sugars and one mole of 3 carbon sugar. The 6 carbon sugars can be recycled into the pathway in the form of G6P, generating more NADPH. The 3 carbon sugar generated is glyceraldehyde-3-phsphate which can be shunted to glycolysis and oxidized to pyruvate. Alternatively, it can be utilized by the gluconeogenic enzymes to generate more 6 carbon sugars (fructose-6-phosphate or glucose-6-phosphate).

Glutathione is the tripeptide **-glutamylcysteinylglycine**. The cysteine thiol plays the role in reducing oxidized thiols in other proteins. Oxidation of 2 cysteine thiols forms a disulfide bond. Although this bond plays a very important role in protein structure and function, inappropriately introduced disulfides can be detrimental. Glutathione can reduce disulfides nonenzymatically. Oxidative stress also generates peroxides that in turn can be reduced by glutathione to generate water and an alcohol. It can also reduce hydrogen per-oxide in to two molecules of water.

Regeneration of reduced glutathione is carried out by the enzyme, ***glutathione reductase***. This enzyme requires the co-factor NADPH when operating in the direction of glutathione reduction which is the thermodynamically favored direction of the reaction.

It should be clear that any disruption in the level of NADPH may have a profound effect upon a cells ability to deal with oxidative stress. No other cell than the erythrocyte is exposed to greater oxidizing conditions. After all it is the oxygen carrier of the body.

The PPP in erythrocytes is essentially the only pathway for these cells to produce NADPH. Any defect in the production of NADPH could, therefore, have profound effects on erythrocyte survival.

Several deficiencies in the level of activity (not function) of ***glucose-6-phosphate dehydrogenase*** have been observed to be associated with resistance to the malarial parasite, *Plasmodium falciparum*, among individuals of Mediterranean and African descent. The basis for this resistance is the weakening of the red cell membrane (the erythrocyte is the host cell for the parasite) such that it cannot sustain the parasitic life cycle long enough for productive growth.

Coris Cycle or Lactic Acid Cycle

In an actively contracting muscle, only about 8% of the pyruvate is utilized by the citric acid cycle and the remaining is, therefore, reduced to lactate. The lactic acid thus generated should not be allowed to accumulate in the muscle tissues. The muscle cramps, often associated with strenuous muscular exercise are thought to be due to lactate accumulation. This lactate diffuses into the blood. During exercise, blood lactate level increases considerably. Lactate then reaches

liver where it is oxidized to pyruvate. It is then taken up through gluconeogenesis pathway and becomes glucose, which can enter into blood and then taken to muscle. This cycle is called cori's cycle, by which the lactate is efficiently reutilized by the body.

Significance of the cycle:

Muscle cannot form glucose by gluconeogenesis process because glucose 6 phosphatase is absent. Unlike Liver, muscle cannot supply Glucose to other organs inspite of having Glycogen.

Fig 2.12 The Cori cycle.

Gluconeogenesis

Gluconoegenesis is the biosynthesis of new glucose from non carbohydrate substrates.

In the absence of dietary intake of carbohydrate liver glycogen can meet these needs for only 10 to 18 hours

During prolonged fast hepatic glycogen stores are depleted and glucose is formed from precursors such as lactate, pyruvate, glycerol and keto acids.

Approximately 90% of gluconeogenesis occurs in the liver whereas kidneys provide 10 % of newly synthesized glucose molecules,

The kidneys thus play a minor role except during prolonged starvation when they become major glucose producing organs.

Reactions Unique to Gluconeogenesis

Seven of the reactions of glycolysis are reversible and are used in the synthesis of glucose from lactate or pyruvate. However three of the reactions are irreversible and must be bypassed by four alternate reactions that energetically favor the synthesis of glucose.

A. Carboxylation of Pyruvate

In gluconeogenesis, pyruvate is first carboxylated by pyruvate Carboxylase to oxaloacetate (OAA).where it is converted to Phosphoenolpyruvate (PEP) by the action of PEP carboxykinase,

Note: pyruvate carboxylase is found in the mitochondria of liver and kidneys, but not in muscle

1. Biotin is a coenzyme of pyruvate carboxylase derived from vitamin B6 covalently bound to the apoenyme through an ε-amino group of lysine forming the active enzyme.
2. Allosteric regulation

Pyruvate carboxylase is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA may signal one of several metabolic states in which the increased synthesis of oxaloacetate is required. For example, this may occur during starvation where OAA is used for the synthesis of glucose by gluconeogenesis,

At low levels of acetyl COA, pyruvate carboxylase is largely inactive and pyruvate is primarily oxidized in the TCA cycle

B. transport of Oxaloacetate to the Cytosol

Oxaloacetate, formed in mitochondria, must enter the cytosol where the other enzymes of gluconeogenesis are located. However, oxaloacetate is unable to cross the inner mitochondrial membrane directly. It must first be reduced to malate which can then be transported from the mitochondria to the cytosol. In the cytosol, Malate is reoxidized to oxaloactate (see figure 2.13)

C. Decarboxylation of Cytosolic Oxaloacetate.

Oxaloacetate is decarboxylated and phosphorylated in the cytosol by PEP-carboxykinase. The reaction is driven by hydrolysis of GTP

The combined action of pyruvate carboxylase and PEP carboxykinase provides an energetically favorable pathway from pyruvate to PEP.

PEP then enters the reversed reactions of glycolysis


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Advantages of Gluconeogenesis

1) Gluconeogenesis meets the requirements of glucose in the body when carbohydrates are not available in sufficient amounts.
2) Regulate Blood glucose level
3) Source of energy for Nervous tissue and Erythrocytes
4) Maintains level of intermediates of TCA cycle
5) Clear the products of metabolism of other tissues(Muscle)

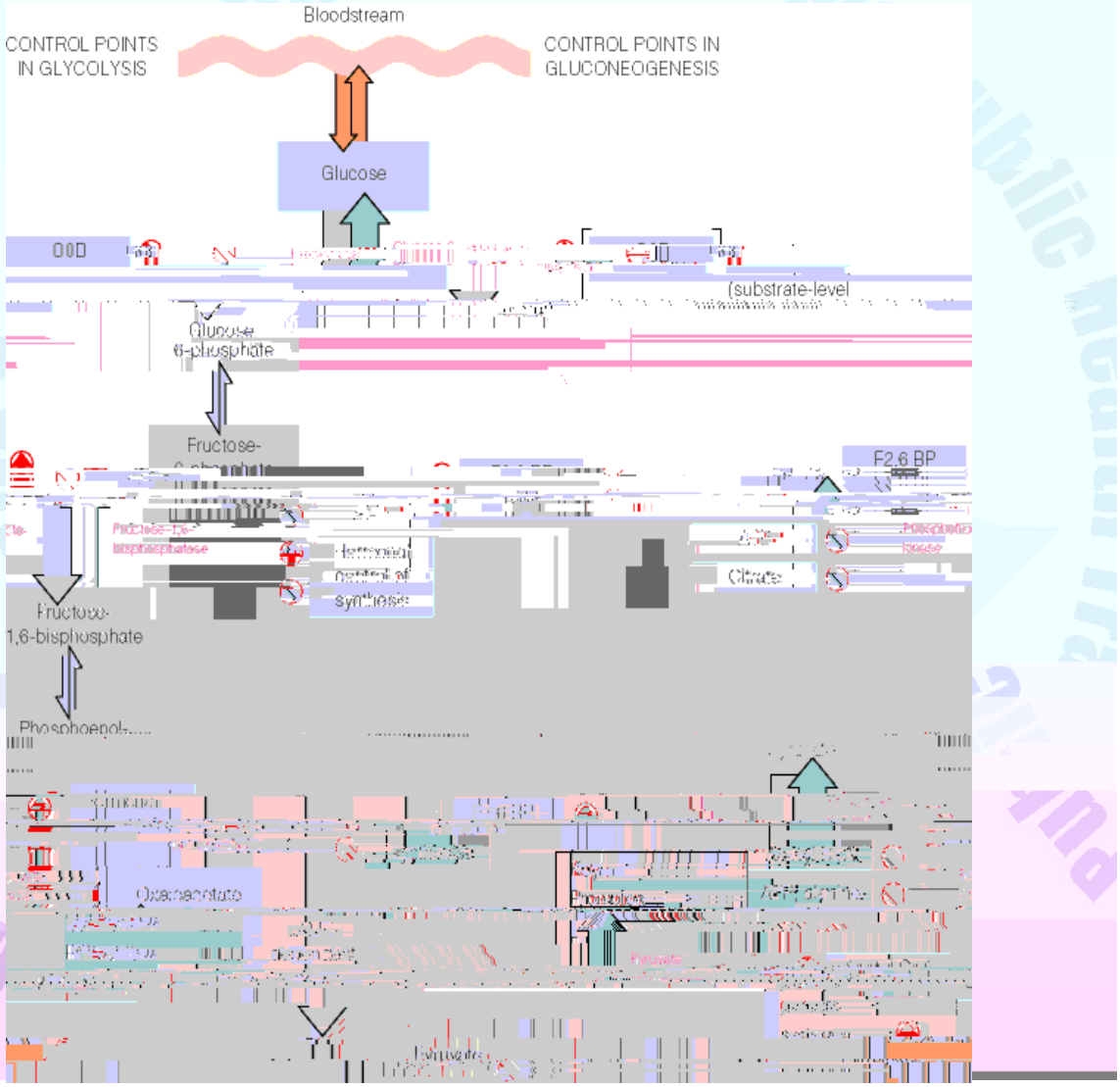

Fig.2.13 Major control mechanisms affecting glycolysis and gluconeogenesis

Homeostasis of Blood Glucose

Homostasis of glucose is due to balance of addition and utilization of glucose. Fasting blood glucose is maintained between 80-120mg %. After a meal it rises by 40-60mg% and returns to normal within 2-3hours.

UNIT THREE

INTEGRATIVE METABOLISM AND BIOENERGETICS

Objectives

1. To enable the students:
2. Identify energy rich dietary constituents
3. Identify cellular sites of energy generation
4. Understand mechanism of cellular ATP formation and utilization
5. Understand ways of regulation of cellular energy metabolism
6. Understand the mechanism and effect of poisons on cellular energy generation

Energy Generation and Utilization in the Living System

I-Introduction

Energy is vital to life. Growth, reproduction and tissue repair require energy. Most organisms obtain energy by oxidation of these fuel molecules Carbohydrates, fats and amino acids. Cellular oxidation of these molecules release energy, part of which is conserved through the synthesis of high-energy phosphate bonds and the rest is lost as heat. The high-energy phosphate bonds are directly utilized for cellular energy requiring processes. ATP (adenosine triphosphate) is the common high-energy phosphate bond that is formed during oxidative processes.

Under cellular conditions energy releasing (oxidative) processes are coupled to energy requiring cellular processes through common energy currency, ATP.

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Glycolysis- partial catabolism

- small amount of energy conserved (ATP, NADH)
- prepares carbohydrates for the next catabolic processes
- sometimes the only life sustaining energy generating process
    - RBC (red blood cells-lack mitochondrion)
    - exercising muscle (oxygen limitation)

b) Fats-digestion or mobilization of stored fat

Fatty acids + glycerol

Transport by albumin

Oxidation by major pathway → Oxidation

Section B- Concept of Free Energy

Definition – Free energy is that portion of the energy of a system available to do work as the system proceeds toward equilibrium under conditions of constant temperature and pressure and volume

The change in free energy content (G) depends on two components

H (change in enthalpy, internal energy heat) and S (change in entropy)

$$G = H - T S$$

Chemical reaction performs work if it can be harnessed in utilizable form of energy. Amount of work performed depends on the efficiency of the machinery.

Free energy change of a biological reactions is reported as the standard free energy change ($G^{0'}$)

$G^{0'}$ is the value of **G** for a reaction at standard conditions for biological reactions (pH 7, 1M, 25^{o}C, 1 atmosphere pressure)

Free energy change is used to predict the direction and equilibrium of chemical reactions

If **G** is negative – net loss of energy (exergonic)

- reaction goes spontaneously

If **G** is positive - net gain of energy (endergonic)

reaction does not go spontaneously

If **G** is zero- reactants are in equilibrium

C - Oxidation-Reduction Reactions

The utilization of chemical energy in living system involves oxidation – reduction reactions. For example, the energy of chemical bonds of carbohydrates, lipids and proteins is released and captured in utilization form by processes involving oxidation- reductions.

I- Oxidation - removal of electron(s) from substance

- usually accompanied by a decrease in energy content of oxidized substance

II-Reduction - addition of electron(s) to a substance

usually accompanied by an increase in energy content of reduced substance

Oxidation reduction reactions are coupled processes

Example: H_2 $2e^- + 2H^+$ – first half reaction

– oxidation of H_2

– release electrons and protons

– requires second half reaction coupled to

– oxidation of H_2

$\frac{1}{2} O_2 + 2e^- + 2H^+$ H_2O – second half reaction

– reduction of O_2

– oxidation of H_2 is coupled to reduction of O_2

III- Reduction Potential (Oxidation-reduction potential, E'_o)-Concept

Definition - Measure of electron donating tendencies

Electrically measured in reference to a standard substance H_2. Determined by measuring the electromotive force generated by a sample half-cell with respect to standard reference half-cell

Anegative E'_o = lower affinity for electrons

A positive E'

In respiratory chain the electrons from NADH are transferred through a series of carriers (organic or inorganic) until they are accepted by molecular oxygen (O_2) releasing energy at different levels. The free-energy change of an oxidation – reduction reaction can be calculated from the difference in reduction potentials of the reactants using the formula:

$G^{O'} = - nF \ E'_o$

Where n= 2 (No of electrons transferred)

F= 23.06 faraday constant

E'_o = + 0.82 – (-0.32) = 1.14 V potential difference

For example consider oxidation of NADH by O_2

$G^{O'} = -2 \times 23.06$ kcal V^{-1} $mol^{-1} \times 1.14$ V

= - 52.6 Kcal/mol → heat

→ 3 ATP (about 40% of efficiency)

Under cellular condition part of the free-energy of oxidation of reducing equivalents is conserved in the form of high-energy phosphate compound, ATP. This occurs by the help of energy conserving system in the inner mitochondrial membrane of eukaryotes or plasma membrane of prokaryotes.

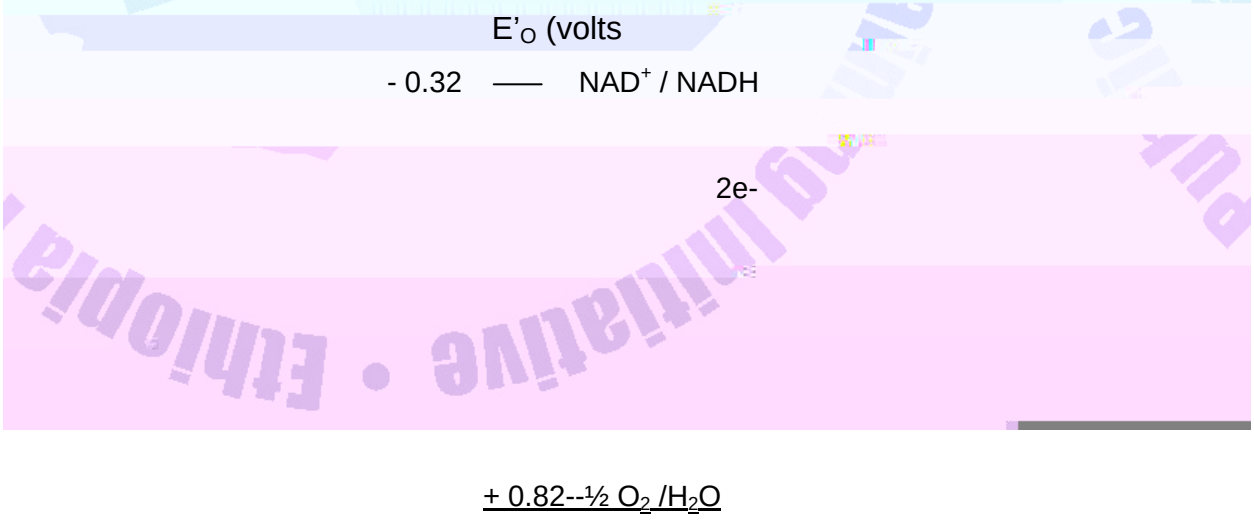


Fig 3.2. Change in free energy as a result of oxidation of NADH

Aerobic Energy-Generation

In aerobic organisms the complete breakdown of fuel molecules, carbohydrates, fats and proteins takes place in mitochondria of eukaryotes and cytoplasmic membrane and cytoplasm of aerobic prokaryotes.

The fuel molecules are metabolized to a common intermediate called aceyl CoA which is further degraded by a common pathway called Kreb's cycle.

This metabolic pathway in addition to providing energy provides building blocks required for growth, reproduction, repair and maintenance of cellular viability.

Mitochondria - it is an organelle where major amount of energy produced. Structurally it is bounded by two separate membranes (outer mitochondrial membrane and inner mitochondrial membrane)

Out membrane - smooth and unfolded
- Freely permeable to most ions and polar molecules
(Contain porous channels)

Inner membrane - folded into cristae-increased surface area
- Highly impermeable to most ions and polar molecules

Contain transporters which access polar and ionic molecules in and out

Cristae are characteristic of muscle and other metabolically active cell types
- Protein-rich membrane (about 75%)

Inter membrane space – space between outer and inner membranes

Matrix-the internal compartment containing soluble enzymes and mitochondrial genetic material

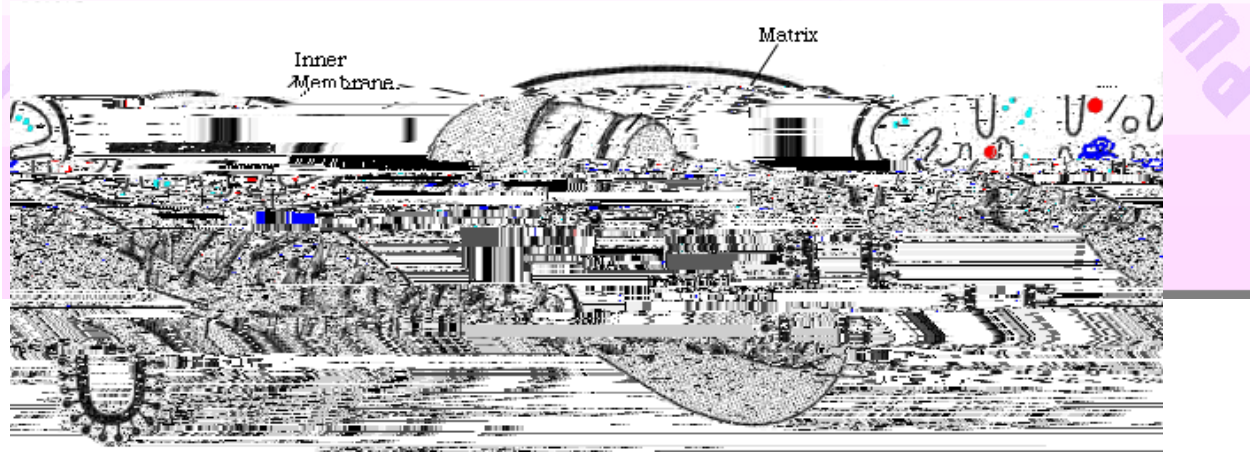


Fig 3.3 Structure of a mitochondria

Oxidation of Pyruvate

Pyruvate is common intermediate of many catabolic reactions. It is still energy rich molecule. It is a cross road molecule which can be converted into different intermediates depending on :-

type of cells-eukaryotes except RBC- acetyl CoA

- aerobic prokaryotes- acetyl CoA
- anaerobic prokaryotes- ethanol or lactate
- in RBC-always lactate

absence or presence of oxygen -lactate, ethanol or acetyl-CoA

- high ratio of $NADH/NAD^+$ favors lactate formation in actively exercising muscle (oxygen limitation)

Alanine

Oxaloacetate **Pyruvate** Lactate

Acetyl CoA

Oxidation of pyruvate into acetyl CoA-aerobic process (O_2 terminal electron-acceptor) which takes place in the mitochondrial matrix of eukaryotic cells Pyruvate is transported into mitochondrial matrix by special transporter.Inside matrix pyruvate is oxidized into acetylCoA by pyruvate dehydrogenase complex which is complex of E_1, E_2 and E_3 enzymes.This enzyme requires s five coenzymes- TPP, Lipoate, CoA, FAD and NAD+

Where: E_1 = pyruvate dehydrogenase,

E_2 = dihydrolipoyl transacetylase

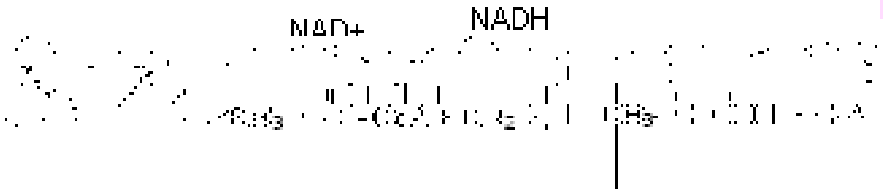
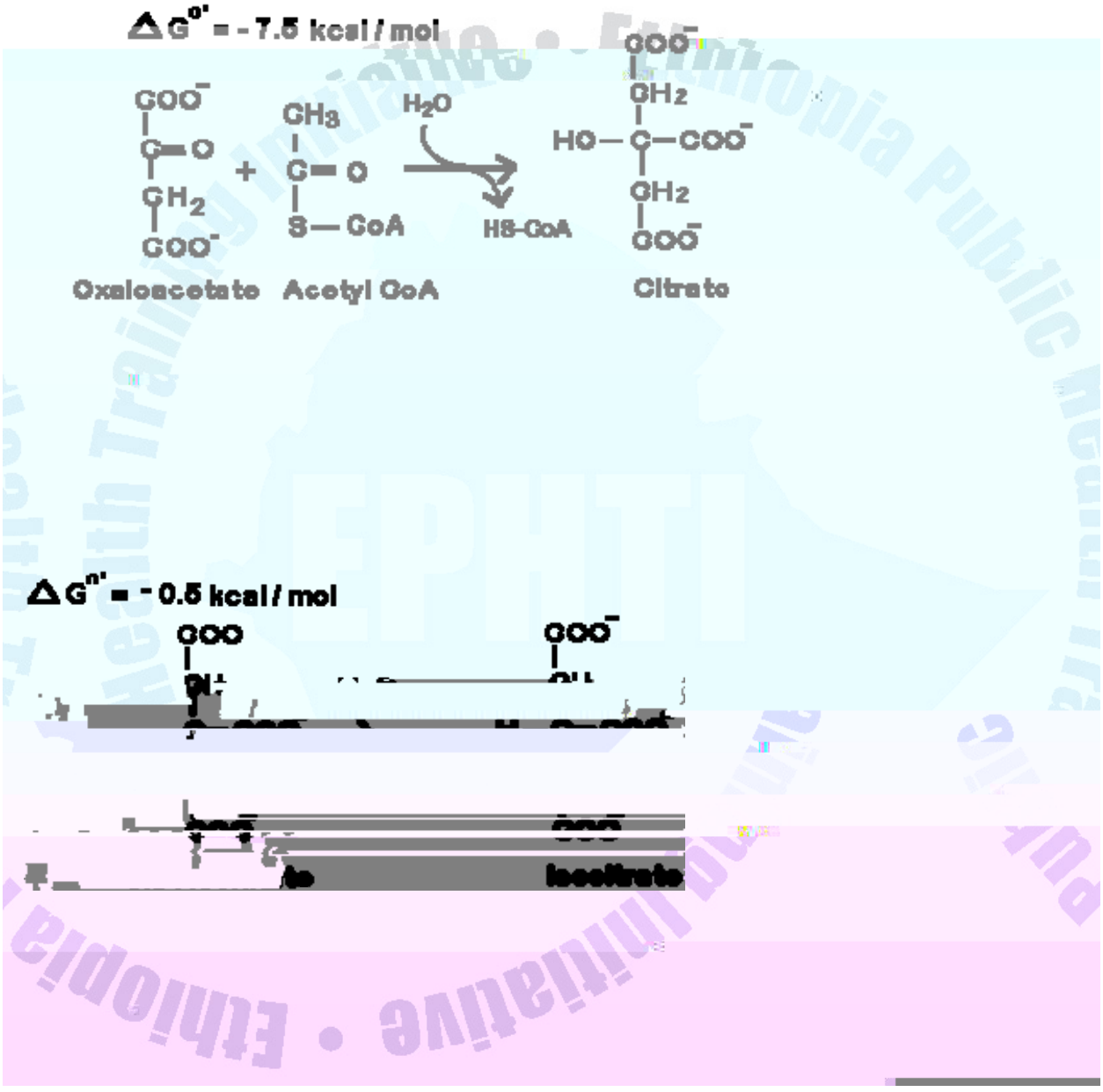


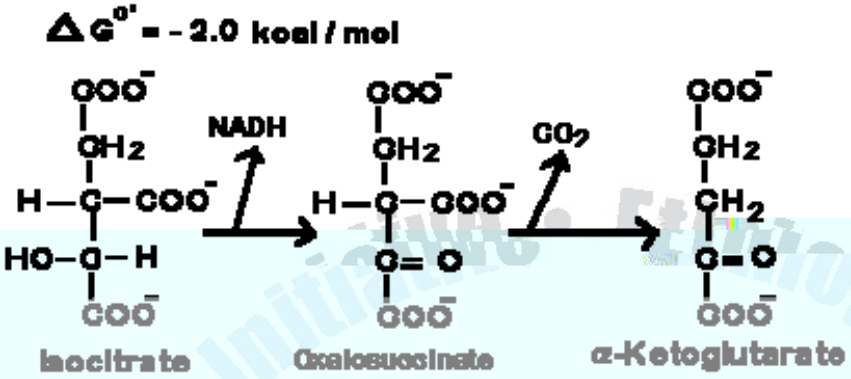
Fig 3.4 The citric acid cycle

Reactions of Kreb's cycle

1. **Condensation of acetyl COA with oxaloacetate by citrate synthase (condensing enzyme) to form citrate.**



3**. Oxidative decarboxylation of isocitrate by isocitrate dehydrogenase**



There are three types of isocitrate dehydrogenases

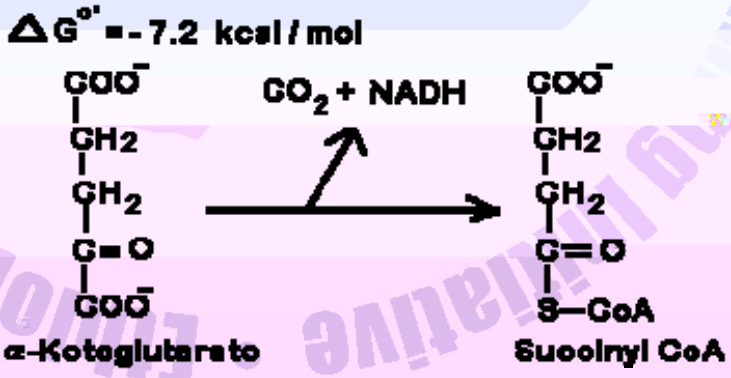
NAD+ - specific – only mitochondrial

NADP+ - specific – cytosolic and mitochondrial

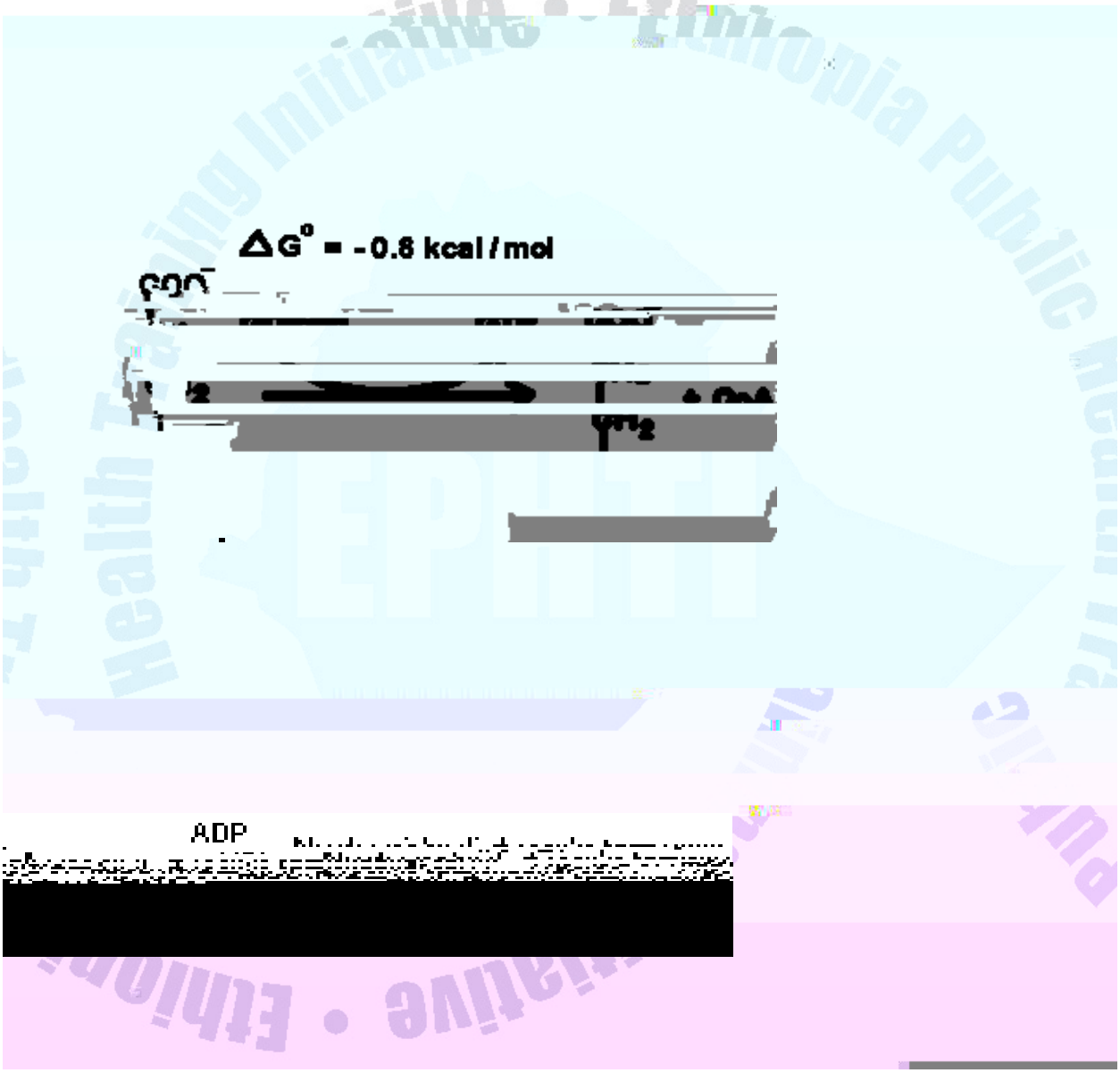
Respiratory chain linked oxidation of isocitrate proceeds almost completely through NAD+ - dependent enzyme

* Cytosolic isocitrate dehydragenase reaction generates NADPH and CO_2

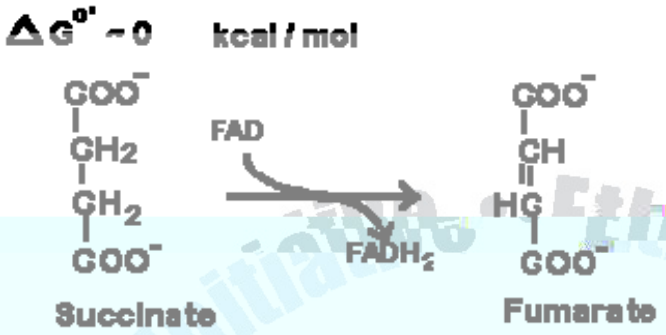
anabolic m3qd



A' (- ketoglutarate dehydrogenase), B' (transu



6**. Oxidation of succinate by succinate dehydrogenase**



- Stereospecific for transfer of H-atoms of succinate (*)
- Succinate dehydrogenese – is integral part of inner mitochondrial membrane
- Contains Fe – S centers (non-heme iron protein) and FAD as prosthetic groups

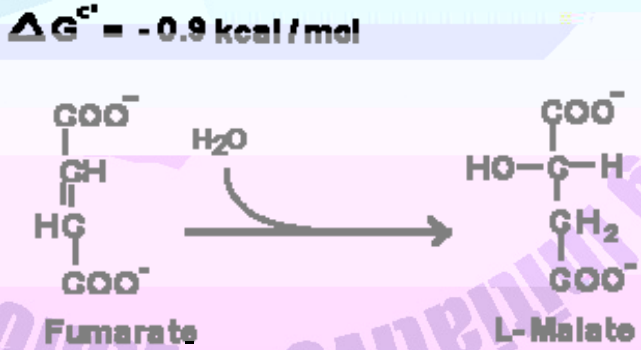
Flavoprotein

- Inhibited by malonate which competes for the active site of the enzyme

-OOC-CH_2-COO- (Malonate)

- Also inhibited by oxaloacetate resulting in succinate accumulation.

7**. Hydration of Fumarate by Fumarase**



- Fumarase is stereospecific for L-malate (catalyzes stereospecific trans addition of H and OH).

8. **Oxidation of L-malate by NAD–linked malate dehydrogenase**

This reaction regenerates oxaloacetate, used in the first reaction.

N.B:

The cycle is aerobic process i.e. regeneration of oxidized coenzymes requires O_2 as terminal electron acceptor.

No net consumption or production of cycle intermediates

Oxaloacetate plays catalytic role in catabolism of acetyl CoA

Energy of acetyl CoA catabolism 792STc-.0 bT ofnservd sAD–H an-6.(d)49( )]TJ/-2.4215301.7268 TD-.00

Provide precursors for – gluconeogenesis (all intermediates)

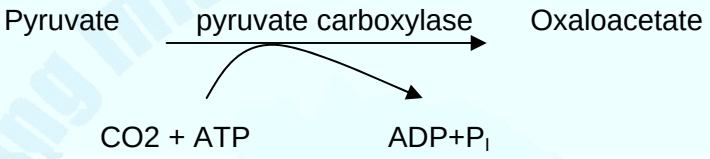
– amino acid synthesis (non-essial amino acids)

– heme synthesis (succinyl CoA)

– fatty acid synthesis (citrate)

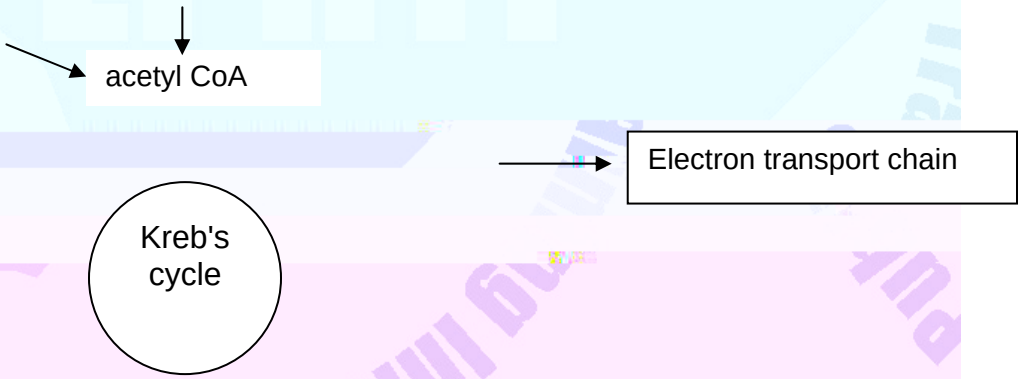
Regulate other pathways – citrate (inhibit phosphofructokinase)

pyruvate carboxylation with formation of oxaloaccetate replenishes the cycle intermediates used for biosynthesis.



Note -different amino acids enter the cycle at different points

- Propionyl CoA (product of odd number fatty acid oxidation) enter the cycle as succinyl CoA



C - Regulation of Kreb's Cycle

Primary function of the cycle is to provide energy, thus rate of the cycle is adjusted to meet an animal cells ATP demand. Increased utilization of ATP increases the rate the cycle because of availability of oxidized coenzymes necessary for the continuation of the cycle (NAD+, FAD) and ADP which is needed for oxidative phosphorylation. High levels of ATP and NADH are inhibitory

indicating high energy status of the cell. ATP inhibits both citrate synthase and isocitrate dehydrogenase where as both are activated by high levels of ADP. NADH inhibits isocitrate dehydrogenase and -Ketoglutarate dehydrogenase. Complementary mechanisms of controlling rate of acetyl CoA formation and rate of acetyl CoA degradation is also involved

III- Electron Transport system and Oxidative Phosphorylation

In aerobic organisms the major amount of ATP is synthesized by phosphorylation of ADP related to electron transport, a process occurring on the inner mitochondrial membrane of eukaryotes. It is a system composed of a chain of membrane associated electron carriers.

a- Components:

flavoproteins – FMN and FAD prosthetic groups. Accept H atoms but donate electrons

nonheme iron-sulfur proteins (Fe: S centers). Carry electrons not H – atoms. They are component of complexes (I, II and III)

Complexes of respiratory chain are designated complex I, II, III and IV (integral parts of inner mitochondrial membrane). CoQ and cytochrome C are mobile electron carriers which act as a link between the complexes.

Complex	Components
I (NADH dehydrogenase)	FMN, Fe-S centers
II (Succinate dehydrogenese)	FAD, Fe-S centers
III (Cytochrome reductase)	cyt.b, cytc, Fe-S center
IV (Cytochrome oxidase)	cyt.a, cyt.a_3 ,copper (Cu^{1+}/Cu^{2+})

Table 2. Components of electron transport chain

Complexes I, III and IC are proton pumps (trans-member and proteins) linked by CoQ and Cyt.c (mobile electron carriers)

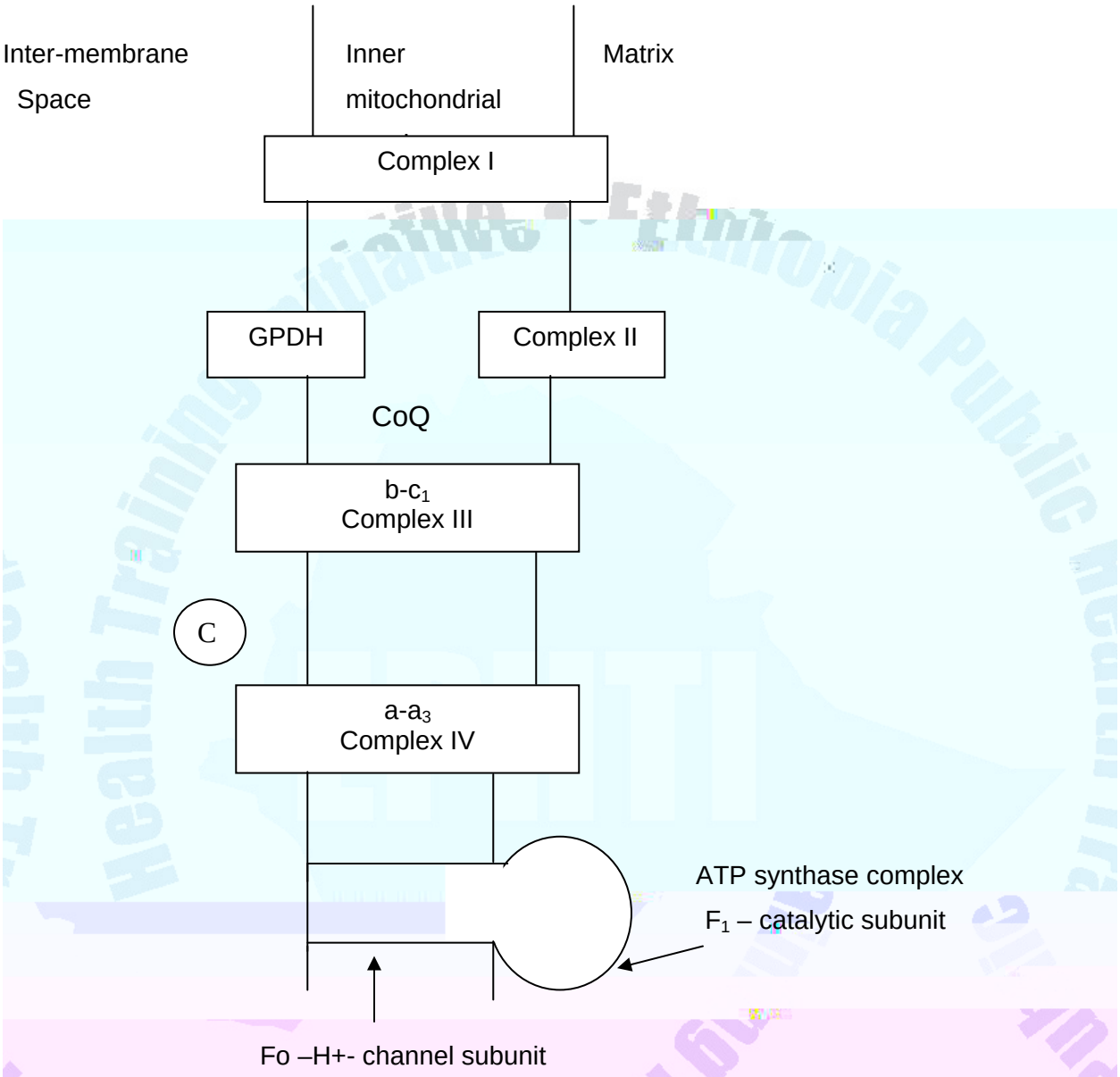


Fig 3.5. Arrangement of complex in electron transport chain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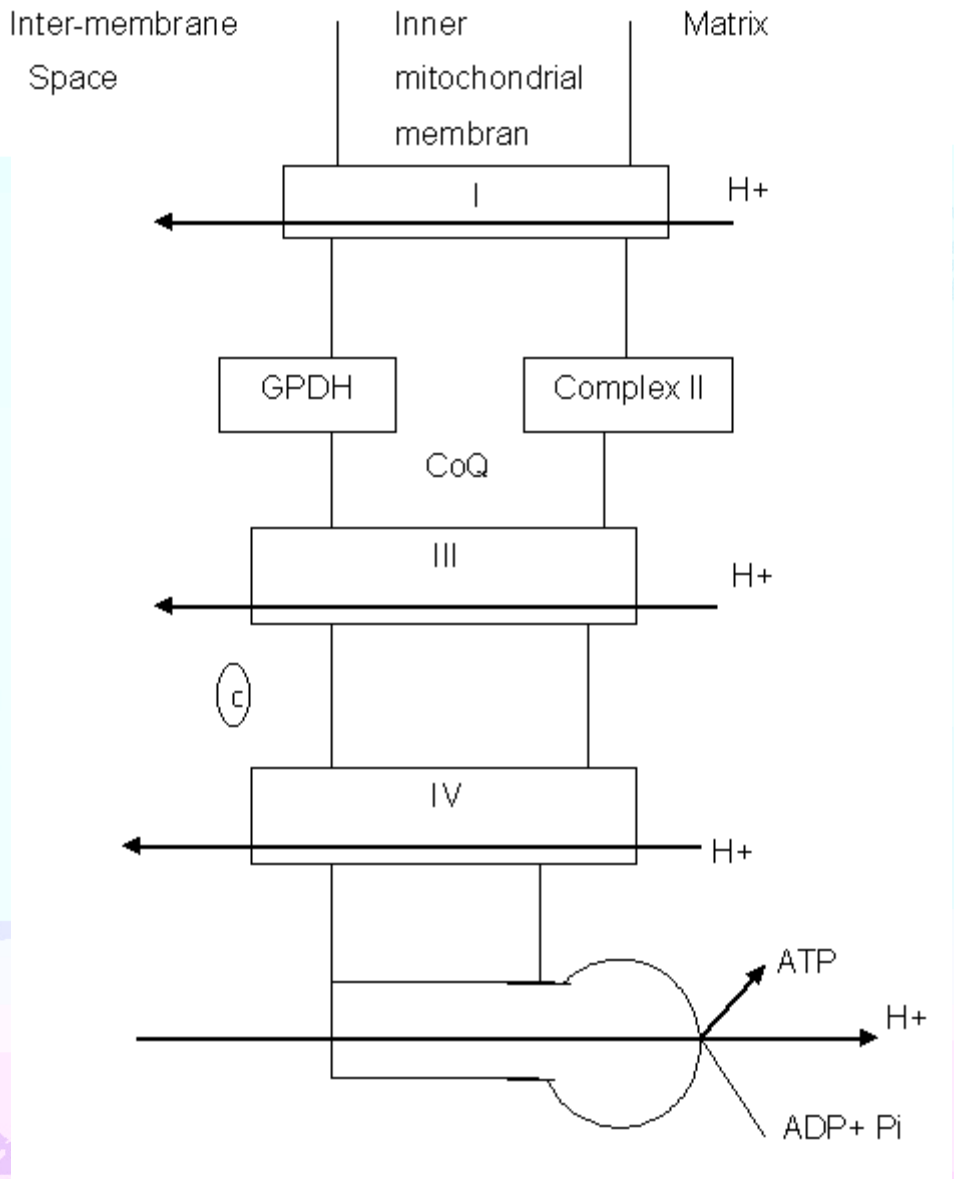


Fig 3.6. **ATP synthesis coupled to electron transport**

Energy of oxidation lost as heat

Types of Uncouplers:

1. Chemical uncouplers – example 2, 4-dinitrophenol (2, 4-DNP) 2, 4, - DNP accepts proton and carries it into matrix through IMM (membrane permeable)
2. Physiological uncoupler – thermogenin (protein)

H^+ -channel in the IMM

Abundant in mitochondria of brown adipose tissue (low level of ATP synthase activity)

Responsible for diet induced thermo-genesis

Brown adipose tissue is absent or reduced in obese individuals

Present in newborns and cold adapted individuals

Thermogenin is opened by fatty acids liberated upon degradation of stored fat by activated hormone sensitive lipase by norepinephrine released in response to drop in body temperature due to cold environment.

Opening of thermogenin allows reentry of translated protons through IMM (no proton gradient formed

No ATP synthesis

Energy of oxidation lost as heat

biological importance - maintain body temperature in newborns

- cold adaptation

f- Respiratory Poisons

Inhibit electron flow through respiratory chain

Inhibit proton translocation

Inhibit proton gradient formation

Inhibit O_2 consumption

Inhibit ATP synthesis

Reducing equivalents remain reduced

Other aerobic oxidation's inhibited

(Kreb's cycle, pyruvate oxidation, fatty acid oxidation)

Inhibitor	*Complex inhibited*
Rotenone	I
Amytal	II
Antimycin A	III
Cyanide (CN^-)	IV
Carbon monoxide (CO) , (Azide (N_3	

UNIT FOUR

LIPIDS

Lipids comprise very heterogeneous group of compounds which are insoluble in water but soluble in non-polar organic solvents such us benzene, chloroform, and ether. They are present in all living organisms. The group includes fats, oils, waxes and related compounds.

General Functions of Lipids

i. They are efficient energy sources.
ii. Serve as thermal insulators.
iii. They are structural components of the cell membrane.
iv. Serve as precursors for hormones (steroid hormones).
v. They also dissolve the vitamins, which are fat-soluble and assist their digestion.

Classification: - There are two ways of classification i.e.,

ü Classification as storage and structural lipids and some other functional lipids.
ü Classification based on lipid composition.

I. **Simple lipids**:- esters of fatty acids with different alcohols.

Fats and oils:- These are esters of fatty acids with glycerol.

Waxes:- Esters of fatty acids with high molecular weight monohydric alcohols

II. **Complex lipids**:- Esters of fatty acids and alcohols together with some other head groups.

A. **Phospholipids**:- Esters of the above type containing phosphoric acid residue.
   a) Glycerophospholipids:- The alcohol is glycerol
   b) Sphingophospholipids:- The alcohol is shingosine.

B. **Glycolipids**:- Lipids containing fatty acid, sphingosine and carbohydrate residues.

C. **Others**:- Include sulfolipids, amino lipids and lipoproteins, which are modified forms of lipids.

III. **Derived lipids:** include the hydrolytic products of the simple and complex lipids. Eg. Fatty acids, cholesterol etc.

The simplest naturally occurring lipids are triacylglycerols formed by esterification of fatty acids with glycerol. Biological membranes are made up of phospholipids, glycolipids and proteoglycans.

FATTY ACIDS

Fatty acids are building block of most lipids, made of long chain organic acids having one polar carboxyl group (head) and a non-polar hydrocarbon chain (tail). The latter makes them water insoluble. They are not found free in nature but found as esterified forms. Most naturally occurring fatty acids have got even number of carbons. They may be saturated or unsaturated, with one or more double bonds. Mostly the double bond occurs at the 9^{th} carbon as we count from the carboxyl group end.

There are two systems of numbering the carbon atoms in a fatty acid

n 3 2 1

$CH_3(CH_2)_nCH = CHCH_2CH_2CH_2COOH$

ω

1. Numbering starts from carboxyl carbon. The last carbon is the "n" carbon
2. The second carbon is the " "and the third the " " Carbon. The last carbon atom is omega.

Eg:- $CH_3(CH_2)_7CH_2CH_2(CH_2)_7COOH$ stearic acid (saturated fatty acid)

Eg:- $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ oleic acid (Unsaturated fatty acid)

Fatty acids can be represented as shown below where the delta indicates the position of the double bond and the next number shows the number of carbon atoms and the last number indicates the number of double bonds. In a different way the position of the double bond(s) can be indicated as shown in the second expression without the delta.

C18:1, Δ^9 or 18:1(9)

C18 indicates 18 carbons, 1 indicates the number of double bonds, delta 9(Δ^9) indicates the position of double bond between 9^{th} and 10^{th} carbon atoms.

- Double bonds in naturally occuring fatty acids are in the cis- configuration and saturated fatty acids of C_{12} to C_{24} are solids at body temperatu3-..t8 6ut the delta. ty

PUFA (Polyunsaturated fatty acids): They have two or more double bonds .they are called as essential fatty acids because they are required in the body and cannot be synthesized. So they need to be include in the diet.

18	Linoleic acid	18: 2; 9 (12)
18	Linolenic acid	18: 2; 9 (12, 15)

These two are called essential fatty acids.

20	Arachidonic acid	20: 4; (5, 8, 11, 14)

Arachidonic acid is semi essential fatty acid because it can be synthesized from the above two essential fatty acids.

Functions:

1. The fluidity of membrane depends on length and degree of unsaturated fatty acids.
   Membrane PL contains essential fatty acids. In case of deficiency of EFA, other fatty acids replace them in the membrane; as a result membrane gets modified structurally and functionally.
2. They are required for the synthesis of PL, cholesterol ester and lipoproteins
3. Poly unsaturated fatty acids are released from membranes, diverted for the synthesis of prostaglandins, leukotriens and thromboxanes.
4. They act as fat mobilizing agents in liver and protect liver from accumulating fats (fatty liver).

TRIACYLGLYCEROLS

These are esters of fatty acids with the alcohol glycerol, which are storage forms of lipids (depot lipids). Triacylglycerols or also called as triacylglycerides, exist as simple or mixed types depending on the type of fatty acids that form esters with the glycerol. Both saturated and/or unsaturated fatty acids can form the ester linkage with the backbone alcohol. Eg. Tripalmitate, Triolein.

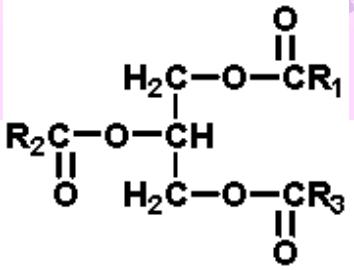


Fig 4.1. Structure of Triacylglycerol.R_1, R2 and R_3 are fatty acids.

Tristearin is a chief component of beaf lipid

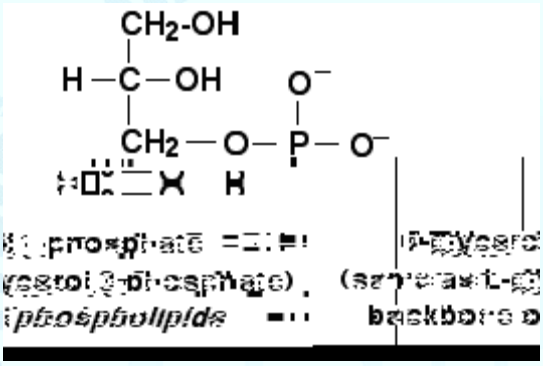
Butter has short chain fatty acids.

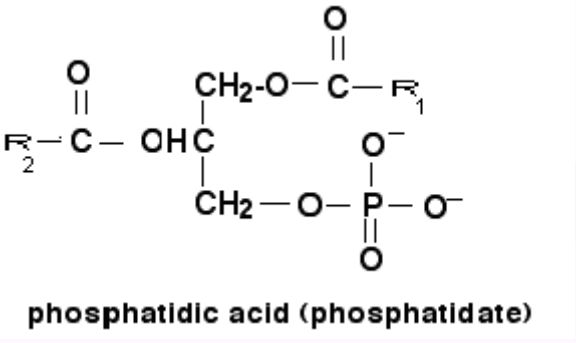
Unsaturated fatty acids are sensitive to air and oxidized to give rancid smell.

Triacylglycerols are mainly found in special cells called adipocytes (fat cells), of the mamary gland, abdomen and under skin of animals.

They produce twice as much energy as that of carbohydrates per gram

STRUCTURE OF LIPIDS





phosphatidic acid (phosphatidate)

R_1 and R_2 are fatty acids

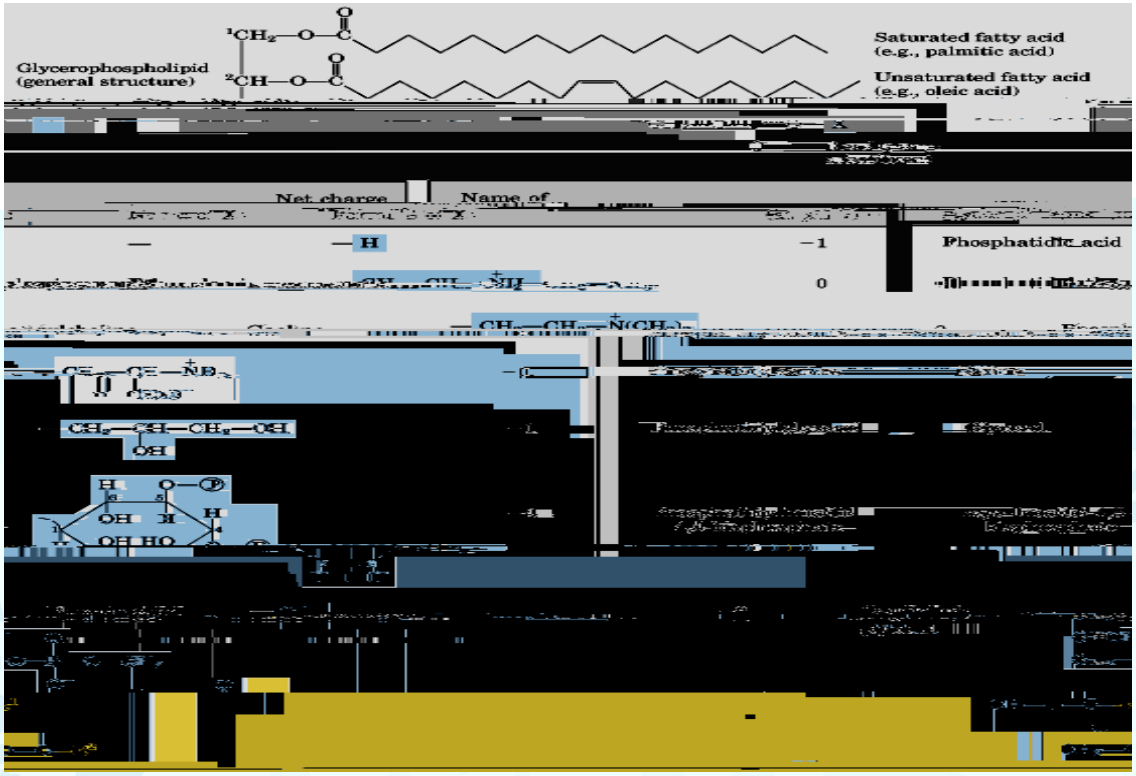


Fig 4.2. Structure of phosphatidate

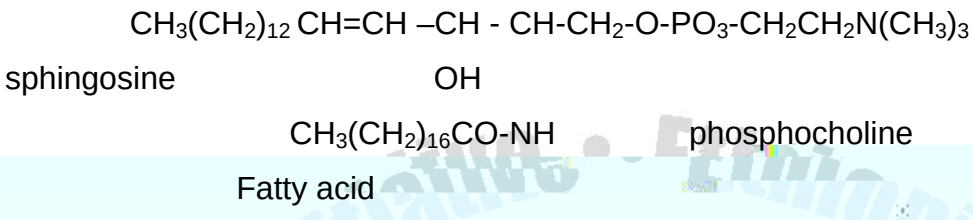
Phosphatidate is the parent compound for the formation of the different glycerophospholipids. To the phosphate group different head alcohol may be attached. If choline is attached it is called phosphatidyl choline (lecithin), if ethanolamine is attached it is called phosphatidyl ethanolamine.

The second largest membrane lipids are sphingolipids, which contain two non-polar and one polar head groups. Their alcohol is the amino alcohol sphingosine.

Sphingolipids have subclasses viz., sphingomyelins, cerebrosides and gangliosides. Out of these only sphingomyelin contains phosphorus.

$CH_3(CH_2)_{12}CH{=}CH{-}CH(OH){-}CH(NH_2){-}CH_2OH$ Sphingosine

Sphingomyelins contain as head group phosphocholine or hosphoethanolamine. Below is an example of a sphingomyelin.



Gangliosides: These are glycolipids most of which are complex containing oligomers of sugars inorf10.98 0

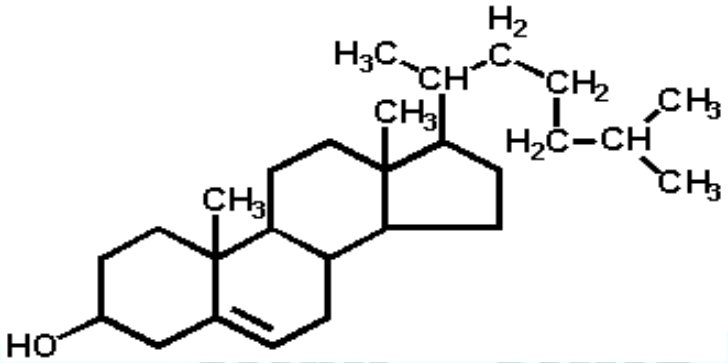


Fig 4.3. Structure of Cholesterol.

Cholesterol is important in many ways:

For the synthesis of bile salts that are important in lipid digestion and absorption.

For the synthesis of steroid hormones that are biologically important like the sex hormones estrogen and progesterone.

For the synthesis of vitamin D_3

As a structural material in biological membranes.

As a component of lipoproteins as transport forms of lipid based energy.

Digestion and Absorption of Lipids

Diet contains triglycerides, cholesterol and its ester, phospholipids, fattyacids etc.Mouth and gastric juice has got lipase. It can hydrolyse fats without emulsification with bile salts.Milk fat and butter fat is digested by the enzyme.

Major part of fats are digested by pancreatic lipase. It acts on emulsified lipids only. The products are monoglyceride and 2 fatty acids. Monoglyceride is further hydrolyzed by another lipase. Thus 3 fatty acids and one glycerol molecule is produced from the digestion of dietary triglyceride.

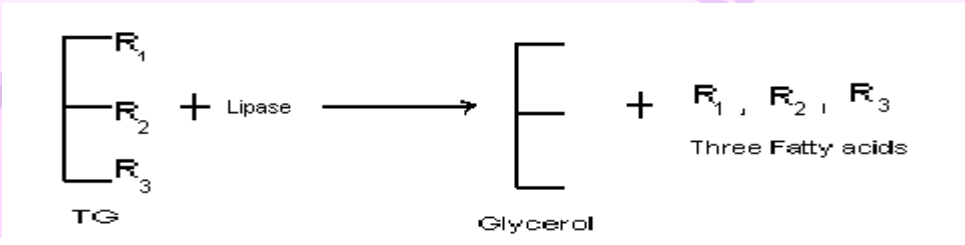


Fig 4.4. Action of lipase on TAG

The bile salts are reabsorbed and reach by enterohepatic circulation to the liver to be used over again. Their absorption is maximum in the ileum and jejunum. The free fatty acids and monoayclglycerols are absorbed through the epithelial cells lining the small intestine and pass to the lymphatic system where they join the systemic blood via the thoracic duct. The intestinal mucosa secretes into the lymph, the absorbed lipids as chylomicrons and VIDL. The former have short life in blood (<lhr) and make plasma milky after rich mea1. The free fatty acids in blood (long chain) are bound to albumin and transported by blood to the liver.

Metabolism of Fatty Acids and Triacylglycerols

The triacylglycerols play an important role in furnishing energy in animals. They have the highest energy content over 9kcal/mole. They provide more than half the energy need of some organs like the brain, liver, heart and resting skeletal muscle.

Mobilization of Fatty Acids from Adipocytes

When the energy supply from diet is limited, the body responds to this deficiency through hormonal signals transmitted to the adipose tissue by release of glucagon, epinephrine, or adrenocorticotropic hormone.

The hormones bind to the plasma membranes of adipocyte cells and stimulate synthesis of cyclicAMP (cAMP). The cAMP activates a protein kinase that phosphorylates and in turn activates hormone-sensitive triacylglycerol lipases (see the mechanism action of Hormones). These lipases hydrolyze the triacylglycerols at position 1 or 3 to produce diacylglycerols (DAG) and fatty acid, which is the rate limiting step in the hydrolysis. The diacylglycerol lipases hydrolyze the DAG to monoacylglycerols (MAG) and a fatty acid. Finally MAG lipases hydrolyze MAG to fatty acid and glycerol.

The free fatty acids (FFA) produced by lipolysis move through the plasma membranes of the adipose cells and endothelial cells of blood capillaries by simple diffusion and bind to albumin in the blood plasma, which are transported to peripheral tissues. The glycerol produced is taken up by liver, phosphorylated and oxidized to dihydroxyacetone phosphate, which is isomerised to glyceraldehydes-3-phosphate, an intermediate of both glycolysis and gluconeogenesis. Therefore, the glycerol is either converted to glucose (gluconeogenesis) or to pyruvate (glycolysis).

D. Transport of Fatty Acids to the Mitochondria

The fatty acids transported to the different tissue cells must first be activated or primed by reaction with CoenzymeA at the expense of ATP. The reaction is catalyzed by AcylCoA synthetase or also called thiokinase, found in the cytosol and mitochondria of cells. The pyrophosphate generated from ATP favors more Acyl CoA formation by further hydrolysis. In order to undergo -oxidation, the fatty acids must enter the mitochondria. But they cannot easily cross it as such by passive diffusion.

There are two fatty acid sources viz., those coming from absorption of FFA and those from hydrolysis of triacylglycerols from adipose tissue. The transport of acyl derivatives across the mitochondrial membrane needs three acyltransferases (shuttles).

1. Specific for short chain acyl groups, does not require carnitine
2. Specific for the long chain acyl groups. The shuttles for long chain acyl groups are carnitine acyltransferase I and II. Therefore, long chain acyl groups cross the mitochondrial membrane in combination with carnitine.

Acyl-CoA + Carnitine $\xrightarrow[\text{Enzyme}]{}$ Acyl carnitine + CoASH

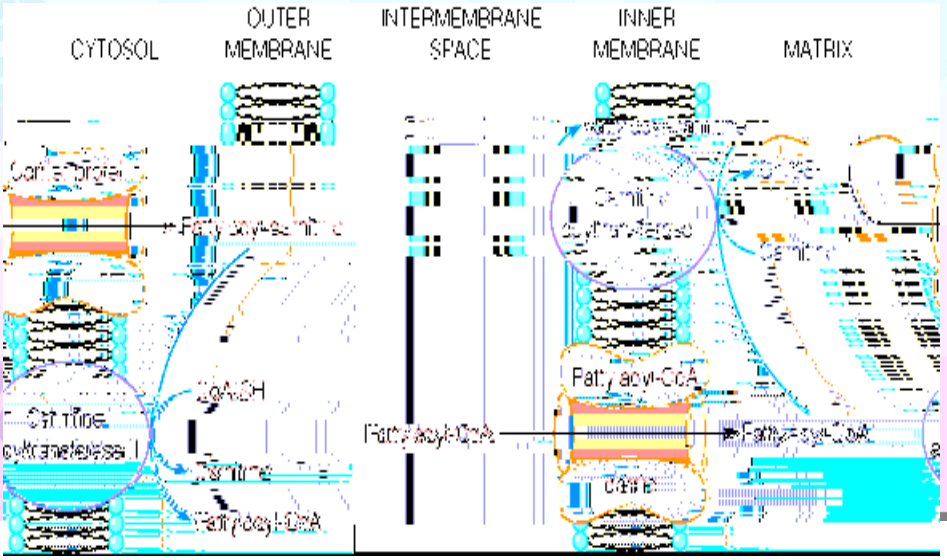


Fig 4.6. Carnitine transport system.

The carnitine pools are in the cytosol and mitochondria, abundant in muscle and it is synthesized from the amino acids lysine and methionine in the liver and kidney. The other name of carnitine is -hydroxy- - trimethyl ammonium butyrate. Carnitine acyl transferase I, found in the surface of the outer mitochondrial membrane, catalyzes the acyl transferase reaction from acylCoA to the carnitine. It passes through the outer membrane to the inner membrane of mitochondrion. In the final stage of the transport, the fatty acyl group is released from the carnitine to the

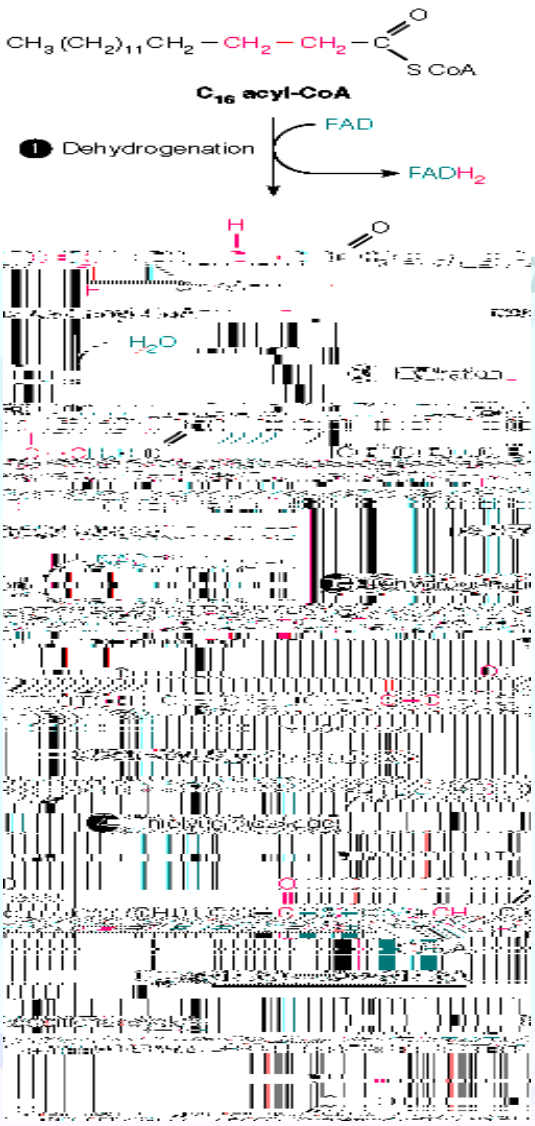


Fig 4.7: β-oxidation of fatty acid

The $FADH_2$ and NADH $+H^+$ join the electron transport chain as high energy electron carriers. The latter donates its reducing equivalents (hydrogens) to NADH dehydrogenase to produce 3ATP per pair of electrons and the former produces only 2ATPS.

Complete oxidation of fatty acid can be divided in to two stages.

A. Formation of acetyl CoA.

B. Oxidation of acetyl CoA to CO2, water via TCA cycle.

Stochiometry of the reaction:

Palmitoyl CoA + 7FAD + 7 NAD +7CoA = 8 Acetyl CoA+7$FADH_2$ +7 NADHH.

Energetics of palmitate oxidation:

Reduced equivalents enter ETC and produce energy rich phosphate bonds. Acetyl CoA release energy through TCA cycle.

7 $FADH_2$ → 7 x 2 = 14 ATPs

7NADHH → 7 x 3 = 21 ATPs

8 Acetyl CoA → 8 x 12 = 96 ATPs

Total ATP produced from one molecule of palmitic acid is 131. Two ATPs (Two energy rich bonds) are utilized, during activation of fatty acid. Therefore total gain of ATPs is 129.

Oxidation of Unsaturated Fatty Acids

The oxidation of unsaturated fatty acids requires two additional enzymes called isomerase and reductase. Most naturally occurring unsaturated fatty acids are in cis- configuration, which are not suitable for the action of enoyl-CoA hydratases and hence they must be changed to their trans isomer by an isomerase. The rest of the enzymes are needed for the oxidation in addition to these two for the oxidation are the same.

Oxidation of Fatty Acids with Odd Number of Carbons

Ruminant animals can oxidize them by B- oxidation producing acetylCoAs until a three carbon propionylCoA residue is left.The acetylCoAs produced are funneled to the Krebs cycle but the propionylCoA produced is converted to succinylCoA by three enzymatic steps. SuccinoyCoA is an intermediate in the Kreb's cycle and it can be metabolized.

The fates of acetyl-CoA formed by b-oxidation of fatty acids are:

1. Oxidation to CO

The HMGCoA formed in the hepatocytes mitochondria by the action of the enzyme HMGCoA lyase is changed to acetoacetate.

The acetoacetate, when its concentration is very high in blood is spontaneously decarboxylated to acetone.

Acteoacetate can be converted to β-hydroxy butyrate by a dehydrogenase enzyme. It is a reversible reaction. See the figure

The odor of acetone may be detected in the breath of a person who has a high level of acetoacetate, like diabetic patients. During starvation and severe diabetes mellitus peripheral tissues fully depend on ketone bodies. Even tissues like the heart and brain depend mainly on ketone bodies during such conditions to meet their energy demand.

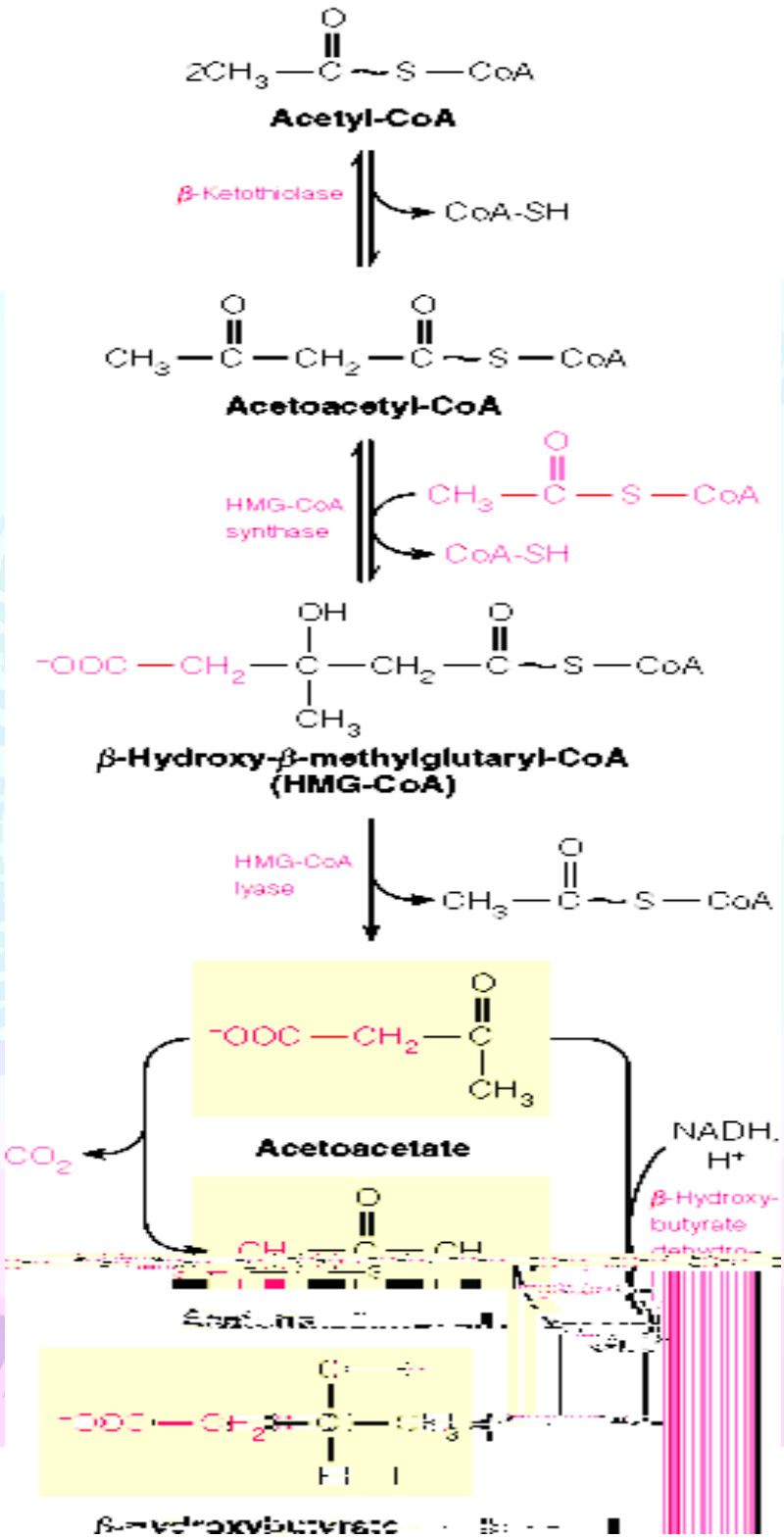


Fig 4.8. Synthesis of ketone bodies.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In Diabetes, there is lack of insulin, which brings about lipolysis and decreased utilization of glucose.

Lipoysis increases free fatty acids in blood, which are oxidized to meet energy requirements. This causes increased production of acetyl CoA, NADH, ATP which in turn inhibits TCA cycle.

Acetyl CoA requires oxalo acetate to enter TCA cycle .Since oxaloacetate is not forming from glucose, acetyl CoA can't enter the cycle. It is diverted to ketone bodies synthesis.

Similarly in starvation, due to hypoglycemia, there is less insulin, lipolysis increases and ketogenesis increases .Oxaloacetate is also diverted to gluconeogenesis, which further depletes TCA cycle .So acetyl CoA can only be converted to ketone bodies.

The Biosynthesis of Fatty Acids

Apart from diet fatty acids can be synthesized in the body.

Denovo synthesis of fatty acids take place in cytosol of liver, lactating mammary gland, adipose tissue and renal cortex.

Main site for TG, fatty acid synthesis is adipose tissue.

* Acetyl CoA is converted to Malonyl CoA by acetyl CoA carboxylase.
* Malonyl CoA and acetyl CoA are attached to acyl carrier protein (ACP).
* Malonyl ACP acetyl ACP get condensed to ketoacyl ACP, by condensing enzyme.
* Ketoacyl ACP gets reduced to hydroxyl acyl ACPby a reductase. It requires NADPHH.
* It loses one molecule of water, forms 2-enoyl acyl ACP. Enzyme is dehydratase.
* It undergoes reduction and forms Butyryl ACP.NADPHH and reductase are needed for the reaction.

The formation of malonyl CoA is the committed step in fatty acid synthesis

For the synthesis, all the enzymes are required in the form of fatty acid Synthase complex.

1. Ketoacyl adipose zn.etohe form of reduct 5at8lS


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HMG CoA is reduced to Mevalonate by a reductase.

Mevalonate undergoes three times Phosphorylation, in the presence of 3 ATPs and various kinases.The product is 3- phosphor-5 pyrophospho mevalonate.

Dephosphorylation, decarboxylation converts it to Isopentenyl pyrophosphate.

It is isomerised to dimethyl allyl pyrophosphate by isomeras.

Isopentenyl pyrophosphate and dimethyl allyl pyrophosphate form Geranyl PP(10C).

Geranyl PP and one more molecule of Isopentenyl PP→ Farnesyl PP(15C).

Two of Farnesyl PP join to form Squalene (30C).

1. Squalene undergoes cyclization, loses three carbon atoms,aquire a double bond,forms cholesterol

Regulation of Cholesterol Synthesis:

Functions of Bile Salts

They lower surface tension ,emulsify fats ,a pre requisite for the action of pancreatic lipase

They activate Lipase.

They shift the pH from 9 to 6

They form micelles with fatty acid,a mono,di,triglyceride and help in absorption

Promote absorption of fat soluble vitamins

Bile salts keep cholesterol in soluble form in gall bladder.

They regulate the breakdown of cholesterol

Cholelitiasis (Gall stones):

Absence of bile salts precipitate cholesterol as gall stones.Solublity of cholesterol depends on the ratio of phospholipids, bile salts to cholesterol.Due to infections bile acids are destroyed which leads to decreases solubility of cholesterol.

Decrease of bile salts can be due to:

A. Failure in enterohepatic circulation
B. Cirrhosis of Liver
C. Disease of ileum.

The patients are treated with chenodeoxycholic acid to solublize the cholesterol or the stones are removed by surgical intervention.

Hypercholesterolemia :

Normal cholesterol level is 150-250mg% in blood.

High concentration leads to hyper cholesterolemia.

Excess cholesterol gets deposited under the skin, tendons as Xanthomas.

In some cases the regulatory enzyme HMG-CoA reductase is not sensitive to feed back regulation. Such people suffer from familial hypercholesterolemia.

Lipid Storage Diseases

Lipid storage diseases are also called as sphingolipidosis. They are degraded by hydrolytic enzymes found in lysosomes .When the degradation is impaired; sphingolipids accumulate in the tissues.

1. **Nieman-pick disease**

Sphingomyelin accumulates in brain, liver and spleen .The condition is due to deficiency of sphingomyelinase .Patient suffers from mental retardation and early death.

2. **Gaucher's disease**.

Glucocerebroside accumulates in liver, spleen, brain and bone marrow, due to the deficiency of glucocerebrosidase .Patient suffers from mental retardation.

3. **Tay-Sach's disease**.

Hexoseaminidase is absent as a result gangliosides accumulate in brain, spleen and retina. Patient suffers from demyelination. Cerebral degeneration, mental retardation and early death.

Fatty Liver:

Excess accumulation triglycerides in liver causes fatty liver,Liver cirossis and failure of liver function.

Causes are:

- Elivated levels of free fatty acid in blood
- Deficiency of lipotropic factors,which help in the mobilization of fat from liver
- Failure in the secretion of lipoproteins from liver

Chemical Composition of Membranes

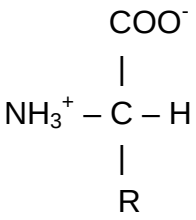
Phospholipids are the major class of membrane lipids. Cholesterol, glycoproteins and glycolipids are also the other components of membranes. Glycerophospholipids like lecithin, cephalin and phosphatidyl serine. Membranes are mainly formed of phospholipid bilayers. Sphingolipids also form membrane structures, especially that of the brain cells and nerve cells.

Structure of Membranes

All membranes have a bimolecular leaf of lipid bilayers. Proteins are found submerged in the sea of the lipid bilayers (intrinsic proteins) or loosely bound (extrinsic proteins) and cholesterol is also found intercalated between the lipid bilayers giving the fluidy nature of membranes. The integral proteins contain sugar oligomers and most of them function as receptors. Some of the characteristic features of membranes are listed below.

Membranes can be regarded as a sea of lipid bilayers and due to the presence of unsaturated fatty acids and cholesterol. This fluidity enables lateral diffusion of molecules such that integral and non-integral proteins span the whole membrane structure. This implies that membranes are not rigid structures but dynamic structures. The modern representation of lipids as fluid and non-rigid ... flip-flo... structurally and functionally. The outer and inner surfaces have different components and different enzymatic activities.

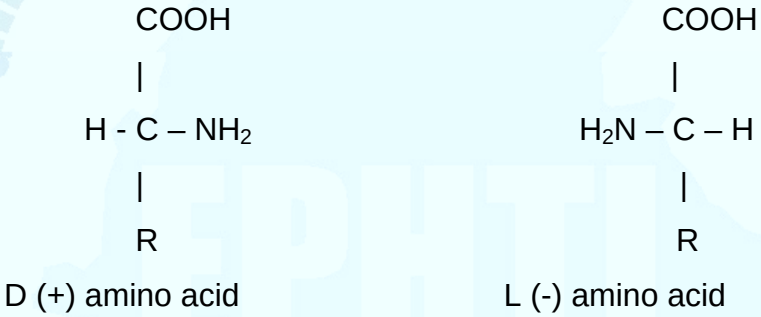
UNIT FIVE



In dipolar (zwitterion) form the amino group is protonated ($-NH_3^+$) and the carboxyl group is dissociated (deprotonated) ($-COO^-$) leading to a net charge zero.

Stereochemistry (Optical activity)

Stereochemistry mainly emphasizes the configuration of amino acids at the carbon atom, having either D or L- isomers.



D (+) amino acid L (-) amino acid

Fig 5.2: D and L- forms of Amino acids

Out of the 20 amino acids, proline is not an amino acid rather an - imino acid. Except for glycine, all amino acids contain at least one asymmetric carbon atom (the - carbon atom).

Classification of Amino Acids

L-Amino acids are the building blocks of proteins. They are frequently grouped according to the chemical nature of their side chains. Common groupings of amino acids are **aliphatic**, **hydroxyl/sulfur**, **cyclic**, **aromatic**, **basic**, **acidic and acid amides**. Links to individual **amino acids** are given below:

I. Structural Classification

This classification is based on the side chain radicals (R-groups) as shown in the table 5.1.Each amino acid is designated by three letter abbreviation eg. Aspartate as Asp and by one letter symbol D.

Table 5.1 : Structural classification of Amino acids

II. Electrochemical classification

Amino acids could also be classified based on their acid – base properties

Acid amino acids (Negatively charged at pH = 6.0)

Example:

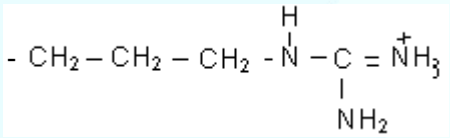
- aspartic acid - $CH_2 – COO^-$
- glutamic acid - $CH_2 – CH_2 – COO^-$

Basic amino acids (positively charged at P^H = 6.0)

Example:

- Lysine - $CH_2 – CH_2 - CH_2 - CH_2 – NH_3^+$

- Arginine



Neutral amino acid

Example:

- Serine - CH_2- OH
- Threonine - CH_2- OH
  |
  CH_3

- Asparagine - CH_2- CO-NH_2
- Glutamine - CH_2- CH_2 - CO-NH_2

E. III. Biological or Physiological Classification

This classification is based on the functional property of amino acids for the organism.

1. Essential Amino Acids

Amino acids which are not synthesized in the body and must be provided in the diet to meet an animal's metabolic needs are called essential amino acids. About ten of the amino acids are grouped under this category indicating that mammals require about half of the amino acids in their diet for growth and maintenance of normal nitrogen balance.

2. Non- Essential Amino Acids

These amino acids are need not be provided through diet, because they can be biosynthesized in adequate amounts within the organism.

Essential and Non essential amino acids are as shown in the Table 5.2:

Table 5.2: Essential and Non-Essential Amino Acids

Essential Amino Acids in Mammals	Non-Essential Amino Acids in Mammals
Arginine*, Histidine*, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine	Alanine, Asparagine, Aspartic Acid, Cysteine, Glutamic Acid, Glutamine, Glycine, Proline, Serine, Tyrosine

3. Semi-essential amino acids

Two amino acids are grouped under semi-essential amino acids since they can be synthesized within the organism but their synthesis is not in sufficient amounts. In that they should also be provided in the diet.

The set of essential amino acids required for each species of an organism can be an indicative of the organism propensity to minimal energetic losses on the synthesis of amino acids. Semi essential amino acids include Arginine and Histidine.

IV. Classification Based on the Fate of Each Amino acid in Mammals.

Fig 5.3. Ketogenic, Glucogenic and Glucogenic-Ketogenic amino acids

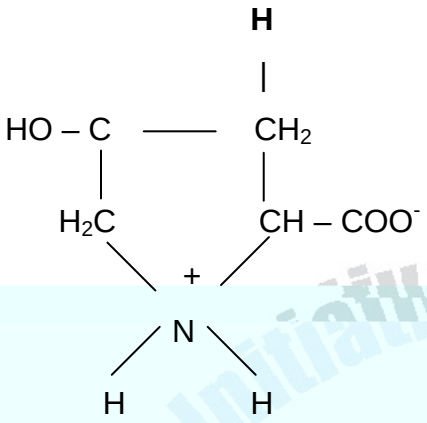
Ketogenic and Glucogenic amino acids are as indicated in the chart except Leucine and Lysine which are exclusively ketogenic.

V. Classification Based on Participation in Protein Synthesis.

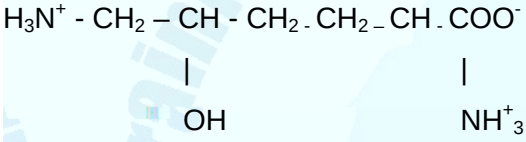
I. Non-Standard Amino Acids

In addition to the 20 standard amino acids, proteins may contain non- standard (proteogenic) amino acids, which are normally components of proteins but created by modification of the standard amino acids.

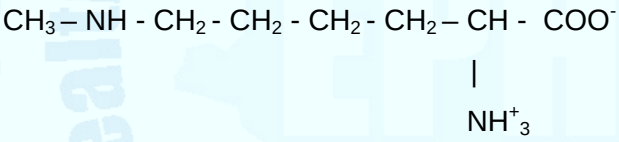
Among the non – standard amino acids 4 – hydroxyproline a derivative of proline, 5-hydroxylysine derivative of lysine where both are found in collagen, a fibrous protein of



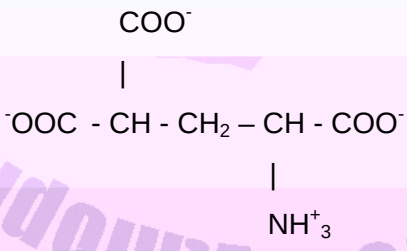
4- hydroxyproline



5 – hydroxylysine



6 – N methyl Lysine



– Carboxyglutamate

Fig:5. 4 **Non standard amino acids**

II. Non – Proteogenic Amino Acids

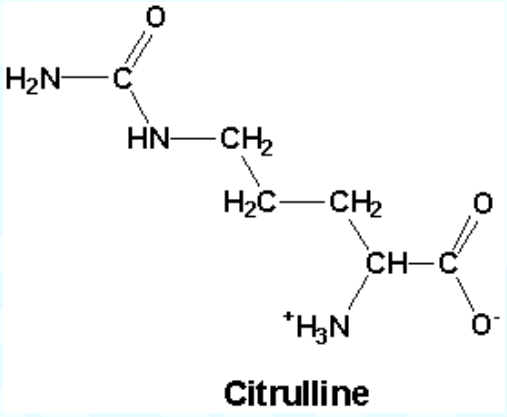
These amino acids occur in free or combined state, unlike in proteins,and play important roles in metabolism in plasma, free amino acids are usually found in the order of 10 to 100 µ mol/L, including many that are not found in proteins.

Citrulline ,for example, is an important metabolite of L. arginine and a product of Nitric - Oxide synthase, an enzyme that produces nitric oxide an important signaling molecule.

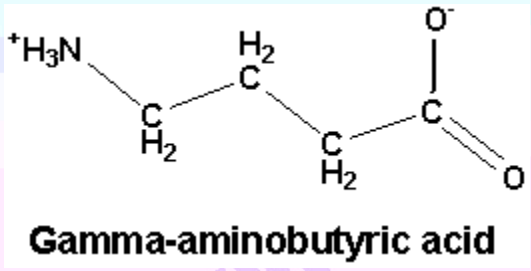
Antibiotics - gramicidin and antimycin D

-aminobutryric acid - which acts as an inhibitory neurotransmitter

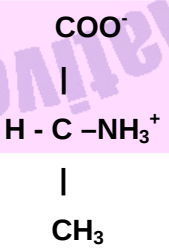
D - Alanine - a component of vitamin, panthothenic acid, are some of the non-proteogenic amino acids.



Citrulline



Gamma-aminobutyric acid



D-Alanine

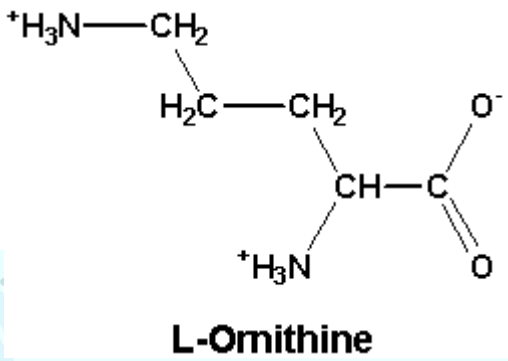


Fig 5.5: Non-Proteogenic Amino acids

Ionization States of Amino Acids

Amino acids are amphoteric molecules, that is, they have both basic and acidic groups. Monoamine and monocarboxylic acids are ionized in different ways in solution, depending on the pH of solution.

At pH 7, the "zwitterions" $H^+_3N - CH_2 - COO^-$ is the predominant species of Glycine in solution and the overall molecule is electrically neutral. At acidic pH the amino group ($-NH_2$) group is fully protonated and positively charged, yielding $H^+_3N - CH_2 - COOH$, while at alkaline pH glycine exists primarily as the anionic $H_2N - CH_2 - COO-$ species, (Negatively charged species).

At the pH intermediate between pka (a measure of the tendency of group to give up a proton, with that tendency, decreasing 10 fold as the pka increase by one unit) of the amino and carboxyl groups, known as isolectric point (PI), the zwitter ionic form of the amino acid has no net charge.

PI can be calculated for each amino acid with mono amine and mono basic groups as follows:

$$PI: (\tfrac{1}{2} PK_1 + PK_2)$$

Example : Glycine (G) PKa_1 - carboxyl = 2.4

PKa_2 - amino = 9.8

$$\text{So, PI} = \frac{Pka_1 + Pka_2}{2}$$

$$\frac{2.34+9.8}{2} = 5.97$$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At any pH below its PI, glycine has a net positive charge and moves toward negative electrode (cathode, when placed in an electric field.)

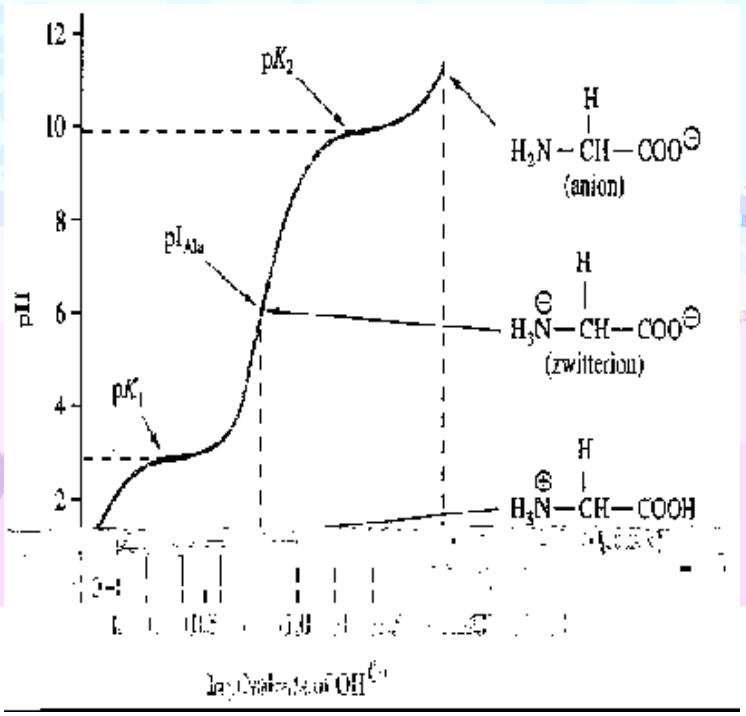
So, all amino acids with a single - amino group and a single - carboxyl group and an R - group that does not ionize, have titration curves resembling that of glycine.

i.e. PK_1 = (the PK of COOH group)

Usually in the range of 1.8 – 2.4

PK_2 (of the PK of $-NH_3$ group)

Usually in the range of 8.8 – 11.0



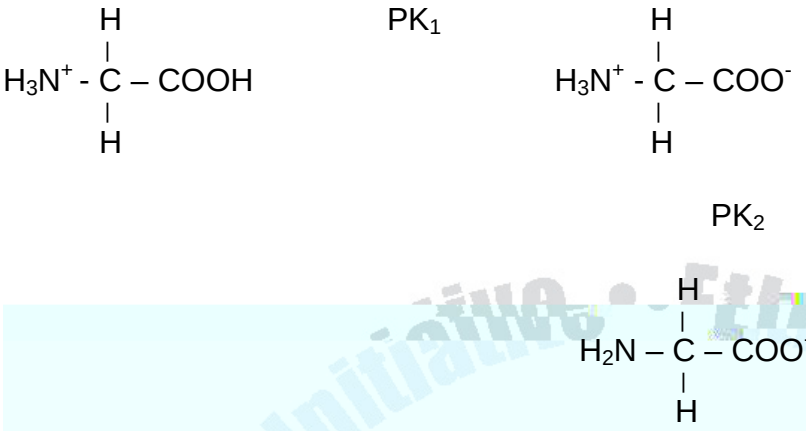
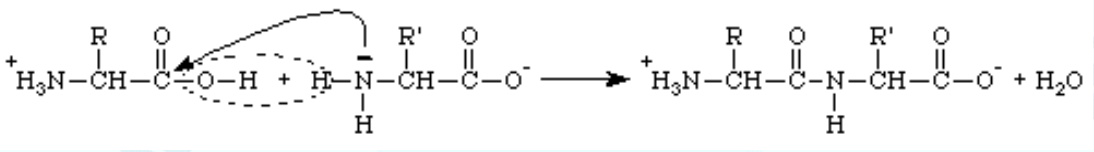


Table 5.3.

Peptides



1. The imino group (-NH-) of the peptide linkage has no significant tendency to ionize or protonate in the pH range 0 – 14
2. The C - N of a peptide linkage is relatively rigid and cannot rotate freely, a property of supreme importance with respect to the three dimensional conformation of polypeptide chains.

In amide linkage of the peptide bond due to the substantial double bond character there exists little twisting. As a result the group of atoms in the peptide bond exist in the cis or trans nature of the peptide bond. It was found out that the trans configuration is usually favored in order to minimize the steric interaction between bulky R groups on adjacent α-carbon atoms. One exception is bonds in the sequence X – Pro, which X is any amino acid followed by Proline. In this case Cis configuration may be favored.

In fact, the **peptide bond** can be considered a resonance hybrid of the forms

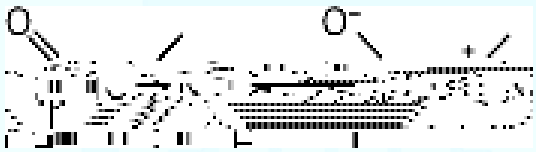


Fig 5.9: A peptide bond

The group of atoms about the peptide bond can exist in either the ***trans*** or ***cis*** configurations:

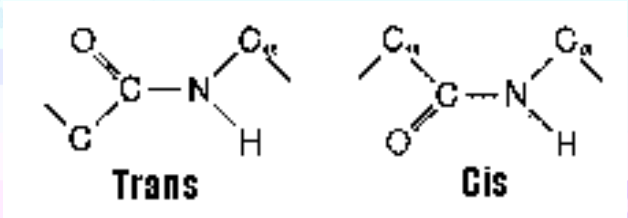


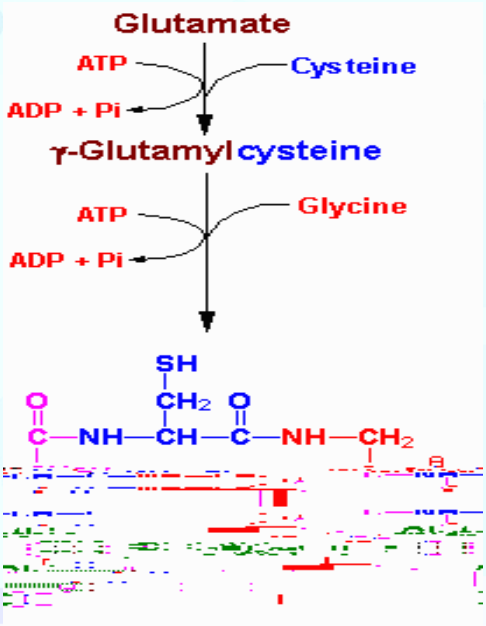
Fig 5.10: Tran and cis conformation of peptide bonds

Peptides of Physiological Significance

Glutathione

Glutathione is a tripeptide formed from amino acids glutamate, cysteine and Glycine, linked together in that order. The glutamate is linked to cysteine through the - carboxyl group and - amino group of cysteine.

Here, the carboxyl group is first activated by

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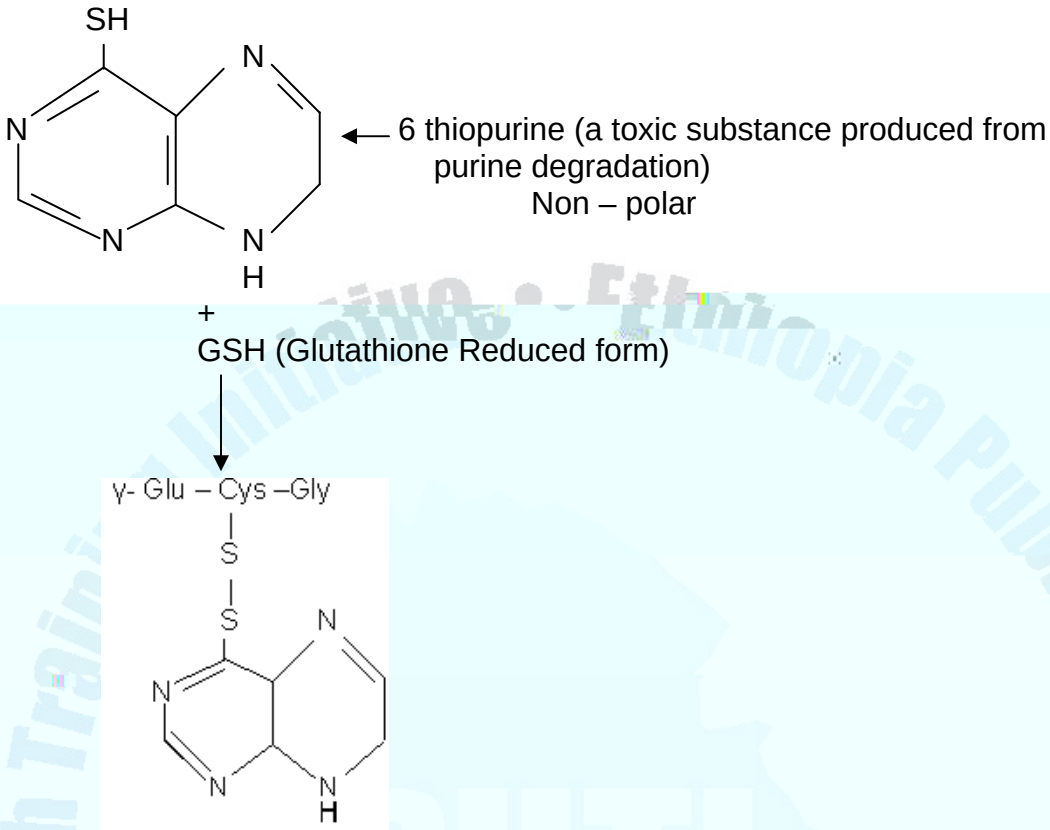


6 thiopurine (more polar and can be excreted out of the body easily)

Fig 5.13: **Transformation of 6-thio purine in to a non toxic form**

b) Scavenging of peroxides by glutathione peroxidase.

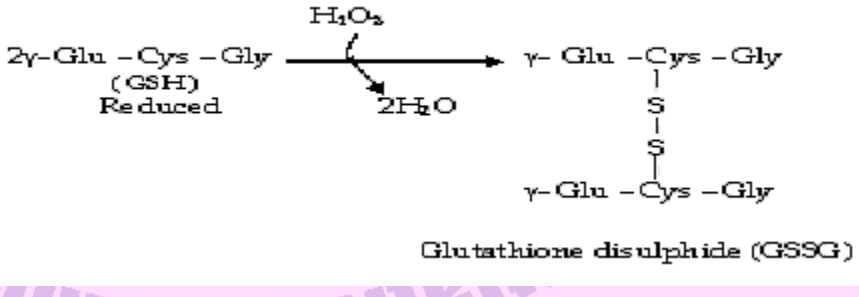


Fig 5.14: Role of Glutathione in scavenging Peroxides or

(2 GSH + H_2O_2 ↔ GSSG + $2H_2O$).

*** Other peptides having physiological role include**:

- Enkephalin (penta peptide)
- Bradykinin (nano peptide)
- Antidiuretic hormone (ADH) (nano peptide), etc.

PROTEINS

The word protein is derived from Greek word, proteious meaning primary. So, proteins are the major components of any living organism.

Proteins are natural substances with high molecular weights ranging from 5,000 to many

B. Conjugated Proteins

Yields amino acids and other organic and inorganic components

E.g. Nucleoprotein (a protein containing Nuclei acids)

Lipoprotein (a protein containing lipids)

Phosphoprotein (a protein containing phosphorous)

Metalloprotein (a protein containing metal ions of Fe^{2+})

Glycoprotein (a protein containing carbohydrates)

II. Solubility

a) Albumins: These proteins such as egg albumin and serum albumin are readily soluble in water and coagulated by heat.

b) Globulins: these proteins are present in serum, muscle and other tissues and are soluble in dilute salt solution but sparingly in water.

c) Histones:

Histones are present in glandular tissues (thymus, pancreas etc.) soluble in water; they combine with nucleic acids in cells and on hydrolysis yield basic amino acids

III. Overall Shape

A. Fibrous proteins

In these protein, the molecule are constituted by several coiled cross-linked polypeptide chains, they are insoluble in water and highly resistant to enzyme digestion. The ratio of length to breath (axial ratio) is more than 10 in such protein. A

IV. On their Biological Functions:

Proteins are sometimes described as the "workhorses" of the cell because they do So many things Like:

Enzymes:	kinases, transaminases etc.
Storage proteins	myoglobin, ferretin
Regulatory proteins	peptide hormones, DNA binding proteins
Structural protein	collagen, proteoglycan
Protective proteins	blood clotting factors, Immunoglobins,
Transport protein	Hemoglobin, plasma lipoproteins
Contractile or motile Proteins	Actin, tubulin

V. On their level of organization

Primary, secondary, tertiary and quaternary.

a) Primary Structure of Proteins

The primary structure cannot represent the 3D-nature of a protein molecule since the extended chain of amino acids is co-planar as the covalent bind of peptide is right.

H O H O H O H

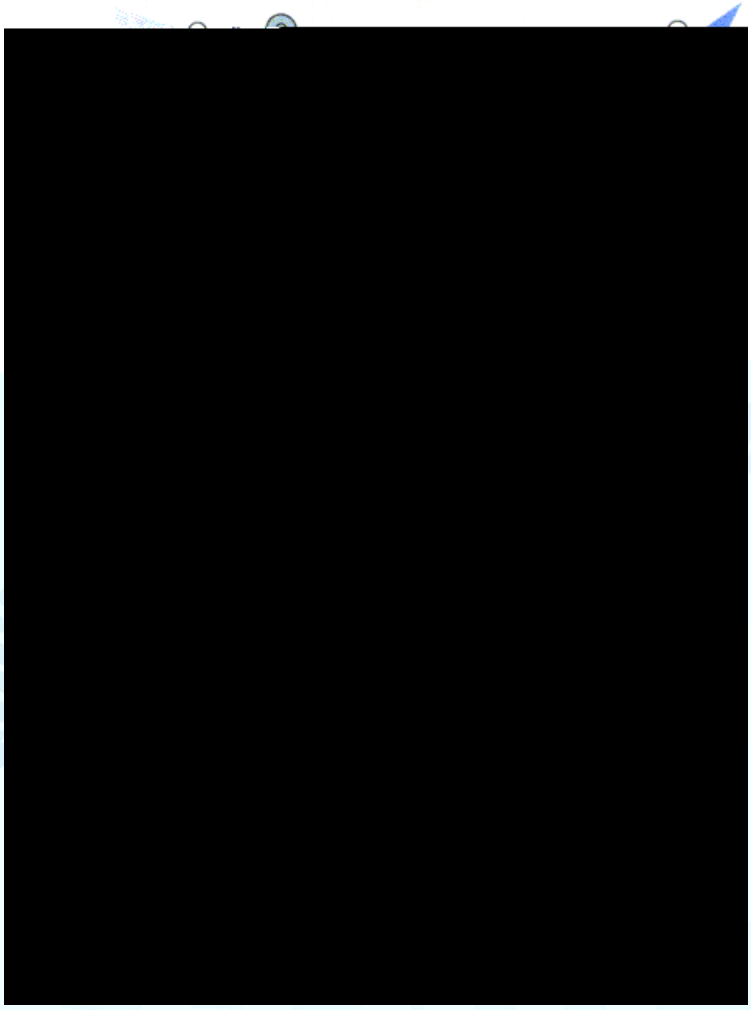


Fig 5.16. **The**

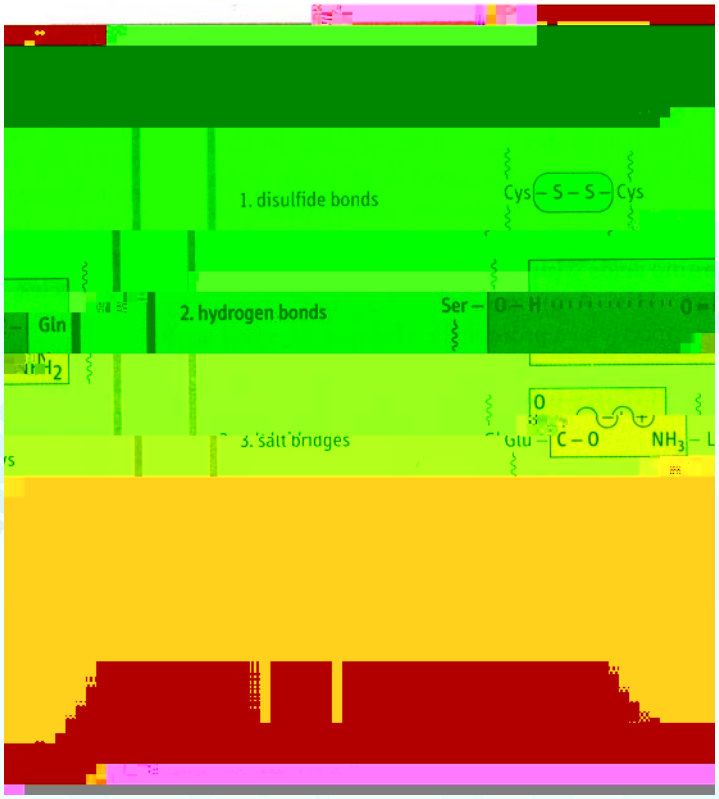


Fig.5.17. **Elements that stabilize the tertiary structure of a protein**

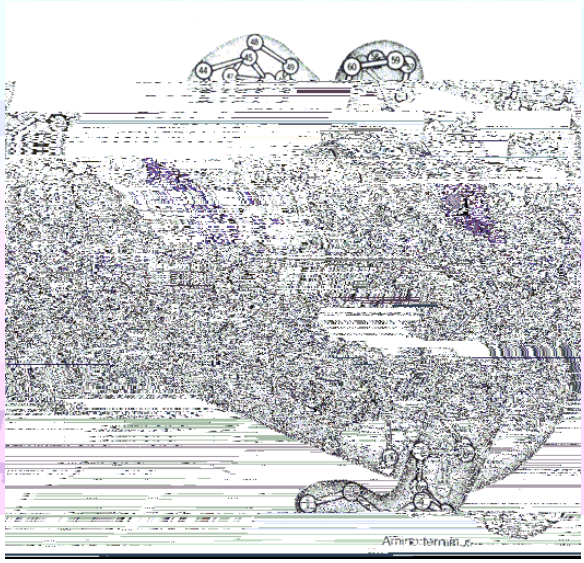
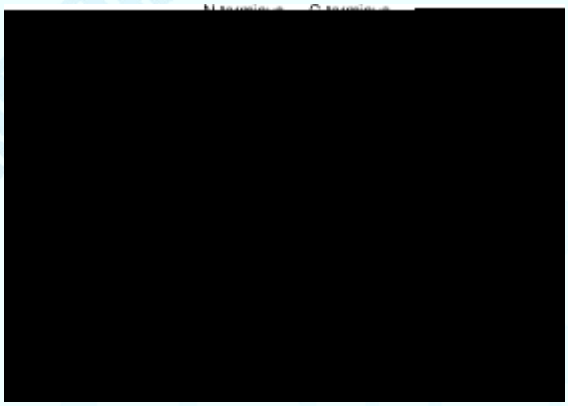


Fig 5.18.**The three dimensional structure of Myoglobin**

d) Quaternary Structure

Quaternary structure refers to a complex or an assembly of two or more separate peptide chains that are held together by non- covalent or, in some case, covalent interactions.

If the subunits are identical, it is a homogeneous quaternary structure; but if there are

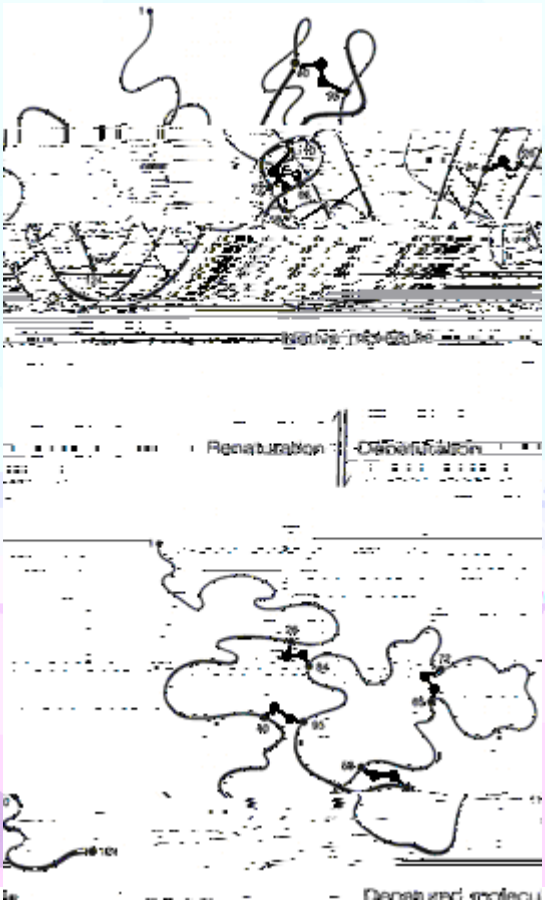


Denaturation of Proteins

Proteins have finite lifetimes. They are also subject to environmental damages like oxidation proteolysis, denaturation and other irreversible modifications.

Denaturation involves the destruction of the higher level structural organization (2^0, 3^0 and 4^0) of protein with the retention of the primary structure by denaturing agents.

A denatured protein loses its native physico-chemical and biological properties since the bonds that stabilize the protein are broken down. Thus the polypeptide chain unfolds itself and remain in solution in the unfolded state. The denatured protein may retain its biological activity by



Factors that Affect Denaturation

Denaturing agents

1. physical factors

   Temperature, pressure, mechanical shear force, ultrasonic vibration and ionizing radiation causes the protein to lose its biological activity.

2. chemical factors

   Acids and alkalis, organic solvents (actone, ethanol), detergents (cleaning agents), certain amides urea, guandidine hydrochloride, alkaloids, and heavy metal salts (Hg, Cu, Ba, Zn, Cd…) Cause the denaturation.

Properties of a Denatured Protein

A. an increase in number of reactive and functional group in the composition of the native protein molecule (side chain group of amino acids, COOH, NH_2, SH, OH … etc)
B. Reduced solubility and pronounced propensity for precipitation

   this occurs due to loss of the hydration shell and the unfolding of protein molecules with concomitant exposure of hydrophobic radicals and neutralization of charged polar groups.

C. Configurational alteration of the protein molecule.
D. Loss of biological activity evoked by the disarrangement of the native structural molecular organization.
E. Access of proteolytic enzymes in comparasion with the native protein

Clinical Application of Denaturation

The amounts of proteins found in the urine, serum, CSF are utilized to asses various pathological conditions. The appearance of proteins like Albumin and Globulin in the urine can be detected by precipitating them using ammonium sulphate. This could be used to asses the degree of kidney impairment and glomerular permeability.

In some disease, abnormal proteins may be present in plasma and be filtered at the glomerule. The most important member is Bence-jons' protein which is most often associated with multiple myeloma. So recognition of such protein in the urine may be useful in the diagnosis of the disease.

This could be done by treating few ml of urine with few ml of hydrochloric acid giving a white ring at the junction of the two fluids.

In the case of CSF protein estimation and analysis, a saturated phenol solution is used where 2 drops of CSF with 2ml of 10gm phenol dissolved in distilled water to check for turbidity. If the

If the iron atom were to become oxidized to Fe^{3+}(Ferric), the Globin would get changed to metmyoglobin or (Met hemoglobin) where heme can no longer interact with O_2 and O_2 transport is compromised.

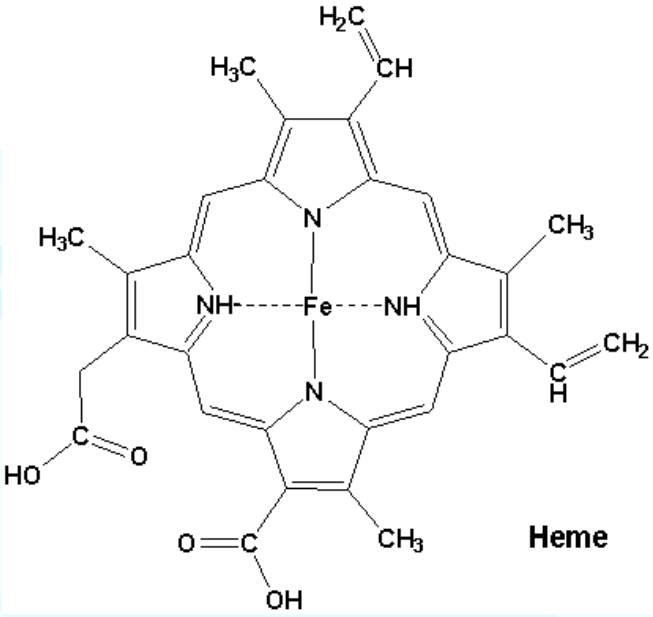


Fig 5.21: Structure of the heme prosthetic group (protophorphyrin IX) ring system.

Heme is non-covalently bonded in a hydrophobic crevice in the myoglobin and hemoglobin molecules.

Ferrous iron is octahedrally coordinated having six ligands or binding groups, attached to it, the nitrogen atoms account for only four ligands. The two remaining coordination sites which lie along the ring contain on the plane of the ring contains one histidine with imidazole nitrogen that is close enough to bond directly to the Fe^{2+} called proximal histidine the other histidine which facilitates the alignment of heme to O_2 and that of Fe^{2+} called distal Histidine.Distal histidine confers important geometrical constraints on the six coordination sites which normally restricts the interaction with CO.

The coordinate nitrogen atoms mainly prevents conversion of the heme iron to the ferric state (Fe^{3+}) due to their electron donating character.

In free heme molecules, reaction of oxygen at one of the two “open” coordination bonds of iron which is perpendicular to the plane of the porphryin molecule above and below can result in irreversible conversion of Fe^{2+} to Fe^{3+}. In heme containing proteins this reaction is prevented by

sequestering the heme deep within a protein structure where access to the two open coordination bonds is restricted polar amino acids are located almost exclusively on the exterior surface of globin polypeptide and contribute to the high solubility of these proteins. Amino acids which are both polar and hydrophobic, such as Threonine, tyrosine and Tryptophan are oriented to the exterior.

Hydrophobic amino acid residues are buried with in the interior where they stabilize the folding of the polypeptide and binding of iron porphyrin ring.

The only exceptions to this general distribution of amino acids residues in globins are the two Histidines that play an indispensable role in the heme binding are oriented perpendicular to and on either side of the planor heme prosthetic group.

In the quaternary stucture of human Hb there exists two - globin and two – β- globin sub units ($_2$ β_2). These subunits are arranged in a tetrahedral array. Experimental analysis of the quaternary structure indicates multiple non-convalent interactions between each pair of dissimilar subunits, that is, at the - β - interfaces. In contrast there are few interactions between identical subunits at the - or β – β interface so hemoglobin is considered more as a heterodmer (β)[W

Adult Hb (HbA*)*

Contains two types of globin two - chains (141 residues each) and two β - chains (146 residue each). The amino acid sequences of the two type of subunits are identical at 27 positions.

Fetal Hb (HbF)

Contains a different type of Hb just after conception fetuses synthesize zeta chain (quite like - chain)

The HbF variant barely detectable and ε- chains just like β - chain later zeta replaced by - and ε- by . HbF contain 2 and 2 subunits in most adult often increases up to 15 - 20% in individuals with mutant adult Hbs, such as sickle cell disease. This is an example of the body's compensatory response to a pathologic abnormality .The direct benefit of this structural change in Hb isoform is a more efficient transfer of O_2 from maternal HbA to fetal(HbF) .

Sickle Cell Hemoglobin (HbS)

HbS, the variant most commonly associated with sickle cell disease, cannot tolerate high protein concentration when deoxygenated. At low oxygen concentrations, deoxy HbS polymerizes, forms fibers, and distorts erythrocytes in to sickle shapes.

The mutation is Glu^6 β -val a surface localized charged amino acids is replaced by a hydrophobic residue as show below

HbA = Val – His – Leu – Thr – Pro – Glu – Glu – Lys

HbS = Val – His – Leu – Thr – Pro – Val – Glu – Lys

Such substitution of Valine (non - polar) for Glutamate (polar) have the following consequence

1. Place A non - polar residue on the outside of HbS which markedly reduce solubility of deoxy HbS. But has little effect on oxy - HbS (causes Hb to clump when deoxygenated)
2. Creates sticky patches on the outside surface of each β - chains (not present HbA)
3. The sticky patches interact with complementary sites of another HbS (oxy) and forms

This observation represents an example of a selective advantage that HbA/ HbS heterozygote exhibits over the HbA/HbA normal or the HbS/HbS homozygte.

Sickled erythrocyte exhibits little or less deformity, they no longer move freely through the micorvasculature and often block blood flow. Moreover this cells lose water, become fragile and have a considerably short life span leading to anemia.

Sickle Cell Disease

Sickle cell disease is caused by an inherited structural abnormality in the β –globin polypeptide. Clinically, an individual with sickle cell disease present with intermittent episode of haemolytic and painful vaso–occlusive crisis. The latter leading to severe pain in bone chest and abdomen. There is also a likely to be impaired growth, increased susceptibility to infections and multiple organ damage.

Digestion and Absorption of Proteins

Proteins are larger polypeptide molecules coiled by weaker bonds in their tertiary structure the digestion of proteins involves the gradual breakdown of this polypeptide by enzymatic hydrolysis in to amino acid molecules which are absorbed in the blood stream. The protein load received by the gut is derived from two sources 70-100g dietary protein which is required daily and 35 - 200g endogenous protein (secreted enzymes and proteins in the gut or from intestinal epithelia cell turnover)

Only 1-2g of nitrogen equivalent to 6-12g of proteins are lost in the feces on a daily basis. Thus the digestion and absorption of protein is more efficient.
The process of protein digestion can be divided, depending on the sources of peptidases.

A. Gastric Digestion

Entry of a protein in to stomach stimulates the gastric mucosa to secrete a hormone gastrin which in turn stimulates the secretion of Hcl by the parietal cells of the gastric glands and pepsinogen by the chief cells.

The HCL thus produced lower the pH of stomach to (pH1.5 – 2.5) and acts as an antiseptic and kills most of the bacteria and other foreign cells ingested along with.

The acid denatures the protein and the whole protein susceptible to hydrolysis by the action other proteolytic enzymes.

Proteases are endopeptidases which attack the internal bonds and liberate large fragments of peptides.

Then pepsinogen having MW 40,000 an inactive precursor or zymogen is converted in to active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These are passed in to the interior of the epithelial cell where other specific peptidases convert almost all of them to a single amino acids that are transported to the blood stream by the opposite side of the cell membrane and carried to liver (primarily) and other tissues for oxidative degradation. This process complete the absorption of 99% of digested proteins. The whole scheme is as shown in the figure below.

Fig 5.22: Digestion and absorption of proteins

II. Transport of Amino Acids in to Intestinal Epithelial cells.

2. Neutral amino acid symporter for aromatic or hydrophobic side chains. Phe, Tyr,
3. lmino acid symporter Pro,and OH – Pro
4. Basic amino acid symporter Lys, Arg and Cys.
5. Acidic amino acid symporter. Asp, Glu
6. β amino acid symporter β-Ala , Tau.

These transporter systems are also present in the renal tubules and defects in their constituent protein structure can lead to disease called Hartnup disease.

Neutral amino Aciduria (Hartnup Disease)

Transport functions, like enzymatic functions, are subject to modification by mutations. An example of a genetic lesion in epithelial amino acid transport is hartnup disease; entry resulting from the defect was first recognized. The disease is characterized by the inability of renal and intestinal epithelial cells to absorb neutral amino acids from the lumen. In the kidney, in which plasma amino acids reach the lumen of the proximal tubule through the Ultra filtrate, the inability to reabsorb amino acids manifests itself as excretion of amino acids in the Urine (aminoaciduria). The intestinal defect results in malabsorption of free amino acids from the diet.

Therefore the clinical symptoms of patients with this are mainly those due to essential amino acid and Nicotinamide deficiencies. The pellagra-like features are explained by a deficiency of Tryptophan, which serves as precursor for nicotinamide. Investigations of patients with Hartnup disease revealed the existence of intestinal transport systems for di - or tripeptides, which are different from the ones for free amino acids. The genetic lesion does not affect transport of

called as aminotransferases. Most of the amino acids undergo these reaction except lysine and threonine

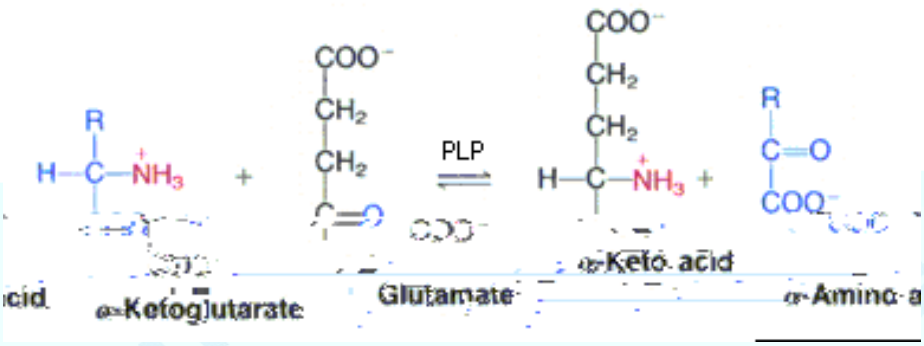


Fig 5.23: Transamination of amino acids

Transminase of Clinical Importance

Transaminase is a name for a category of enzymes involved in exchange of an oxygen from an -keto acid (such as -ketoglutarate) and an amine from an amino acid

The two most important transminase reactions of high clinical important are Alanine transminase and Aspartate transaminase catalyzed reactions.

Alanine + **-Ketoglutarate** <-> **Pyruvate** + **Glutamate**

Oxaloacetate + **Glutamate** <-> **Aspartate** +-ketoglutarate (**Urea cycle**)

In addition to their roles as building blocks of proteins, the carbon skeletons may be used to produce energy in oxidative metabolism by the end stages of glycolysis (such as pyruvate from Alanine) and tricarboxylic acid (such as oxaloacetate from Asparate) thereby providing a metabolic fuel for tissues that requre or prefer glucose. In addition, the carbon skeletons of certain amino acids can produce the equivalent of acetyl-CoA or Acetoacetate termed Ketogenic, indicating that they can be metabolized to give immediate precursor of lipids or ketone bodies.

Alanine transaminase (ALT) also called as glutamate pyruvate transaminase (GPT) and

825 Tw[(bfJ-S8g7.0TT1cating th)5.4(at e AlaT* -ea2(u20r)13]TJ24.0328 0 TD7 ta)2g t4.0328 -1- Tw.0328 a

Deamination of serine and threonine is slightly different because of the *beta*-hydroxyl groups. Dehydratases are involved:

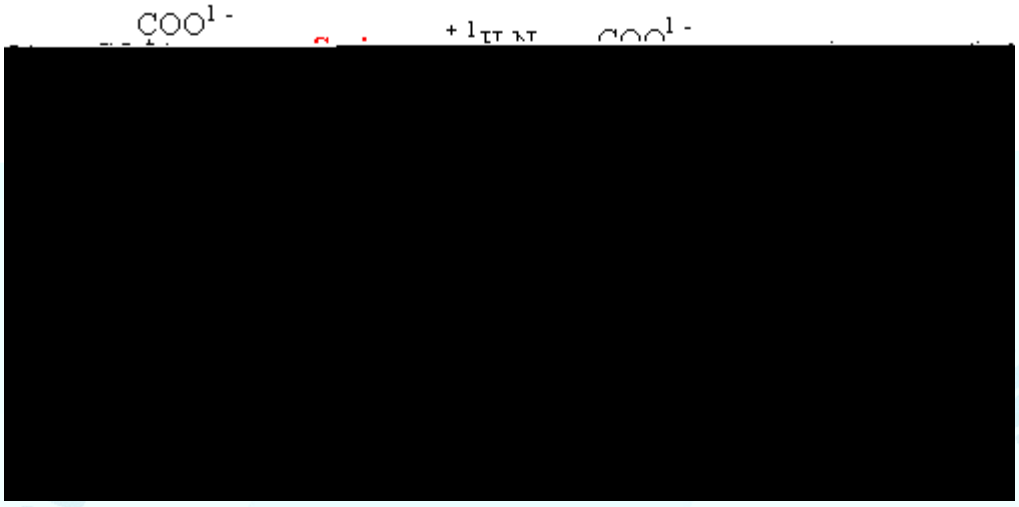


Fig 5.25: **Dehydration of amino acids**

Nitrogen Balance:

A healthy adult eating a varied and plentiful diet is generally in “Normal Nitrogen Balance” a state where the amount of nitrogen ingested each day is balanced by the amount excreted resulting no net change in the amount of the body Nitrogn. In a well fed condition, exreted nitrogen comes from digestion of excess protein or from normal turnover.

Protein turnover (Synthesis and degradation) is involved.

not essential in adults but essential in children because they are synthesized from Methionine and ornithine. These amino acids are readily available in adults but limited in children.

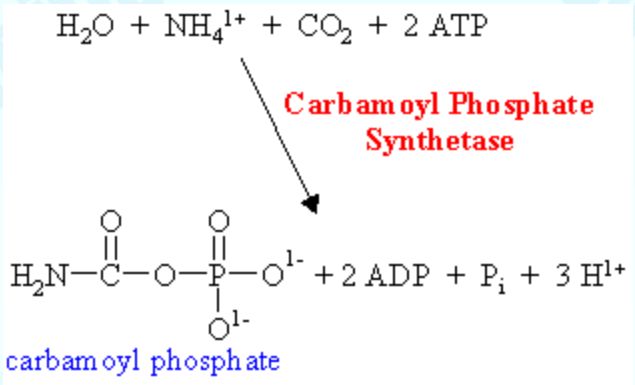
Negative Nitrogen balance occurs in injury when there is net destruction of tissue and in major trauma or illness.

Nitrogen Excretion and the Urea Cycle:

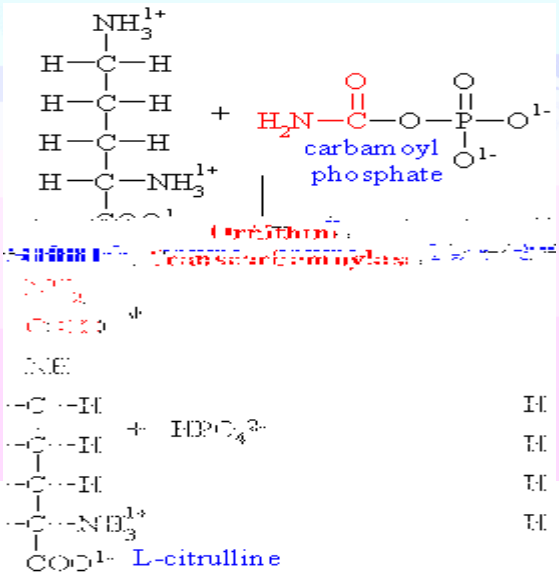
Excess amino Nitrogen from amino acids is removed as ammonia, which is toxic to the human body. Some ammonia is excreted in urine, but nearly 90% of it is utilized by the liver to form urea, which is highly soluble and is passed in to circulation for being excreted by the kidneys.

The reactions are as follows:

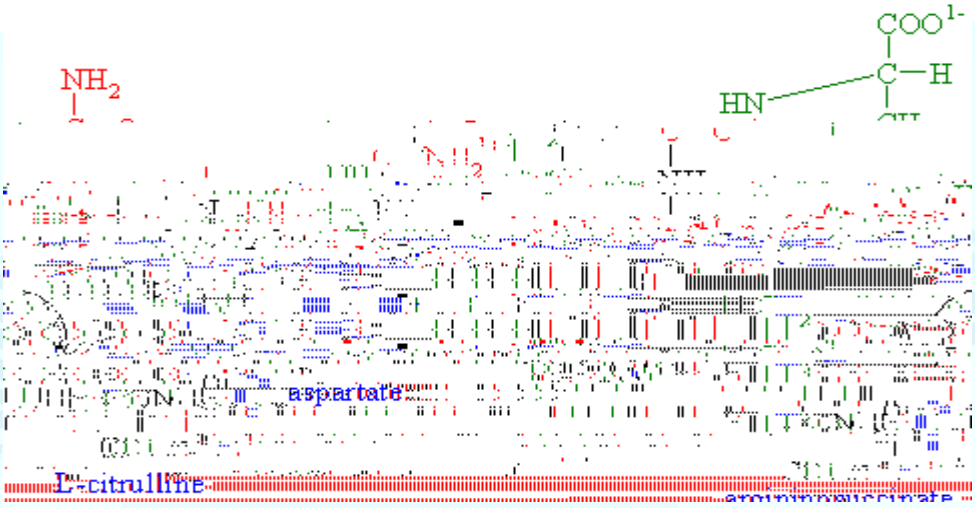
Step 1. CO_2 from bicarbonate and NH_4 from the two sources mentioned above combine together in the liver mitochondria to form carbamoyl phosphate in presence of ATP and Mg^{2+} by the enzyme Carbamoyl phosphate synthetase I (CPSI).



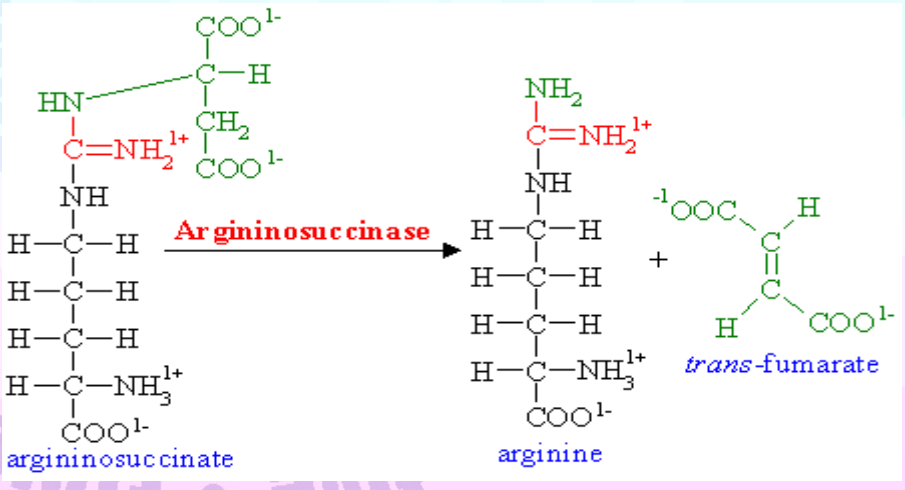
Step 2. Carbamoyl phosphate reacts with ornithine transferring the carbamoyl moiety to produce citrulline: by the enzyme i.e. ornithine transcarbomylase.



Step 3. Argininosuccinic acid is formed by the reaction of Aspartic acid and citrulline: the NH_2 group of the former is linked to – CO group of the latter. The enzyme reqused is argininosuccinic acid synthase.



Step 4. Argininosuccinic acid is cleaved to form Arginine and fumerate by the enzyme Arginiosuccinate lyase. Fumerate goes to the pool of TCA-cycle.

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1) The major toxic effects of ammonia in brain probably involve changes in cellular P^H and depletion of certain TCA cycle intermediates.
2) More and more ammonia might deplete

Tyrosinemia

It is also called (richner-Hanhrt syndrome) caused due to the failure of tyrosine transaminase giving a raised level of tyrosine in blood and urine clinical symptoms include moderate mental retardation, characteristic eye and skin lesions and disturbance in fine coordination. Other metabolites excreted in urine are called tyramine, N-a cetyl tyrosine, P-OH- phenyl acetate PO^4 - phenyl pyruvate.

Alkaptonuria (Black urine disease)

A second inherited defect in the phenyl a larine – tyrosine pathway involves a deficiency in the enzyme that catalyses the oxidation of homogentisic acid (an intermediate in the metabolic breakdown of tyrosine and phenyalanin). This condition occurs 1 in 1,000,000 live birth homogentisic acid accumulates and gets excreted in urine where the urine turns black on standing. There is a form of arthritis in late cases and generalized pigmentation of connective tissues; this is believed to be due to the oxidation of homogentisic acid by polyphenol oxidase

Amino acid derived Nitrogenious compounds.

Creatine and creatine phosphate:

Synthesis of creatine and creatine phosphate creatine is produced by the liver, kidney and pancreas and is transported to its site of usage principally muscle and brain. Creatine is derived from glycine and Arginine by the enzyme Amidinotransferase where ornithine and Guandioacetate are generated.

Further Guanidoacetate gets transmethylated by S- adenosine Methionine removing Adenosine and generating Homocystine and creatine. By creatine kinase, creatine undergoes phosphorylation to form creatine phosphate.

- Creatine phosphate is an important energy reservoir in skeletal muscles. Where at the site of muscle contraction creatine phosphates prevents the rapid depletion of ATP by providing a readily available high energy phosphate which can be used to re generate ATP from ADP.
- High levels of ADP formed in the myofibrils during contraction favors the reverse reaction namely formation of ATP at the expense of creatine phosphate cleavage to creatine.
- Creatine phosphate is formed from ATP and creatine at times when the muscle is relaxed and demands for ATP is not so great.
- Creatine is an end product of nitrogen metabolism, and as such, undergose no further metabolism, but excreated through the urine.

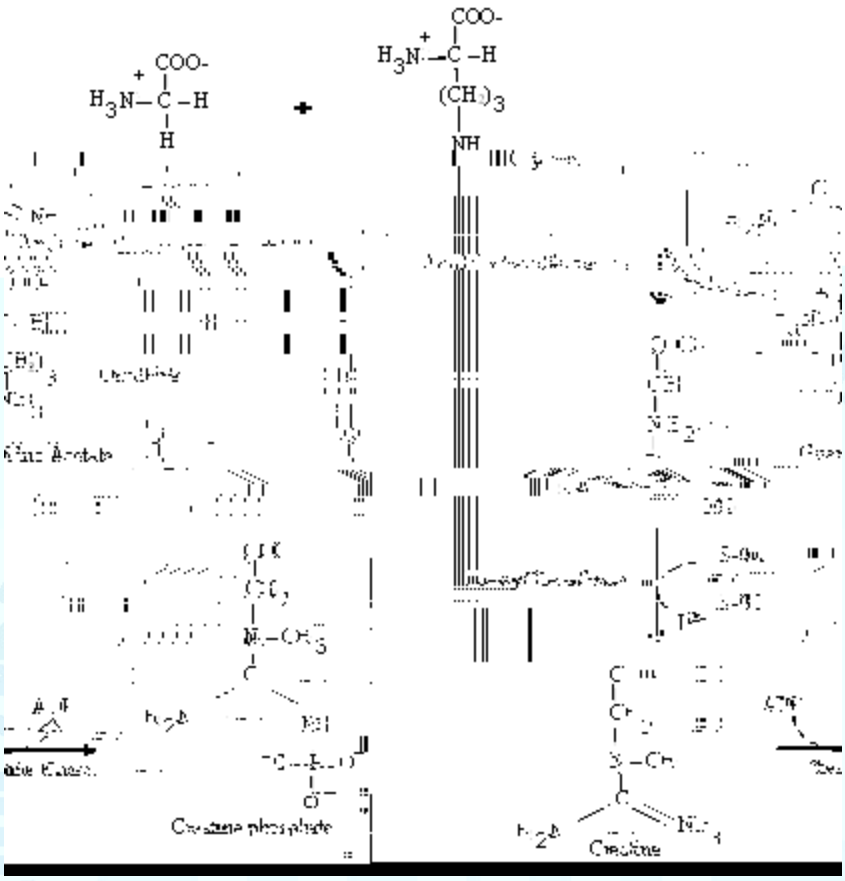


Fig 5.29: Synthesis of Creatine and creatine phosphate

Serotonin

Serotonin is synthesized from Tryptophan. It is a Neurotransmitter that helps the body control satiety, the feeling of fullness after eating. It plays multiple roles in the nervous system, including neurotransmission and a precursor of melatonin, which is involved in regulation of sleepiness and wakefulness, vegetative behaviors like feeding, mood, sexual arousal etc.

In the intestine, serotonin regulates intestinal peristalsis. It is also a potent vasoconstrictor, which helps regulate blood pressure.

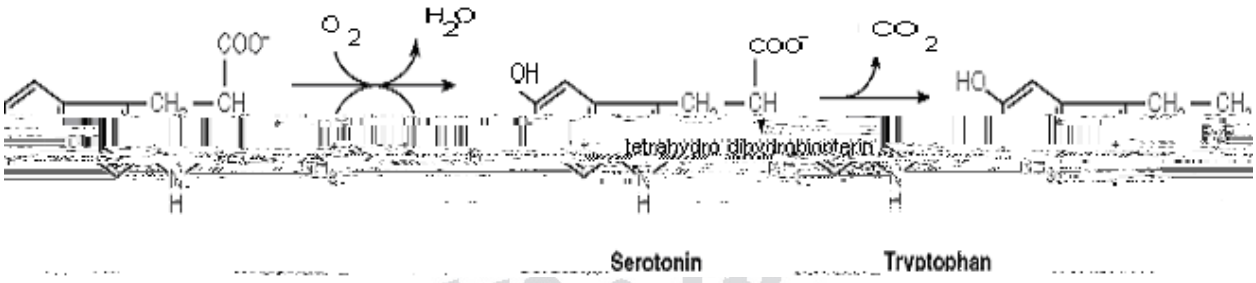


Fig 5.30: Biosynthesis of Serotonin

Catecholamines:

The term catecholamine comes from the aromatic dialchol, catechole. Tyrosine gives rise to a family of catecholamines that include Dopamine, Norepinephrine and epinephrine. The levels of these catecholamines are related with changes in the blood pressure of animals.

Dopamine

The importance of Dopamine in neural transmission is emphasized by the number of major neurological disease that is associated with improper Dopamine regulation.

Dopamine levels are abnormally low in a particular region of the brain of patients with Parkinson's disease

Parkinson's disease commonly occurs in elderly, it can occurs in younger individuals. It is a progressive disease caused by the death of dopamine-producing cells in the substantia nigra and locus ceruleus. This disease is associated with tremor of arm, occasional muscle cramping. The drug, which usually alleviates the disorder that contains L. dihydroxyphenylalanine and monoamine oxidase inhibitor.

Epinephrine

Epinephrine, also known as adrenaline is the principal hormone governing the fight or flight response to various stimuli. In addition it stimulates glycogenolysis (breakdown of glycogen), and a variety of physiological event, such as increasing depth and frequency of heartbeats.

Norepinephrine (nor-adrenaline)

It is a precursor of epinephrine. It causes greater constriction of the blood vessels of muscles, as a result of which the arterial pressure is raised higher than is caused by adrenalin. It acts as a neuro transmitter between sympathetic synthesis of catecholamines in nervous system and smooth muscles.

A drug cimitidine (tagamet) is a structural analogue of histamine, mainly used to alleviate hyperacid secretion by interfering on the mechanism action of histamine.

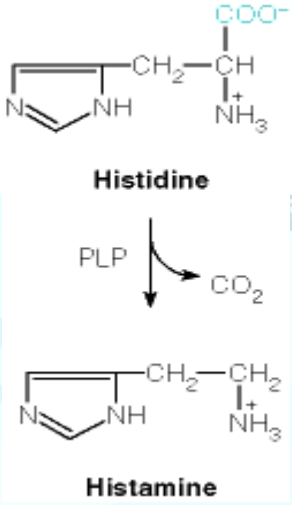


Fig 5.32 : Biosynthesis of Histamine:

Melanin:

Conversion of Tyrosine to Melanine requires Tyrosinase, a copper containing Enzyme. The two step reaction uses DOPA as a cofactor internal to the reaction and produces Dopaquinine, commonly called as Melanin.

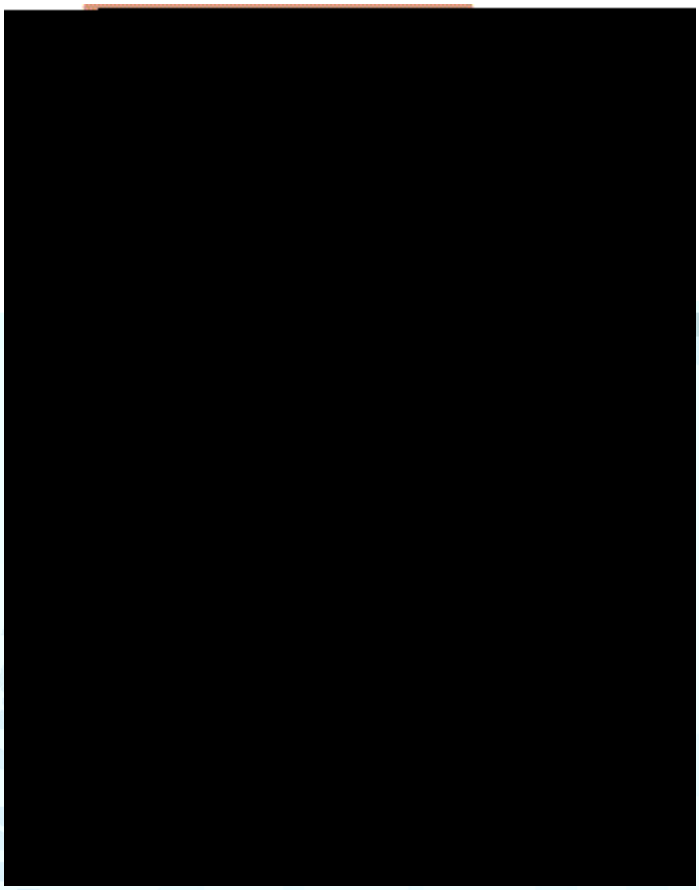


Fig 5.33 **: Melanin Biosynthesis**

Clinical problems

I. Explain with reasons whether high protein diet plans serve to reduce weight especially in obese people.

II. An otherwise healthy 64 year-old women noticed that she occasionally had a tremor in her left arm and occasional muscle cramping in her left leg. She was given a medication that contained L- dihydroxyphenylanin and monoamine oxides .Comment on the pathological condition arose on the women.

III. An apparently healthy 5-month old female's infant was brought to a pediatrician's office by her mother with a complaint of periodic bouts of vomiting and failure to gain weight. The mother also reported that the child would oscillate between periods of irritability and lethargy.

Laboratory results revealed as

- marked by increased plasma ammonia concentration (323 µ mol/L [550 µg/dl)

  Normal range = 15-88 µ mol/L {25-150 µg/dl}

  - Greater concentration of glutamine

  - Low concentration of Insulin.

  Orotate, a pryimidine nucleotide precursor was noted to be excreted in the urine.

IV. A full term infant born to a normal and healthy mother and father, was observed to have a marked lack of pigmentation: had blue eyes and many white patches on his hair.Comment on the pathological symptoms of the child and outline of this pigment forming pathway.

V. Growing children and patients recovering from trauma,surgery and major burns require more high quality protein rich in essential amino acids in addition they excrete less nitrogen than they consume. Explain

VI. Patients with gastric or duodenal ulcer, or both, often experience chronic Recurrence, in these cases, what treatment would you choose?

VII. A 68 year old man's hands shake uncontrollably. He finds it difficult to start walking and, once he has managed to start, he cannot stop easily. He cannot control his gait, suffers from uncoordinated sight and speech. Explain with reason what might happened to the person, with the means to alleviate the condition. Outline the pathway that is related to the disorder that the old man is suffering from.

Failure of growth

UNIT SIX

VITAMINS AND COENZYMES

Vitamins

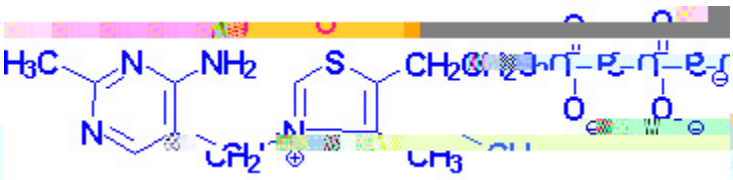
Introduction:

Vitamins are all organic compounds which, as originally defined, cannot be synthesized in the human body and must be provided in the diet. They are essential for the normal processes of metabolism, including growth and maintenance of health. It is known that the body is able to produce part or even all of its requirements for some of the vitamins, Example: Vitamin D from cholesterol and niacin from Tryptophan.

The Water soluble Vitamins

Include the B- Vitamins and Vitamin C. They share few common properties besides their

Thiamine is Vitamin B1. Addition of a pyrophosphate to **thiamine** (from ATP) converts it to thiamine pyrophosphate, a molecule that is the coenzyme for all decarboxylations of



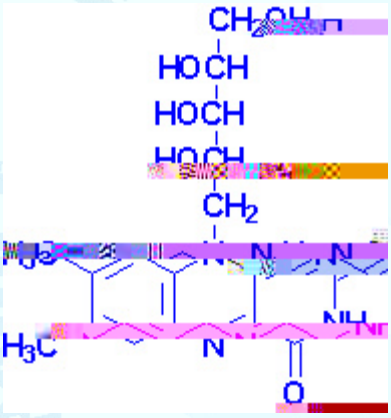
Symptoms: **There are two types** Dry beri-beri not associated with edema and wet beri-beri with edema, probably due to congestive cardiac failure and low plasma albumin. Symptoms include Peripheral Neuropathy, Exhaustion and Anorexia. The signs may progress to edema and Cardiovascular disorders, Neurological & muscular degeneration.

Wernicke Korsakoff syndrome which is frequently found in Alcoholics is associated with Thiamin deficiency.

Diagnostic parameters: Erythrocyte transketolase activity decreases.

Thiamine excretion in Urine and Blood thiamine concentration decreases.

Riboflavin (Vit B_2).



Riboflavin, also known as **vitamin B2**, is a component of the flavin coenzymes, FAD and FMN. It is composed of an isoalloxazine ring system linked to ribitol. The ability of the ring system of **riboflavin** to exist as a semiquinone allows the flavin coenzymes to accept electrons either singly or in pairs. NAD^+ and $NADP^+$ can **only** accept electrons in pairs.

It is mainly used in the energy metabolism of Sugars and Lipids. The activation of FMN and FAD is an ATP-dependent.

Source: Meats, Nuts, Legumes, Milk, fish, egg etc.

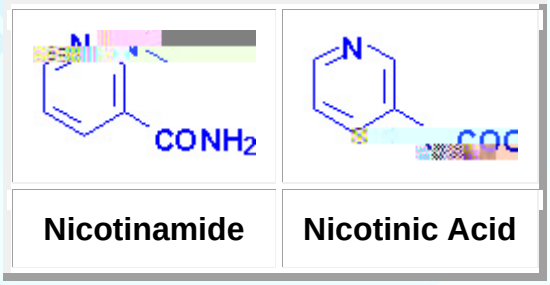
RDA: 1.5-2.5mg for adults, infants 0.6mg, children 1.0-1.8mg

Deficiency: Lack of riboflavin in the diet causes a generally non fatal syndrome of inflammation of the corner of mouth (angular stomatitis), painful glossitis of tongue (Purple) and Scaly dermatitis.

A degree of photophobia may be due to its light sensitivity, because Riboflavin is colored, fluorescent and decompose in visible light but heat stable.

Erythrocyte enzyme activity measurements (Glutathione reductase) is used to determine Nutritional status of Riboflavin.

Niacin



Nicotinamide	Nicotinic Acid

Niacin is not a vitamin in a strictest sense of the word, since it can be synthesized from Tryptophan. However, conversion of Tryptophan to Niacin is relatively inefficient (60 mg of Tryptophan is required to produce 1mg of Niacin) and occurs only after all the body requirements for Tryptophan is met. Thus most people require dietary sources of both Tryptophan and Niacin.

Niacin contains a substituted Pyridine ring and when gets activated forms NAD^+ and its phosphorylated derivative is $NADP^+$ .which are co enzymes of many dehydrogenases.

Source: Milk, Lean meat, Unrefined grains, cereals and from Metabolism of Tryptophan.

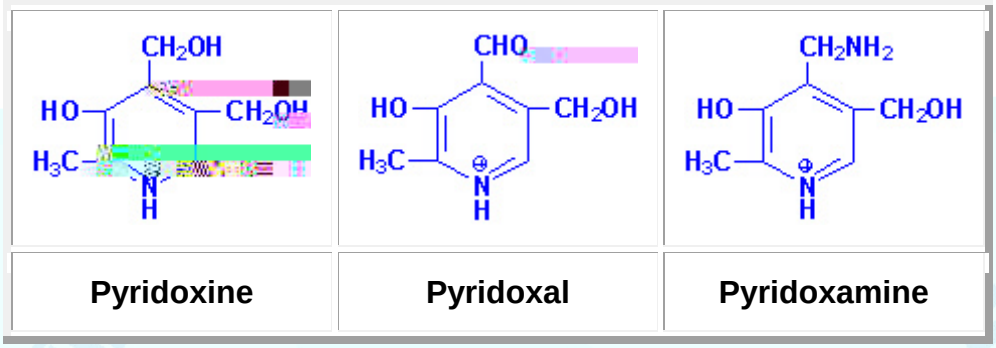
RDA: Adults 17-21mg, infants 6mg.The requirement increases with increased intake of calories, illness, severe injury ,infection ,burns, high corn (maize) diet, pregnancy and lactation.

Deficiency: Deficiency leads to Pellagra, a disease involving GIT and CNS.

The disease is characterized by intense irritation and inflammation of the mucous membranes of the mouth and other parts of the GIT, leading to gastro- intestinal hemorrhage, Dermatitis, Dementia & Diarrehea. (the “3-D’s” cardinal features). Skin lesiondevelop when exposed to sunlight, become redend, thickened and becomes scaly. The patient develops gingivitis and stomatitis (Tongue gets swollen) General effects of deficiency are Failure of growth, loss of weight and anemia.

The case will be severe in Alcoholics.

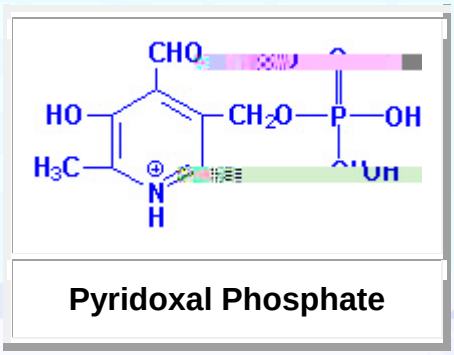
Vit B_6 (Pyridoxine)



Pyridoxine	Pyridoxal	Pyridoxamine

Exists in three forms: Pyridoxine, Pyrodoxal & pyridoxamine and their corresponding phosphates.

Pyridoxal phosphate (PLP)



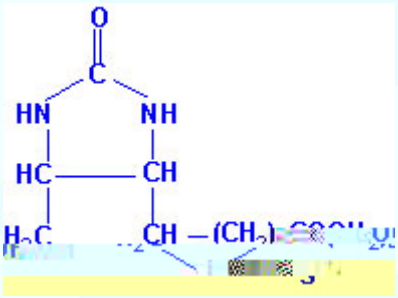
Pyridoxal Phosphate

Pyridoxal phosphate participates in transaminations, decarboxylations, racemizations, and numerous modifications of amino acid side chains. All **pyridoxal phosphate-**requiring enzymes act via the formation of a Schiff base between the amino acid and coenzyme. A cation (a metal or a proton) is essential to bridge the phenolate ion of the coenzyme and the imino nitrogen of the amino acid. This bridging maintains the planarity of the structure, which is essential for catalysis. The most important catalytic feature of the coenzyme is the electrophilic nitrogen of the pyridine ring, which acts as an electron sink, drawing electrons away from the amino acid and stabilizing a carbanion intermediate.It is also used for the synthesis of Neurotransmitter, Serotonin and Nor-Adrenalin. Used as a component of Sphingolipids necessary for myelin formation and Heme synthesis as well. Hypochromic microcytic anemia since PLP is required

for Heme synthesis.Deficiency in infants cause convelsions due to inactive glutamate decarboxylase, GABA not formed there by impaired neurotransmission.

It is an essential component of Glycogen phosphorylase;it is covalently linked to a lysine residue and stabilizes the enzyme.The conversion of Tryptophan to NAD also requires this co-factor.

Sources



RDA: 100-200µg/day. Requirement increase in pregnancy and lactation. Patients on oral antibiotics for a long period of time require more of this vitamin.

Deficiency:

Rare, since it is found in almost all food stuffs. But large consumption of raw egg white may lead to deficiency of Biotin. Avidin, a glycoprotein in egg white binds tightly to biotin and makes it unavailable for the necessary carboxylation reactions.

The symptoms in this case are: Dermatitis, Glossitis, Muscle pain, depression, alopecia (Loss of hair), Loss of appetite and Nausea.

Vit B_{12} (Cobalamin).

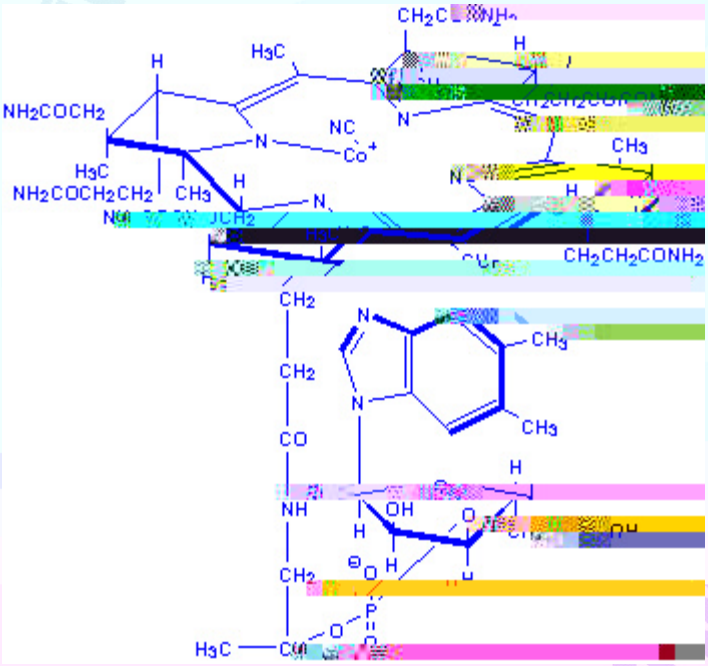


Figure: Structure of Cobalamin

The metal cobalt in **vitamin B12** is coordinated with a tetrapyrrole ring system, called a corrin ring, which is simiilar to the porphyrin ring of **heme** compounds. The cyanide attached to the cobalt in the structure is an artifact of the isolation and is replaced by water or a hyrdoxyl group in cells. The presence of cobalt and amide nitrogens gives **B12** compounds the name cobamides or cobalamins. Only two reactions occur to a significant extent in mammalian metabolism: the synthesis of **methionine** from **homocysteine**

B12- requiring reactions involve either (1) methyl group transfer or (2) adenosylcobalamin-dependent isomerizations. The isomerizations exchange a carbon-bound hydrogen with another carbon-bound functional group.

Pernicious anemia arises from a B12 deficiency. Gastric tissue secretes a glycoprotein called intrinsic factor, which complexes with ingested **B12** in the digestive tract and promotes its absorption through the small intestine into the blood stream. Pernicious anemia results from insufficient secretion of intrinsic factor. Outlines a probable explanation for why failure to absorb **B12** leads to the deficiency of red blood cells that define anemias.

1. When **B12** levels are low, flux through the methionine synthase reaction decreases but, because adequate dietary methionine is usually available, protein metabolism is not immediately disturbed.
2. Reduction of **5,10-methylenetetrahydrofolate** to **5-methyltetrahydrofolate** continues because this reaction is virtually irreversible.
3. Because methionine synthase is the only mammalian enzyme known to act on 5-methyltetrahydrofolate, the decreased intracellular activity of this enzyme causes 5-methyltetrahydrofolate to accumulate, at the expense of depleted pools of the other tetrahydrofolate coenzymes. Thus, even though total folate levels may seem ample, there is a functional folate deficiency, with insufficient levels of the formyl and methylene derivatives needed for synthesis of nucleic acid precursors.

The action of B12 and folic acid, are interrelated. Deficiency of both produce similar signs and symptoms and Anemias.

Source: Synthesized by Microorganisms

RDA: 3mg/day.

Deficiency: As discussed above

Folic Acid.

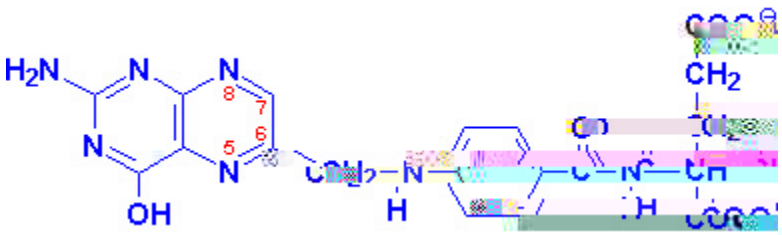


Figure: Structure of Folic Acid

The active form of folic acid is Tetra hydro folate (THF)

Coenzymes derived from the vitamin **folic acid** participate in the generation and utilization of single-carbon functional groups, methyl, methylene, and formyl. The vitamin itself was discovered in the 1930s, when it was found that people with a certain type of megaloblastic anemia could be cured by treatment with yeast or liver extracts. The condition is characterized, like all anemias, by reduced levels of erythrocytes. The cells that remain are characteristically large and immature, suggesting a role for the vitamin in cell proliferation and/or maturation.

Chemically, **folic acid** is formed from three distinct moieties: (1) a bicyclic, heterocyclic pteridine ring, **6-methylpterin** (**p-aminobenzoic acid ,PABA)**, which is itself required for the growth of many bacteria; and (3) **glutamic acid**. Naturally occurring **folates** may differ from this compound in the number of glutamate residues per molecule of vitamin, which ranges from three to eight or more. These residues are linked to one another, not by the familiar peptide bond but rather by a modified peptide bond involving the α-amino group and the γ-carboxyl group.

Source: The vitamin is abundant in leafy green vegetables such as spinach, so is named folic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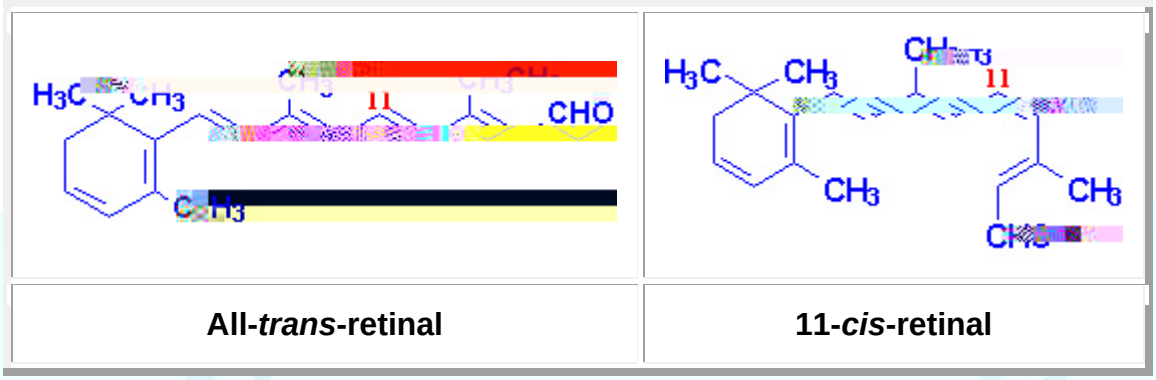
good leaving group in nucleophilic displacement reactions. Thus, the acyl group is readily transferred to other metabolites, as occurs, in fact, in the first reaction of the citric acid cycle.

Some of the common metabolic reactions involving **Coenzyme A** are shown below.

1. Pyruvate + **NAD^+** + **CoA-SH** <=> **Acetyl-CoA** + **NADH**

Vitamin A



All-*trans*-retinal | **11-*cis*-retinal**

Figure.6.1 Vit A Structures

The vitamin is present in the diet as retinol or as β-carotene some of which is hydrolyzed in the intestine to form retinol. It is a generic term for a collection of three forms of Vitamins, retinol, retinal and retinoic acid (Retinoids) all of which are found from animal and plant sources.

Pre-Albumin and specific binding proteins on cell surface membranes are involved in the uptake of Vitamin A ester from the plasma in to the tissues. Owning to the fat soluble nature ,transport is effected by a specific proteins – serum retinol binding protein(SRBP), cytosolic retinol binding protein(CRBP) and Albumin as well as

A specific retinoic acid binding protein (RABP). The vitamin is stored in the liver, mainly as its ester. Some other derivatives of Vit A are stored in the Liver as retinol palmitate.

In natural sources VitA is present as esters of fatty acids .These as well as their precursors are readily absorbed from the intestine via the lymphocytics.

Pancreatic lipase liberates the free Vitamin from the ester during digestion, but it is re-esterified in the intestinal mucosa. Carotenone are converted to vitamin in the liver.

Source: A rich source is Liver, but leafy vegetables and some fruits provide the largest amount of β-carotene

Liver, egg yolk, butter and milk are good sources of β-carotene.

Functions

β-carotene has an antioxidant role and prevents the development of diseases in which the action of free radicals is implicated .

It plays a protective role against Cancer and cardiovascular disease.

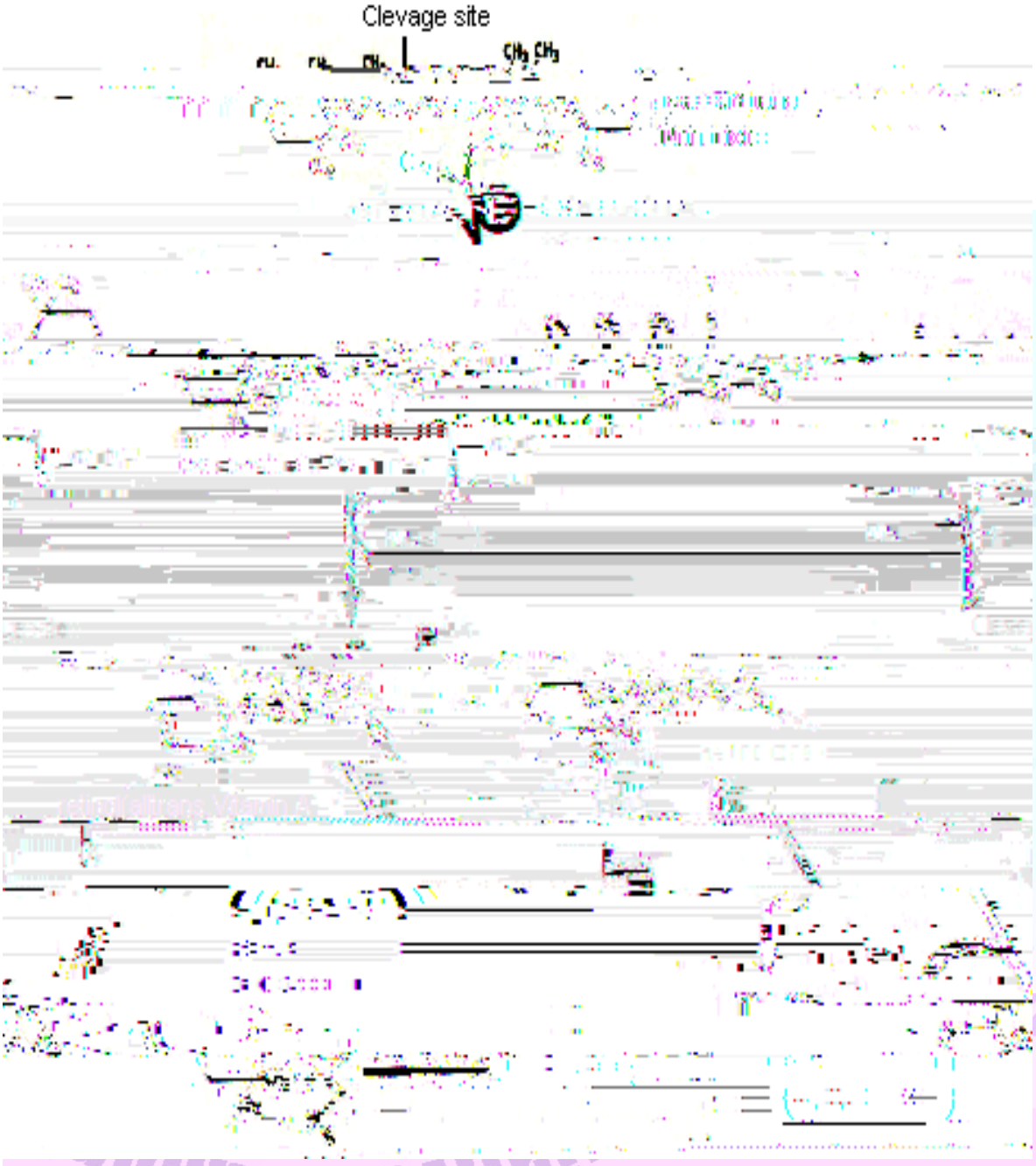


Fig.6.2 Vitamin A structure, function and Metabolism

Retinol: It gets phosphorylated and serves as an anchor for the growing chain of oligosaccharides.It prevents fetal resorption, promote spermatogenesis.

Retionic acid: It is translocated to nucleus and control gene expression. It resembles steroid hormone because of this property.It promotes differentiation of epithelia. Acts as carrier of oligosaccharides for glycoprotein synthesis.

Vit A deficiency

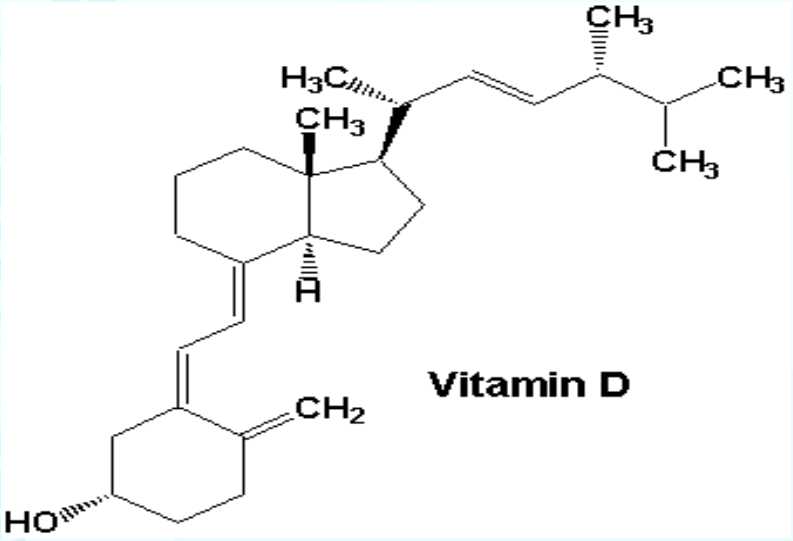
Vit A affects growth and differentiation of epithelial cells leading to defective epitheliazation, a condition affecting the cornea of the eye. It produces softening and opacity. Severe Vit A deficiency leads to progressive keratinization of the cornea and possibly permanent blindness.Another form, retinoic acid, induces differentiation of epithelial cells .Vit A deficiency predisposes to gastrointestinal and respiratory tract infections.

Hypervitaminosis:

Excessive intake of vitamin A, in humans cause head ache, nausea, vomiting and dizziness.This might be related to increased spinal fluid pressure. Patien suffers from dry itchy skin, alopasia, cracking of lips etc. On withdrawl of vit, patient feels relief.

It is virtually impossible to develop e vit.A toxicity by ingesting natural foods.When people consume supplements, there might be hypervitaminosis.

Vitamin D:



Vitamin D

Bound to specific D -binding protein, chloecalciferol moves via circulation directly to the liver.

Hydroxylation at C_{21} takes place in the endoplasmic reticulum of hepatocytes in a non-regulating process. The 25 (OH)-chloecalciferol is a potent Vit.D_3 and is also produced in a smaller proportion in the kidney.Vit D_3 is also found in the diet where its absorption is associated with other fats, and is transported to the liver by chylomicrons

A significant proportion of 25 (OH)-D_3 is excreted in the bile and is reabsorbed in the small bowel, producing an enetrohepatic circulation.

Disturbance in enetrohepatic circulation can thus lead to deficiency of this vitamin.

The main site for further hydroxylation at the 1 position is in the renal tubules. Although bone and placenta can also carryout this reaction.Increase of serum calcium and phosphates Autoregulate the synthesis. Hypocalcemia, Phosphatemia stimulates the release of PTH which enhances the synthesis of Vitamin D. On the other hand, high calcium, phosphate inhibit the synthesis.

Sources: Fish oils, egg yolk are naturally rich sources of Vit D.

Functions: Target organs are bone, Kidney and Intestine. Calcitriol promotes bone mineralization.

Intestine: This vitamin promotes absorption of calcium, phosphates. The mechanism of action resembles that of steroid hormone.It crosses cell membrane bind to cytoplasmic receptor to form a complex, which is translocated to the nucleus.Here it binds to chromatin, induces the synthesis of calcium binding protein. Thus calcium absorption is stimulated.Defect in cytoplsmic receptor may lead to Rickettes.

Kidney: Reabsorption of calcium, phosphate are enhanced.

Bone: It promotes synthesis of osteocalcin which is needed for bone mineralization. It also promotes bone collagen synthesis.

Catabolism of Vit D:

Hydroxylation at C_{26} takes place in Liver by oxidases .This compound has no Vitamin D acticvity.

Deficiency:

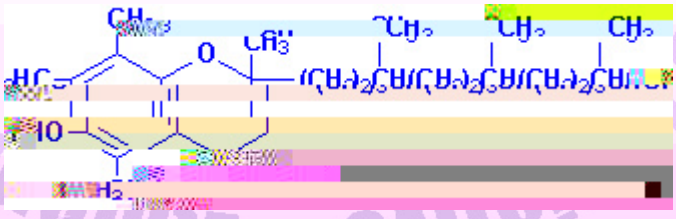
Usually deficiency of Vit D are due to insufficient exposure to sunlight, inadequate dietary intake, GI disorder, obstructive jaundice and Partial gastrectomy.

Ricketes is characterized by the production of soft pliable bones due to defective mineralization secondary to calcium deficiency.

Vit D deficiency is also characterized by low concentration of calcium in blood in association with increased serum alkaline phosphatase.

Type I vit D dependent rickets

Is casued by an inherited defect in the conversion of 25(OH)- Di(e)8(a, t)5.6( )]TJ0 -132732 TD.0605 Tc.0302 T



Source: The reachest source is vegetable oils and nuts

Chemically Tocopherols are derivatives of an alcohol, tocol having a substituted Chromanone nucleus, with their poly isoprenoid side chain of variable length usually three carbons.

Functions

The main function of Vit E is as an antioxidant, in particular a membrane antioxidant associated with lipid membrane structure. It provides protection from the action of peroxides by converting them to a product that is conjugating with glucuronic acid and excreted in bile. This protective phenomenon is very much evident in the prevention of hemolysis of RBCs by H_2O_2 .

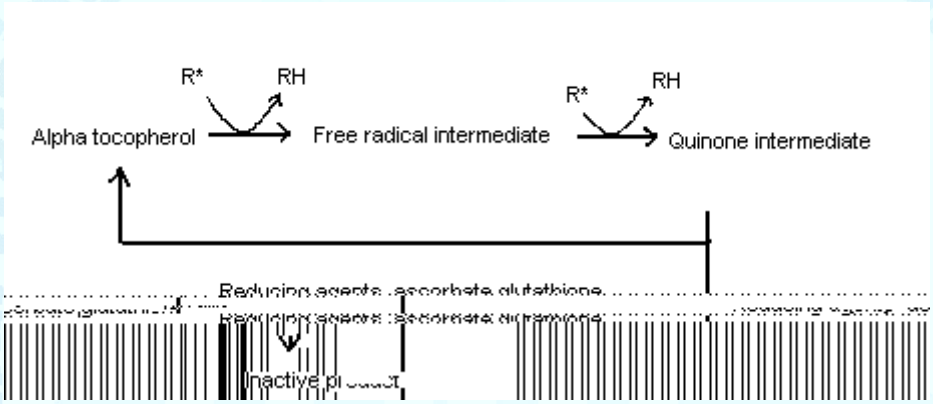


Fig 6.5: Antioxidant action of Vitamin E

R*=free radical, RH= inactivated free radical

If peroxides are formed in excess, in the presence of vit E, selenium containing Glutathione peroxidase destroys them before any damage is caused to the membrane.

Also acts as scavenger of free radical damage to polyunsaturated fatty acids in cell membranes and help prevent oxidation of low –density lipoprotein (LDL) Oxidized LDL may be more atherogenic than native LDL, and there is some evidence that Vit.E may protect against atheromatous coronary heart disease.

Source:

The richest source is vegetable oil, and nuts

Deficiency

Vit E deficiency is a rare but found in complication of prolonged and severe steatorrhoea, and of prolonged parenteral nutrition.

Deficiency of Vit E causes anemia in children with cystic fibrosis of pancreas are found to be tocopherol deficient as a result of stetorrhoea.

Neurological consequence has also been described.Generally deficiency is investigated by measuring plasma [Vitamin E].

Vitamin K

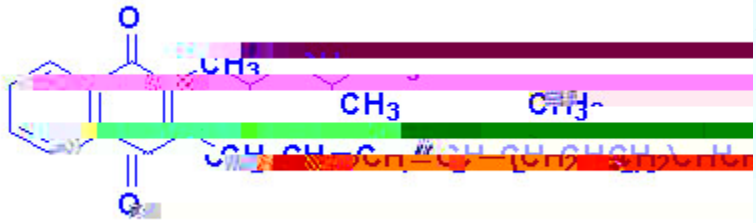


Fig 6.6 Structure of Vitamin K

It refers to a group of related compounds, varying the number of isoprenoid units in its side chain. There are three types, Menaquinone (K_2)present in animals ,Phylloquinone (K1) present in Plants. Menadione a synthetic water soluble vitamin is available for treatment.

Like vit E, the absorption of Vitamin k is dependent on appropriate fat absorption.

Functions

It is the only one acting as co-enzyme from the group of Fat soluble vitamins.

UNIT SEVEN

MINERAL METABOLISM

Large number of elements are needed for the functioning of the body. Some elements are needed at high concentrations, required more than 100mg per day. They come under macroelements. Example. Sodium, Potassium, Calcium, Magnesium and Chloride.

Sodium and Potassium: They are important in cell, muscle physiology, transmission of messages and other biological processes.

Sodium is the principal cation of extra cellular fluid. It is commonly found in all types of foods. Recommended daily allowance (RDA) is 5-10 gms. It is excreted in the urine. The concentrations are maintained by Aldosterone.

Potassium is intracellular cation; daily requirement is 1 gm/day. Its excretion is through kidney, linked to sodium excretion.

Since both are widely distributed, deficiency of the two elements is rarely found.

Functions:

Sodium maintains osmotic pressure of extra cellular fluid and ECF balance.

It has a role , along with others, in the neuro muscular excitability

Sodium is exchanged with Hydrogen in renal tubules to acidify urine.

Sodium pump keeps sodium in far higher concentration outside the cell .This results high polarization, create resting membrane potential.

Sodium and Potassium maintain the degree of hydration of plasma proteins, and there by viscosity of blood.

Potassium is critically important for the functioning of cardiac muscle.

Hypernatremia: It occurs nearly always due to water deficiencies rather than Na^{2+} excess.

Increased sodium is found in ECF. It may be due to increased sodium in the body, decreased body water. It is usually seen in patients with dehydration, on steroid therapy or excess sodium intake.

Hyponatremia: It is common in patients who are in diuretics or excessive sweating, kidney disease, diarrhea and congestive heart failure.

Hyperkalemia is found in patients who are on excess intake orally or given intravenous drip. Other causes are decreased excretion by the kidney, diseases like Anuria, tissue damage or Diabetes Mellitus.

Hypokalemia: Low potassium is not due to dietary deficiency but due to conditions like vomiting, diarrhea. Habitual users of laxatives are prone to the condition.

Calcium and Phosphate: Major parts (90%) of them are found in the form of crystal lattice in the bone. Rest is found in the soft tissues, teeth and ECF.In plasma they have important role.

Sources: Milk, milk products, green leafy vegetables are rich in calcium.

Phosphate is widely distributed in nature.

Calcium: RDA 500mg for adults and 1200mg for children, 1500mg for post-menopausal women. People, who get enough sunlight, exercise regularly, on high protein diet, require 300-400mgs per day.

Absorption: It is influenced by

Acidic pH solubilizes Calcium salts, promote absorption.

High protein diet favors absorption

Certain plant products, high fiber diet, oxalates interfere with absorption.

Vitamin D promotes absorption.

PTH, Calcitonin favors absorption while Glucocorticoids decrease intestinal transport.

Normal blood concentration is critically maintained at 9-11 mg %.

Functions:

Calcification of bones and teeth. Bone formation requires Calcium continuously.

It is important for blood coagulation

Neuromuscular transmission.

Muscle contraction

Acts as secondary messenger in hormone action.

Clinical conditions:

Hyper- calcemia; may be due to hyper parathyroidism, endocrine causes, renal failure and malignancies. Hypo- calcemia (below 8.5mg %) due to

Inadequate dietary intake.

Hypoalbuminemia

Hypo parathyroidism

Renal disease/ failure

Vitamin D deficiency

Chronic deficiency leads to loss of bone mass (bone resorption) and osteoporosis, bone fractures

Phosphorus: Dietary sources are cheese, milk, nuts. Eggs and organ meats.

Absorption and regulation is similar to that of Calcium.

Functions:

Constituent of bone and teeth

Needed for the synthesis of energy rich molecules like ATP and Creatin phosphate.

It forms Phosphate buffer in blood.

Constituent of phospholipids, biomolecules and coenzymes (TPP).

Trace elements

Daily requirements of some elements is very very less .Such elements are included in trace elements.

Iron

In body it is found in Haemoglobin, Myoglobin, ferritin, hemosiderin, transferrine and enzymes like cytochromes etc.

RDA is 10-20mgs. Sources are meat, fish, eggs, cereals like wheat & Teff, green leafy vegetables. Milk is deficient in Iron.

Absorption is through intestinal mucosa.

Requires acidic pH of stomach. Ascorbic acid and Ceruloplasmin promotes absorption.

It combines with intracellular binding protein Apoferritin to ferritin. Almost 300 ferric ions can bind to one molecule of apoferritin..

For transport, free iron binds to Apo transferrin, in blood to form transferrin.It is the major transport form of iron. It also prevents toxicity of free iron.

Excessive binding of iron causes denaturation of ferritin molecule. It undergoes aggregation, to form hemosiderin. Mobilization of iron from hemosiderin is very slow. Thus there is accumulation of hemosiderin, the condition is called hemosiderosis.

Massive deposits of hemosiderin in tissues lead to hemachromatosis. If this takes place in liver, it causes cirrhosis. In pancreas, it damages β cells, result in Bronze diabetes. The skin of the patient has bronze coloration. Oxidative damage to cardiac muscle is a biggest concern.

Iron is stored in liver, spleen and bone marrow.

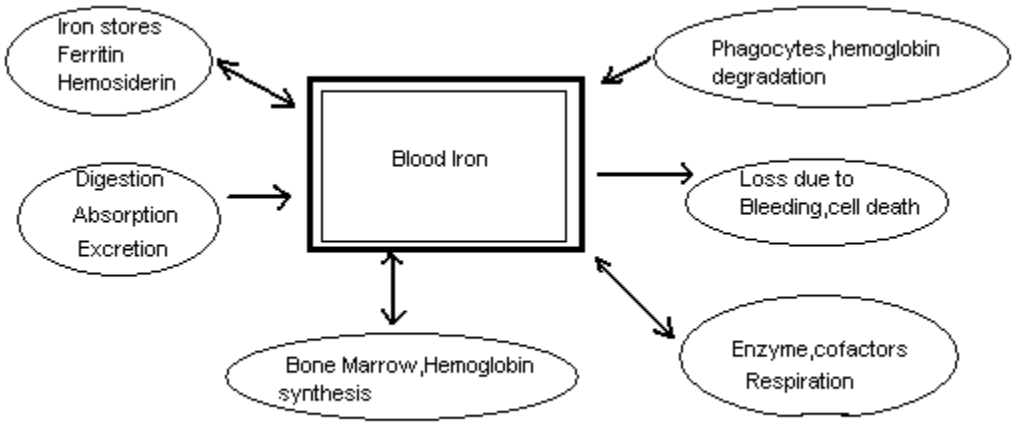


Fig 6.7. Homeostasis of Iron in blood

Causes of iron deficiency:

Storage depletion

Reduced dietary intake.

Malnutrition

Hemolysis

Children who are on milk diet only are prone to iron deficiency.

Chronic bleeding, irregular menstrual cycles

Peptic ulcer, piles

Hook worm infection

Repeated malarial infections.

Deficiency leads to Iron deficiency anaemia or hypochromic microcytic anaemia.

It is associated with low hemoglobin and ferritin.

Copper

Humans contain around 100 mgs of copper. Liver, brain, kidney and heart are rich in copper. Free copper is 4%, 96 % is bound to Ceruloplasmin in body.

Sources: cereals, legumes, raisins, nuts etc

Functions:

There is dysfunction of lenticular region of brain

Defective tubular reabsorption in kidney leads to aminoacidurias.

Copper deposition in the eye, as golden brown or green ring around the cornea.

Patients are treated with Pencillamine, which binds to tissue copper and mobilizes it.

Magnesium:

It is an intracellular ion, essential for life.

Sources: Widely distributed in vegetables, chlorophyll, cereals, beans, potatoes, cheese and animal tissues.

Maximum concentration is found in bones, little in Extra-cellular fluid (ECF) and soft tissues.

2/3 in blood is in ionic form, rest is bound to protein.

It is absorbed from the small bowel.

It is excreted through feces, urine and sweat.

Functions:

Role in enzyme action. It is a cofactor for peptidases, ribonucleases, glycolytic enzymes etc.

Its action is similar to that of calcium in neuromuscular irritability.

High levels depress nerve conduction, low levels may cause Tetany.

Major part is found in bones. In teeth, it is present as dentin and enamel.

Magnesium deficiency occurs rarely in man.

Fluorine

It is solely derived from water, tea, and fish

Daily intake should not be more than 3mg.

Excess is toxic, lethal dose is 2.5 Gms.

It is absorbed by diffusion from intestine.

Mostly it is found in the bones and teeth.

It is eliminated in the urine.

It is bound as complex of protein Metallothionein. The sulfur groups of the protein chelate zinc.

The body does not store Zinc to any appreciable extent in any organ, urinary excretion is fairly constant at 10 mol/day.

Functions:

Zinc is important for the activity of a number of enzymes like

Carbonic anhydrase

Alkaline phosphatase

Alchol dehydrogenase

Porphobilinogn synthase

Leucine aminopeptidase

Carboxy peptidase

Aldolase in glycolysis

DNA, RNA polymerases as zinc has crucial role in DNA.

Release of vitamin A from liver requires Zinc. Retenene reductase (zinc enzyme) participates in the regeneration of rhodopsin (visual cycle).

Insulin is secreted, stored as a complex of Zinc

It is important for wound healing.

Deficiency of Zinc:

Patients requiring total parentral nutration, pregnancy, lactation, old age and alcoholics have been reported as being associated with increased incidence of Zinc deficiency.

It is usually associated with protein energy malnutrition (PEM)

It is caused by diuretics, chelating agents and anti-cancer drug treatment

results in dwarfism and hypogonadism

Delayed sexual development

It decreases spermatogenesis in males and irregular menstrual cycles in females.

It stimulates ribonuclease activity; thereby it affects the synthesis of mononucleotides and nucleic acids.

Hepatosplenomegaly

- The Toxicity symptoms are Hair loss,failing of nails, diarrhea,weight loss and gaslicky odour in breath(due to the presence of dimethyl selenide in expired air).

Halogenated aromatic hydrocarbons are useful in the treatment of Selenosis.

UNIT EIGHT

Definition

Hormones are chemical messengers secreted into blood by endocrine or ductless glands. However many hormones are secreted by organs which are not ductless glands. Hormone means to arouse or to excite.

Major endocrine glands are pituitary, hypothalamus, thyroid; adrenals, pancreas, ovaries and testes.Others are Thymus, Pineal gland and gastro intestinal hormones.

Hormones can be classified based on their structure, mechanism of action, based on their site of production etc.

Classification of hormones based on their structure.

1. Follicle stimulating hormone (FSH)	P	Adenohypophysis.
2. Leutinising hormone (LH)	P	"
3. Somatotrophin (GH)	P	"
4. Prolactin	P	"
5. Thyrotrophin (TSH)	P	"
6. Corticotrophin (ACTH)	PP	"
7. Vasopressin (ADH)	PP	Neurohypophysis.
8. Oxytocin	pp	"
9. Triiodothyronine (T3)	O	Thyroid.
10. Thyroxine (T4)	O	"
11. Calcitonin (CT)	PP	"
12. Parathyroid hormone (PTH)	PP	Parathyroid
13. 1, 25-Dihydroxy cholecalciferol	S	Kidneys.
14. Noradrenaline (Nadr)	O	Adrenal medulla.
15. Adrenalin (Adr)	O	
16. Aldosterone	S	Adrenal cortC S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Biosynthesis of Hormones

Biosynthetic mechanisms are many. Some protein hormones are synthesized as precursors, which are converted to active form by removal of certain peptide sequences.

E.g. Insulin is synthesized as pre-proinsulin (m.wt11500).Removal of some amino acids, peptides produce insulin (m.wt 5734).

Thyroxine, a single amino acid hormone. It is synthesized as a glycoprotein precursor called thyroglobulin, which has 115 amino acids.

Other hormones like glucocorticoids/ minerolacorticoids from Adrenal gland are synthesized and secreted in their final active form.

Pro-hormones: Some hormones are synthesized as biologically inactive or less active molecules called pro-hormones. Usually they are polypeptides/ proteins.

Eg.Pre-proinsulin → Proinsulin.

Storage

Hormones are stored in secretory granules within the cytoplasm of endocrine cells. eg. Thyroid hormones are stored in follicles filled with colloid particles. Catechoamines of Adrenal medulla are stored in secretory granules of cytoplasm.

Storage always protects the molecule from untimely inactivation.

Steroid hormones are not stored in significant quantities.

In response to stimulus they are synthesized and released immediately.

Release:

When the target cells require free hormones, they are released immediately.

The deficit in the bound form is replaced by the secretion of the endocrine gland. Feed back inhibition/stimulation controls hormone release (Fig.7-1).

Protein, polypeptide hormones are released by exocytosis or pinocytosis. It involves fusion of granules and cellular membrane, followed by secretion in to blood stream.

Stimulus excites the endocrine cell.

The specific enzymes in the storage vesicle activate the hormone before release.

Disruption of the process by certain drugs interferes with exocytosis.

The secretory process is linked to the release of neurotransmitters.

Transport:

Some hormones are soluble and do not require transport proteins.

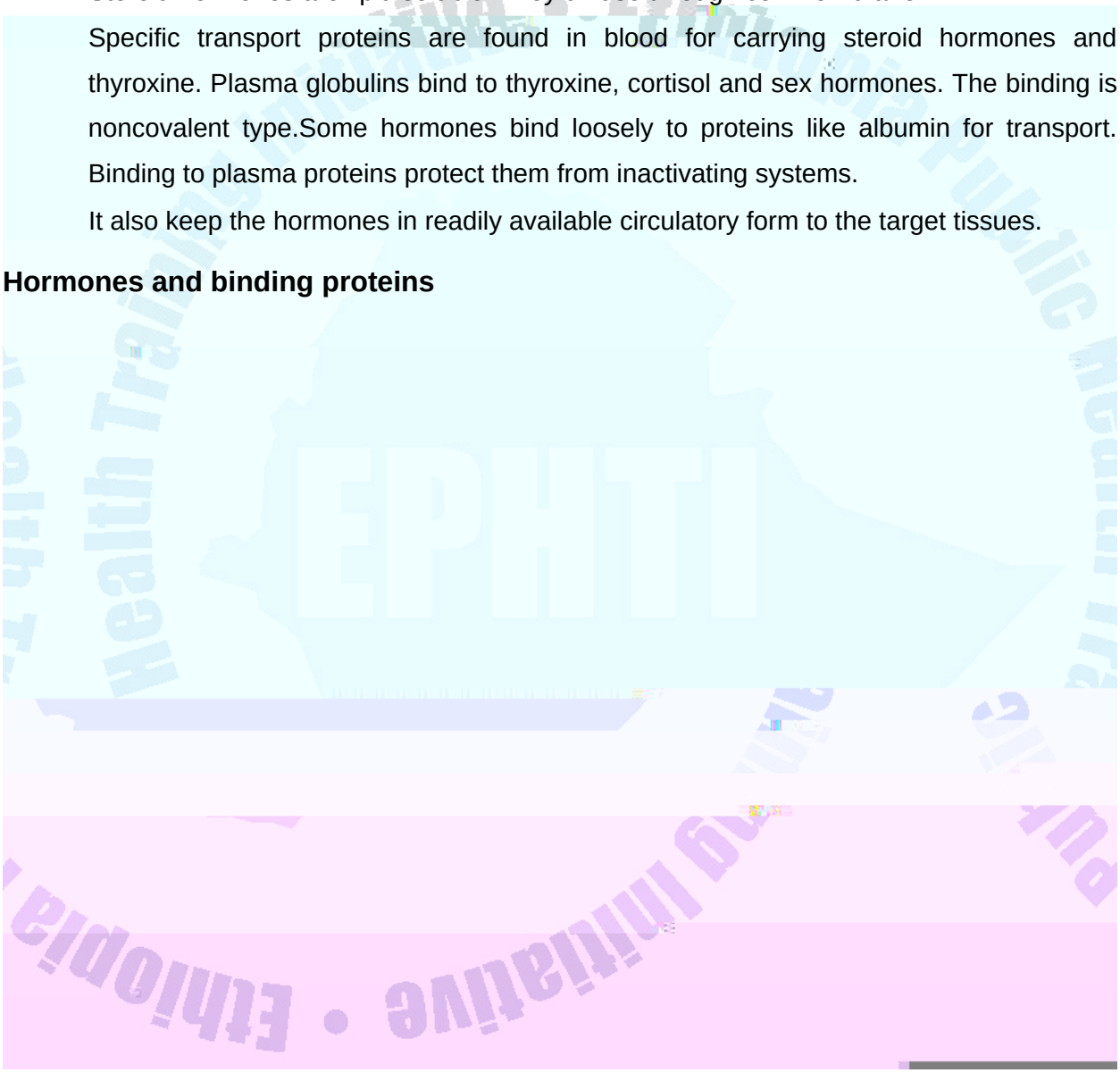
Free hormone is the fraction available for binding to receptors and therefore represents the active form. Free Hormone concentration correlates best with the clinical status of either excess or deficit hormone.

Steroid hormones are lipid soluble. They diffuse through cell membrane.

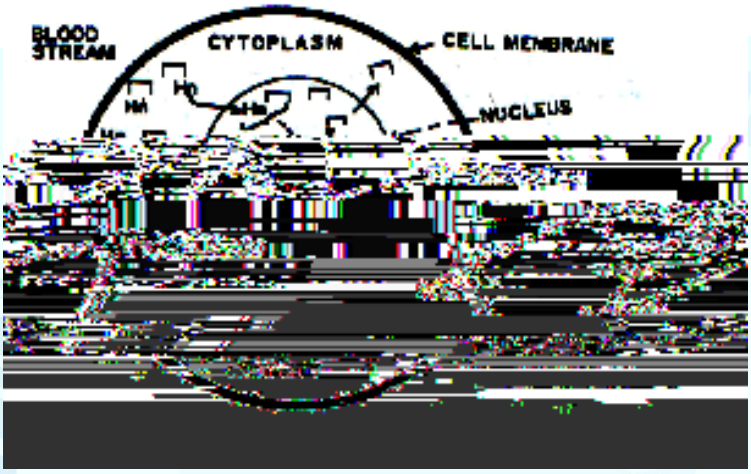
Specific transport proteins are found in blood for carrying steroid hormones and thyroxine. Plasma globulins bind to thyroxine, cortisol and sex hormones. The binding is noncovalent type.Some hormones bind loosely to proteins like albumin for transport. Binding to plasma proteins protect them from inactivating systems.

It also keep the hormones in readily available circulatory form to the target tissues.

Hormones and binding proteins



They also regulate post transcriptional processing of proteins.



* The hormone binds to surface receptors (SR) present on the plasma membrane of target cells.
* The message is carried through cascade of protein-protein interactions.
* Hormone is the first messenger. Then H-R complex sends signal across the membrane.
* It elevates the concentration of intermediary molecules called second messengers.
* These messengers act as signal conducting molecules and bring out the effects of a hormone. Second messengers are:-
  * cAMP

Á Calcium

Á

Binding of hormone to receptor leads to:

Conformational change in the receptor and G-protein (, β, subunits).

It cleaves the trimeric form into activated -GTP complex.

G-protein is a peripheral protein; which diffuses along the inner surface of the plasma membrane to reach the effector protein.

Through allosteric modification the message is conveyed to the effector protein.

Fig.7.4.Receptor for protein hormone

RS= Hormone site for stimulation

RI= Hormone site of inhibition

Effectors are intracellular enzymes like adenylate cyclase, phospholipase-C.

On activation they produce second messengers like cAMP, phosphoinositides (Phosphatidyl Inositol) and diacylglycerol.

cAMP is formed from ATP by adenyl cyclase action .

In turn it activates protein kinase A which phosphorylates intracellular proteins.

This leads to activation of key enzymes like glycogen synthetase, phosphorylase kinase, ultimately resulting in stimulation of glycolysis and inhibition of glycogenesis.

Abnormalities in the hormone-Gprotein-adenyl cyclase axis may result in the impaired action of hormones. *On the other hand the inhibitory system comprises of receptors (Ri) and inhibitory regulatory complex (Gi).

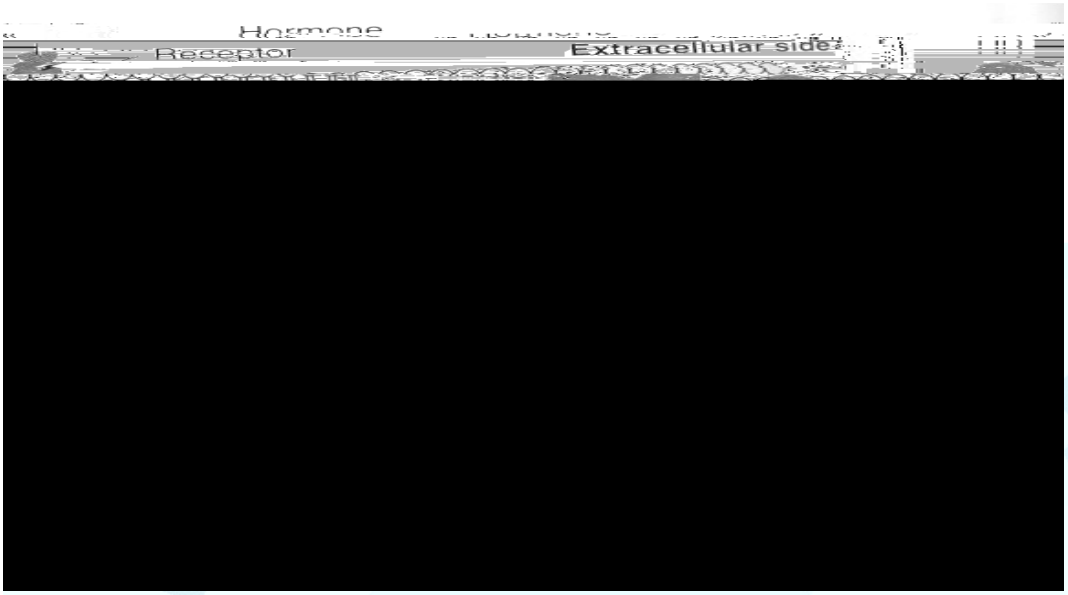


FIG.7.5. Regulation of adenyl cyclase by

Cell surface receptors with second messenger as c-AMP found for the following hormones.

ADH, HCG, LH, FSH, TSH, MSH, ACTH, CRH, Calcitonin, Glucagon,

Parathyroid hormone, somatostatin, angiotensin.

Regulation of receptors.

There are number of specific receptors in the target cells. Prolonged exposure to high concentration of hormone leads to decreased receptors, called as desentitization. There are two mechanisms for regulation.

Down regulation: There is internal distribution of receptors such that few receptors are available on the cell surface. This leads to decreased response in target tissue. More receptors

Second messengers:

Second messengers: calcium, phosphatidyl inositiols are identified for GnRH, TRH, Acetyl choline, Angiotensin-II, Vasopressin.

Insulin, GH, Prolactin, Oxytocin have unknown intracellular messengers.

Hormone itself is first messenger. The message is communicated to the cell Via. Second messengers.

1.

AMP is generated. This causes profuse secretion of water and electrolytes, causing fatal diarrhea.

Pertusis toxin from Bordetella pertusis modifies subunit of Gi, via ADP ribosylation. Consequently, Gi loses its capacity to inhibit adenylcyclase. The enzyme remains active permanently; produce large amounts of cAMP.Ultimate result of infection is Whooping cough.

2. **Calcium, as second messenger:**

Hormones exert their action via Ca, PI, or both (Fig.7-3).Intracellular Ca is increased by

a) Entry of Ca from extra cellular region when stimulated.

b) Inhibition of Ca pumps, which pumps out Ca ions in exchange for H ions.

c) Release of Ca ions from intracellular reseveroirs like mitochondria, endoplasmic reticulum.

3. **Phosphatidyl inositol 4, 5 bisphosphate**


EPHTI

a. Insulin secretion is increased by increase in glucose. B-cells of pancreas have sensors to glucose. High levels of glucose stimulate insulin secretion and decrease glucagon release.

b. High levels of amino acids in the plasma induce the secretion of insulin.

c. Gastrointestinal hormones like secretin and others are released in response to intake of food. They induce anticipatory secretion of insulin, before the rise of glucose in the portal vein. Therefore when glucose is given orally it induces more insulin secretion than when given intravenously.

d. Glucose stimulates secretion of insulin and inhibits the release of glucagon.

e. Synthesis, release of insulin is decreased when there is scarcity of dietary fuels.

Metabolic Role of Insulin

Carbohydrate metabolism: Insulin produces lowering of blood glucose and increases glycogen stores. This is achieved at several metabolic stages. *There is increased uptake of glucose, galactose by various tissues like muscles, adipose, mammary glands etc .It is due to increased translocation of glucose transporters from Golgi to plasma membrane.

* Insulin induces the synthesis of glucokinase which phosphorylates and decreases the intracellular glucose in liver.

* Insulin enhances glycolysis by inducing the synthesis of phosphofructokinse and pyruvate kinase.

* Pyruvate dehydrogenase complex is activated via dephosphorylation of enzyme molecules which lead to increased production of acetyl- CoA from pyruvate.

* Insulin stimulates protein phosphatase-1 which dephosphorylates and activates key enzyme glycogen synthase. This leads to increased synthesis of glycogen.

* Insulin reduces gluconeognesis by repressing at gene level, PEP (Phosphoenol pyruvate) carboxykinase, and it inhibits F-1, 6 bisphosphatase via F- 2, 6 bis phosphatase inhibition.

Fig.7.7. Paradoxycal action of insulin

* Insulin stimulates protein phosphatase-1 which dephosphorylates and activates key enzyme glycogen synthase. This leads to increased synthesis of glycogen.

* Insulin reduces gluconeognesis by repressing at gene level, PEP carboxykinase, and it inhibits F-1, 6 bisphosphatase via F- 2, 6bis phosphatase inhibition.

* Insulin decreases glycogenolysis by dephosphorylating glycogen phosphorylase (inactivate) and also repressing glucose - 6phosphatase.

* It stimulates HMPshunt by inducing the enzymes glucose-6 phosphate dehydrogenase, 6-phosphogluconate dehydrogenase.

Lipid metabolism: Insulin causes lowering of free fatty acids level in blood and increases the stores of triacylglycerol.

It decreases lipolysis by inactivating triacylglycerol lipase by dephosphorylation.

It increases fatty synthesis by making available acetyl - CoA, and acetyl - CoA carboxylase.

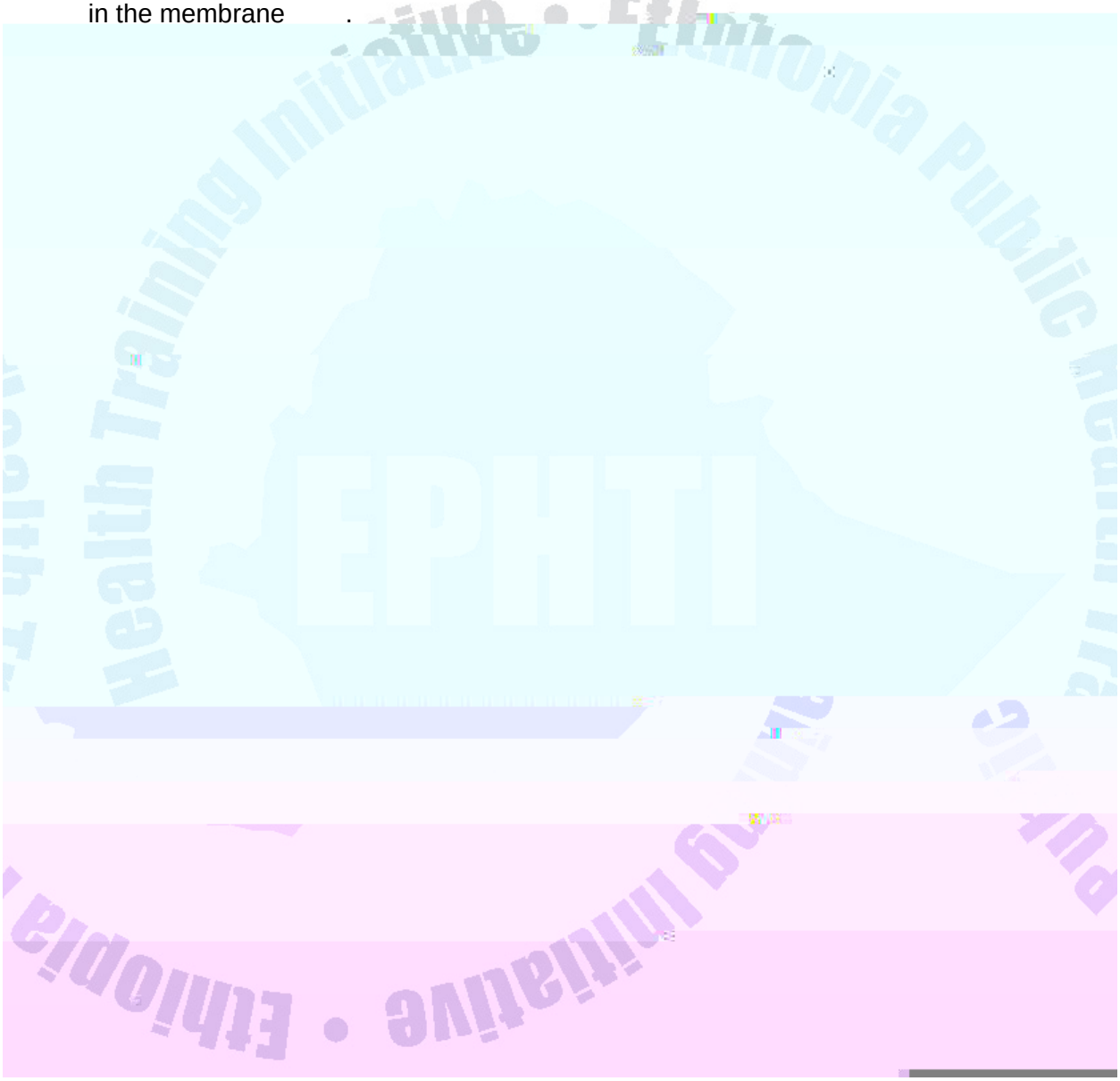
Triacylglycerol synthesis in the adipose tissue is increased by providing more of - glycerophosphate from glycolysis. It also induces the synthesis of lipoprotein lipase

which releases more fatty acids from the circulating lipoproteins. This provides more acyl- CoA for TG synthesis.

Protein Metabolism:

Insulin promotes protein synthesis by:

Increased uptake of amino acids through increased synthesis of amino acid transporters in the membrane .



(Polyuria: Large amounts of glucose may be excreted diuresis, causing excretion of large quantities of urine.Polydypsia: Loss of fluid lead to excessive thirst.Polyphagia: Intake of food is more and craving for sweets is common.) Tissues get enough supply of glucose but can't utilize. This leads to weakness.

C. Some women may present symptoms during pregnancy (stress).
D. Patient derives energy from the break down of free fatty acids. Increased cholesterol synthesis in these patients may lead to atherosclerosis.
E. Patient suffers from increased break down of tissue proteins, which accounts for loss of weight.
F. Increased breakdown of fatty acids lead to ketosis, Diabetic Keto acidosis and hyperventilation. If not treated, patient will slip in to coma and die.

Chronic complications of diabetes:

Uncontrolled diabetic patients develop cataract. It is related to hyper glycemia. There is glycosylation of lense proteins or Glucose gets metabolized to sorbitol in the lense.The associated osmotic changes ultimately result in fibrosis and cataract formation.

Diabetic damage of kidney is called diabetic nephropathy. It manifests initially as proteinuria, subsequently renal failure.

Snynthesis: It is synthesized as pro-glucagon in -cells. Carboxy peptidase B, trypsin like peptidase in the lysosomes of -cells, hydrolyze it to produce active glucagon and some inactive peptides.

Role of glucagon:

* Carbohydrate metabolism:
* It increases glucose by Glycogenolysis in liver. It has no effect on muscle due to the absence of receptor. It induces synthesis of glucose-6 phosphatase.
* It increases gluconeogenesis in liver by inducing the synthesis of key enzymes. Enzymes like PEP carboxy kinase, pyruvate carboxylase, F-1, 6-bisphosphatase are synthesized to promote gluconeogenesis.
* The hormone promotes protein break down in liver to supply glucogenic amino acids.

Lipid metabolism: It promotes lipolysis of Triacyl glycerol in liver.

* Promotes β- oxidation of Fatty acids in adipose tissue.
* It decreases fatty acid synthesis by inactivating acetyl - CoA carboxylase.

Protein metabolism:

* It depresses protein synthesis.
* It promotes breakdown of proteins in liver.

Effect on mineral metabolism:

It increases potassium, and calcitonin release which in turn causes calcium lowering effect.

Clinical aspects.

* Glucagon is used in the treatment of insulin induced hypoglycemia.
* Long acting Zn-glucagon is used in inoperable tumors of pancreas.
* It is used in acute pancreatitis for, it inhibits excessive secretion of pancreas.

Thyroxine

Follicular cells of thyroid produce T4 (thyroxine) and T3 (triiodothyronine). Para follicular cells of thyroid produce calcitonin.T3, T4 are iodinated amino acids of tyrosine, and are synthesized from thyroglobulin and iodine.

Thyroglobulin is a dimeric glycoprotein with two protein chains. There are 115 tyrosine residues in each molecule. A large part (70%) of iodine in thyroglobulin exists as inactive monoiodotyrosine, diiodotyrosine and rest is in the form of T3, T4.If iodine content is normal, the ratio of T3 to T4 is 7:1.In case of iodine deficiency, the ratio decreases.T3, T4 are stored in the thyroglobulin . The peptide bonds are broken before they are released into capillaries.

Synthesis of Thyroglobulin:

* The acinar cells of thyroid synthesize and store thyroglobulin as colloid in follicles.
* They also collect (Iodine trap) and transport iodine for the synthesis of hormone.
* They help in the secretion of T3, T4 into circulation.
* Thyroglobulins are packed into vesicles and pinched off from Golgi cisternae.
* These vesicles fuse with plasma membrane release their contents into the colloid of thyroid follicles.

Dietary iodine comes from vegetables, fruits, grown on sea shore. Sea fish is very rich in iodine. Total iodine in the body is 50 mgs and only.10-15mgs is in the thyroid. Daily requirement is 100-200µ g. In the kidneys 97% of filtered iodine is reabsorbed.

Thyroid concentrates iodine from circulation and transports to colloid. The required transporter pump is located on the plasma membrane which works along with sodium pump. The activity of the pump is stimulated by TSH. The iodine pool in acinar cells exists as exchangeable iodide in blood and unused iodine as iodotyrosine.

Oxidation of iodine is carried out by thyroperoxidase. The enzyme binds iodide to thyroglobulin at specific sites on the molecule.The iodine is added to the 3^{rd} position of aromatic ring in tyrosine .It forms monoiodotyrosine (MIT) then it is iodinated at 5^{th} position to form diiodotyrosine (DIT).

When 2 molecules of DIT undergo oxidative condensation in the presence of thyroperoxidases, T_4 is synthesized. The liberated iodine from thyroglobulin is reutilized. De-iodination converts T_4 to T_3 in other organs than thyroid.

TSH stimulates the synthesis of thyroglobulin and thyroxine.

T_3, T_4:

* 80 % of T_4 is converted to T_3.

* T_3

Here the size of the gland is 2-3 times more than normal. Each follicular cell secretes thyroxine by several fold.

Symptoms:

Patient has protrusion of eye balls; in a condition called exophthalmoses. Eyeballs undergo edematous swelling.

Patient suffers from rapid heart rate, increased BMR, loss of weight, and has marked nervous excitability, increased sensitivity to heat.

Excessive sweating is common.

Hyper glycemia, glucosuria and reduced glucose tolerance due to increased absorption of carbohydrate from intestine. Hyperthyroidism is treated with radioactive isotope like I^{131} or anti thyroid drugs improve the condition of the patient.

Anti thyroid drugs inhibit thyroid function by:

1. Interfering 'iodide trapping'
2. Inhibiting iodination and coupling during synthesis of hormone.
3. Inhibiting hormone release.
4. Inhibiting conversion of T_4 to T_3 in target tissues.

Hypothyroidism: Occurs due to insufficient free T3 or T4, mainly because of thyroid failure. It could be due to diseases of pituitary ,hypothalamus or autoimmunity.

- Patient has decreased BMR.

Body temperature below normal.

Heart rate is decreased, sluggish behaviour.

In children it causes cretinism and in adults it causes myxedema.

Cretinism, is due to failure of growth and mental retardation. Cretinic child has congenital defects like short stature and stunted growth. It can be due to congenital absence of thyroid gland or from lack of iodine in the diet.

Goitre: It means enlarged thyroid gland. When there is iodine deficiency in the diet, patient develops endemic goitre. Iodine deficiency prevents the production of T_3, T_4 but does not stop production of thyroglobulin. So TSH is released in large quantities, which in turn stimulates secretion of thyroglobulin in to colloid of follicular cells. So the gland enlarges 20 times to that of normal.

Symptoms:

Sleeping for long hours .

Muscular sluggishness.

Increased body weight, mental sluggishness, husky voice, scaly skin.

The patient develops nonpitting, edema all over body, in a condition called Myxedema. There is increased level of hyaluronic acid and chondroitin sulfate bound to protein, which forms excessive tissue gel in the interstitial spaces.

Symptoms of this condition are progressive mental retardation, slowing of body processes, weight gain, thinning of hair and swelling of tongue. Endemic goitre is treated with supplementation of diet with iodized salt. Simple goitre (deficiency of Iodine) may be treated with exogenous thyroid hormones.

Catecholamines

Synthesis: Epinephrine is synthesized, stored in adrenal medulla while nor- epinephrine is synthesized in sympathetic nervous system. They act as neurotransmitters. A small concentration is synthesized, stored in adrenal medulla. These two hormones are synthesized in Pheochromomcytes or neuroglial cells, from tyrosine. See the details of synthesis from amino acid chapter.

Metabolic role:

In liver epinephrine stimulates glycogenolysis via c AMP, & increases Calcium levels.

Norepinephrine has no effect on blood glucose, lactic acid levels. Glycogenolysis is increased in muscle by epinephrine.

The same increases cardiac output, & glycogenesis in heart muscle. * Both the hormones promote lipolysis via c AMP.

Epinephrine has inhibitory effect on insulin release.

Both hormones promote metabolic rate through cutaneous vasoconstriction, which decreases heat loss and increases body temperature and muscular activity.

Pheochromocytoma:

It is caused by Chromofin tissue tumors in the adrenal medulla.In this condition both the hormones are increased.

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On examination, his eye balls were bulged, blinking was infrequent. Heart rate was rapid at 140/min, pulse irregular. Thyroid was enlarged by three times to that of normal.

Lab Investigations	**Result**	**Reference Range**
SerumT_3	260µg/100ml	70-180 µg/100ml
SerumT_4	18.1 µg/100ml	5.5-12 µg/100ml
Serum TSH	15.1U/L	5.7U/L
Serum cholesterol	161mg/dl	150-250mg/dl
Plasma Glucose	78mg/dl	<100mg/dl

Pituitary was found normal.

Q. What is the likely diagnosis?

Ans. It is a classical picture of Thyrotoxicosis. It is conformed by very high levels of T_4 .However there is elevated levels of TSH in spite of high thyroxin. The reason could be excessive TSH secretion by pituitary tumor. MRI investigations excluded this possibility. It could be due to loss of feed back sensitivity of pituitary to Thyroid hormones. Thus failure of feed-back inhibition of anterior pituitary by thyroid hormone is the pathological basis of the patient's condition.

Excercises

Q1. Why, an obese diabetic gets benefited by weight reduction?

Ans. Obesity and insulin receptors: Fat people have more of adipocytes. They contain fewer insulin receptors, thus respond poorly to insulin. Obese patients will respond well to restricted diet. Reduction to ideal body weight is the most important aim of nutritional therapy. Weight reduction lead to increased number of receptors per cell.

Q2. Following a normal overnight fast and a cup of black coffee, a diabetic woman feels slightly nauseous and decides to skip breakfast. As per schedule, she decided to lake her insulin shot what is the result of her action?

Ans. Patient of IDDM, improves by insulin treatment. It controls hyper glycemia and glucosuria. Insulin should be given only when blood glucose level can be maintained by dietary or stored glycogen. When blood glucose is low, if insulin is given, severe hypoglycemia might result, further it can lead to insulin shock. Hypoglycemia of 20mg% or less than that deprives brain from glucose. Patient will suffer from convulsions. In the present case, patient has not taken breakfast, insulin shot might result in hypoglycemia.

Ingestion of sugar will prevent serious consequences. If insulin shock occurs, then administration of intravenous glucose will save the patient.

UNIT NINE

MOLECULAR GENETICS

INTRODUCTION

This subject teaches us that the genetic information is stored in DNA or RNA (RNA viruses). And it teaches us the central dogma; that is the flow of genetic information from DNA to DNA from DNA to RNA, and then to protein.

NUCLEIC ACIDs

Nucleic acids are present in nucleus and Mitochondria

They are found in two basic structural forms, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

THE STRUCTURE OF DNA

Deoxyribonucleic acid (DNA) is polymers of deoxyribonucleotides attached to each other by phosphodiester linkages. Each deoxyribonucleotide is composed of deoxyribonucleoside & inorganic phosphate group. Each deoxyribonucleoside is composed of nitrogen bases and a sugar deoxyribose. The nitrogenous bases are purines and pyrimidines.

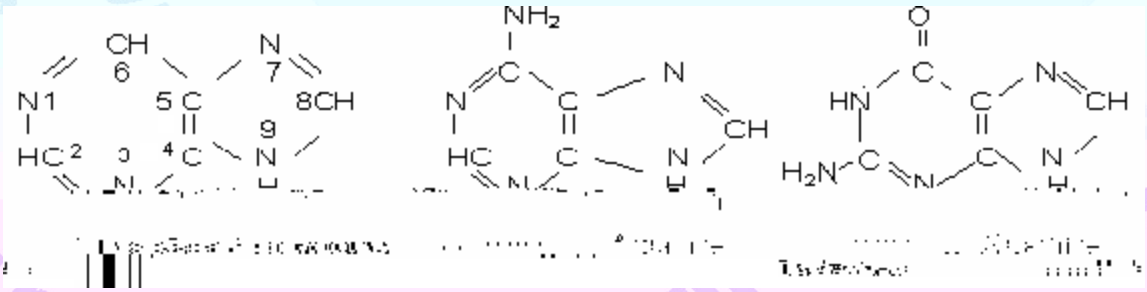


Fig 8.**1** Purine **bases**

The two purine bases are adenine and guanine. They are derived from their parental compound.

The three pyrimidine bases are cytosine, thymine and uracil. It is important to know that thymine is found in DNA and uracil is found in RNA but the other above mentioned bases are found in both DNA and RNA.

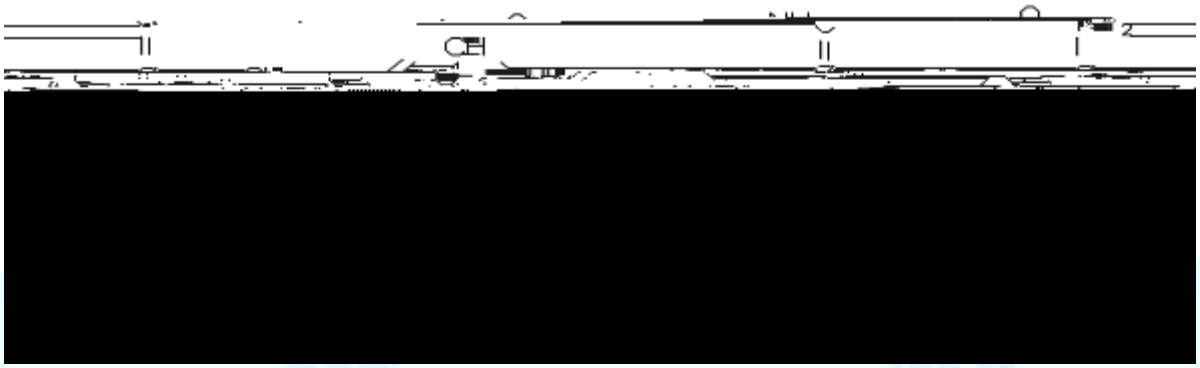


Fig 8.2 Pyrimidine bases

The linkage in purine nucleotide is between 1 of sugar ribose and 9 of purine bases.

The linkage in pyrimidine nucleotide is between 1 of sugar ribose and 1 of pyrimidine bases.

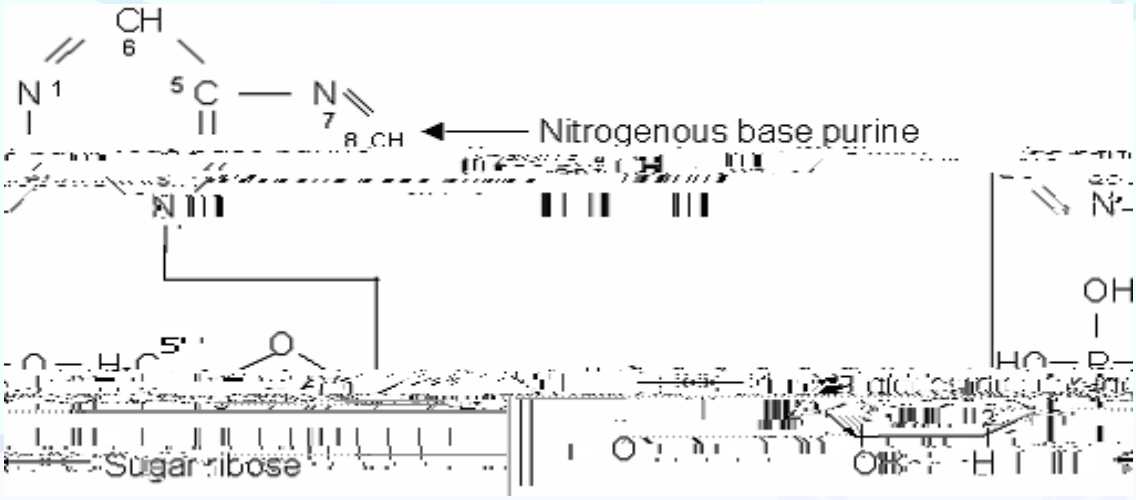


Fig 8.3 Purine deoxyribonucleotide

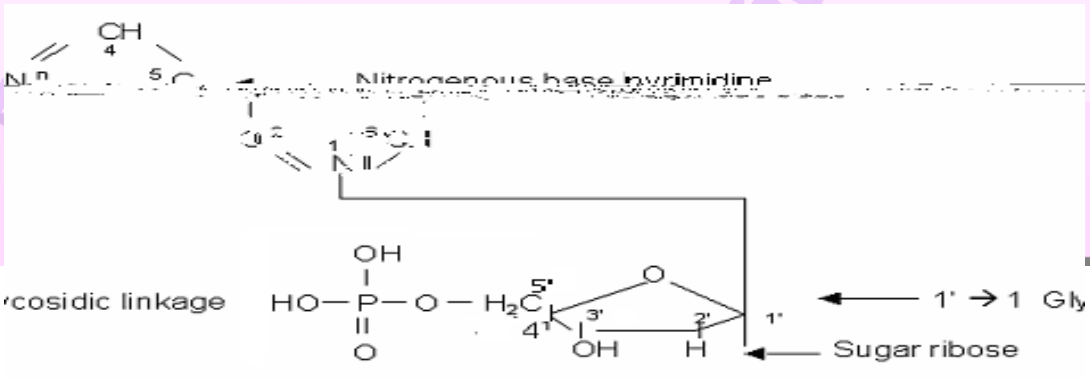


Fig 8.4 Pyrmidine deoxyribonucleotide

N.B. Nitrogenous bases + Pentose = Nucleosides

Nucleoside + Pi = Nuclotides. These are important biomolecules, central to maintenance & propagation of life.

1. ATP, GTP, CTP, UTP, NAD, FAD and CoA are important ribonucleotides which act as coenzymes.
2. Deoxyribonucleotides are required for DNA replication and repair. Ribonucleotides are

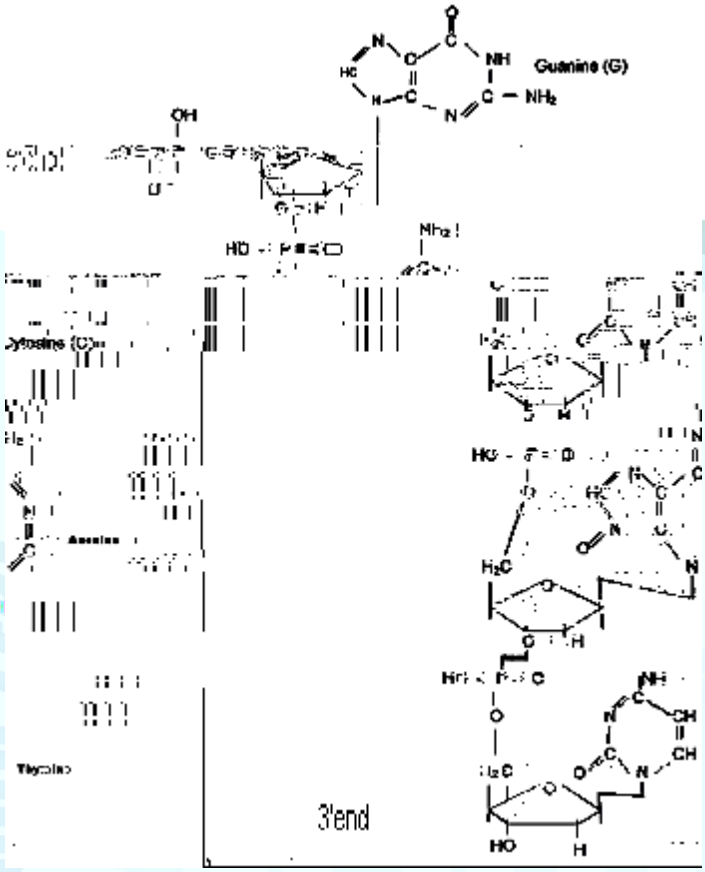


Fig 8.5 primary structure of DNA

1. Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite directions, (anti parallel)
2. The two antiparallel polynucleotide chains are not identical, but they are complimentary.
3. The purine, pyrimidine bases are on the inside of the helix, the phosphate and deoxyribose groups are on the outside. The planes of the sugars are at right angles to that of the bases.
4. The diameter of the helix is 20 A^0,adjascent bases are separated by 3.4 A^0
5. The helical structure repeats after 10 residues on each chain.
6. The two chains are held together by hydrogen bonds between pairs of bases. Adenine is always paired with thymine, Guanine always paired with cytosine. A to T is bonded by two hydrogen bonds (A= T), Guanine is bonded to cytosine by three hydrogen bonds G≡T .
7. The double helix is stabilized by interaction between stacked bases of the same strand.
8. Watson - Crick Model of DNA is also referred as B-DNA, which is the most stable one under physiological conditions.

The mitochondrial DNA is circular and there can be formation of Z-DNA and C-DNA which can be performed during either replication or transcription.

The Structure of RNA

The building unit of RNA is ribonucleotide. Ribonucleotide differs from deoxyribonucleotide in that ribonucleotide contains “O” in the carbon 2’ sugar ribose.

Uracil is found in RNA while Thymine is found in DNA.

The nuclear DNA is in secondary structure, but RNA is the primary structure. Only t-RNA after post transcriptional process can be changed to tertiary structure.

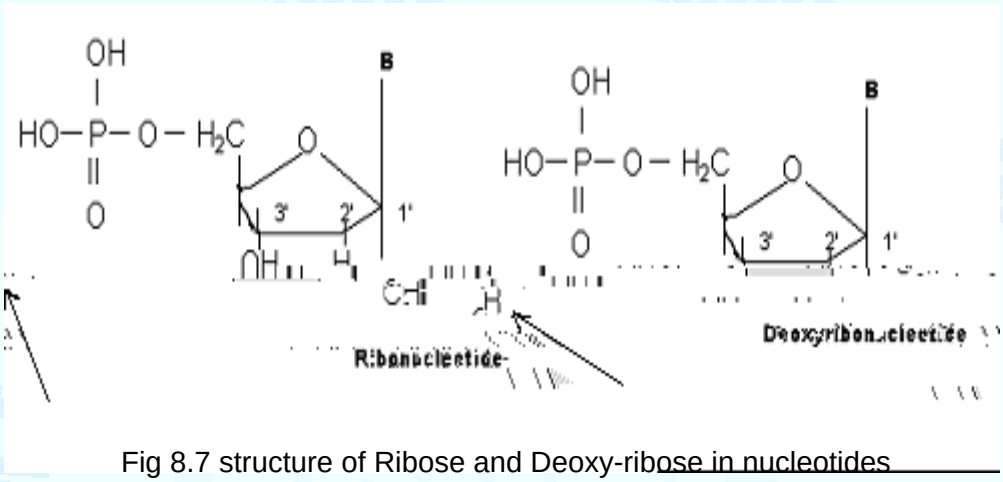


Fig 8.7 structure of Ribose and Deoxy-ribose in nucleotides

Differences between RNA and DNA

Both have adenine, guanine and cytosine. Both have nucleotides linked by phosphodiester bond, in 3’-5’direction. Both have important role in protein synthesis.

	DNA	RNA
1. Uracil	absent	Present
2. Sugar	Deoxyribose	Ribose
3. Site	Nucleus, mitochondria	Nucleus, ribosome, cytosol,

7. DNA can synthesize RNA by transcription		Usually RNA can't form DNA, except by reverse transcriptase.
8. Number of Bases	equal	Not equal
9. Thymine	Present	Absent

The three RNAs that have important role in protein synthesis are:

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA)

Messenger RNA (mRNA)

mRNA in all eukaryotic cells contain cap at the 5' end of the chain. Cap characterizes 7-methylated guanosine tri phosphate. These mRNAs contain poly-A at 3'- end of the chain. Poly-A characterizes about 200 successive adenylate residues. It is illustrated on the given diagram. Poly-A also serves to protect the mRNA form exonuclease attack.

Transfer RNA (tRNA)

All tRNA have 4 arms.

1. Amino acid arm: the one that carries amino acid
2. DHU arm: the one that binds with active center of the enzyme aminoacyl tRNA synthetase.
3. T C arm: the one that binds to ribosome during protein synthesis.
4. Anticodon arm: which pairs with the codon of mRNA during protein synthesis.

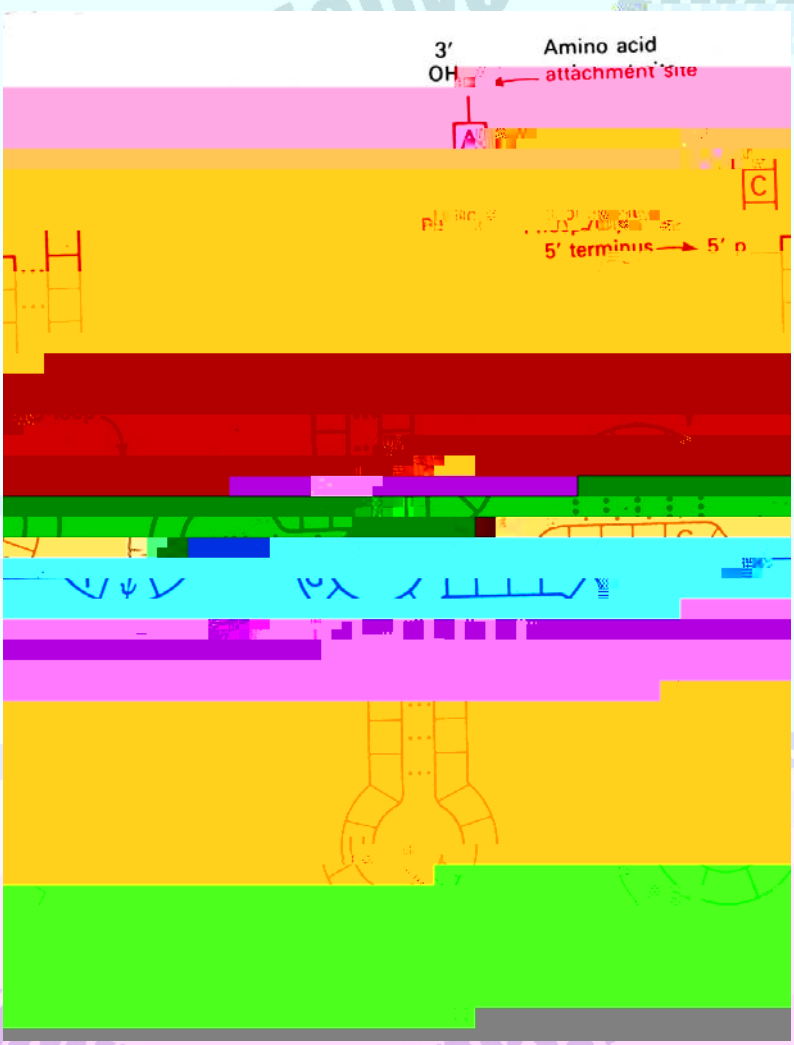
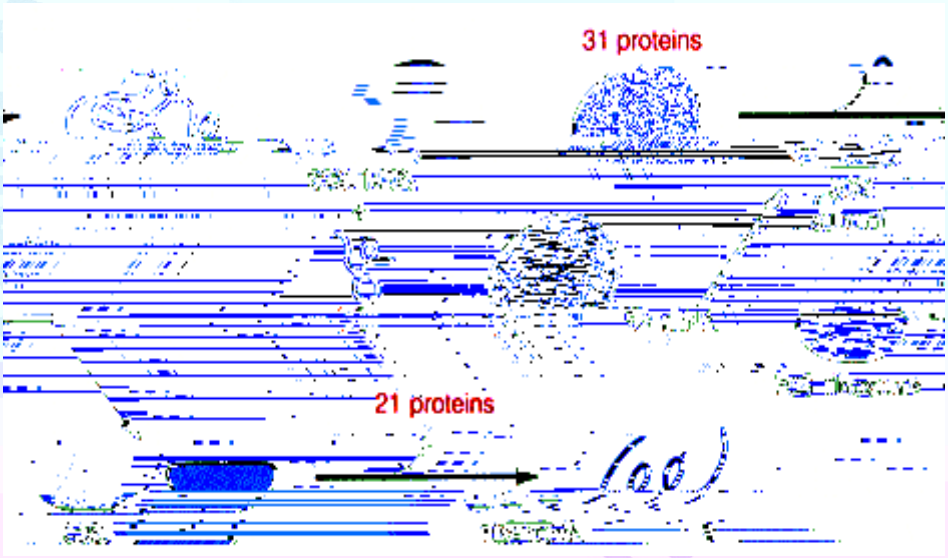


Fig 8.9 Transfer RNA (tRNA)

Ribosomal RNA (rRNA)



Catabolism of Nucleic acids

Pancreatic enzymes called nucleases hydrolyze both DNA and RNA to nucleotides in the intestinal tract. Nucleic acid is also hydrolyzed by lysosomal enzymes inside tissues. Here in the intestine, the nucleotide is also hydrolyzed to nucleoside and phosphoric acid. The nucleoside is absorbed in to blood and transported to peripheral tissues. Excess nitrogen bases are further degraded. Finally adenine and guanine are converted to uric acid in our body which is excreted through urine. Since uric acid has a precipitation character, excess uric acid in kidney causes kidney stone and in joints causes gout.

The degradation of pyrimidine bases are converted to:

1. Cytosine and uracil to ammonia, carbondioxide and beta-alanine
2. Thymine to NH_3, CO_2, H_2O and -methyl β alanine.

DNA REPLICATION

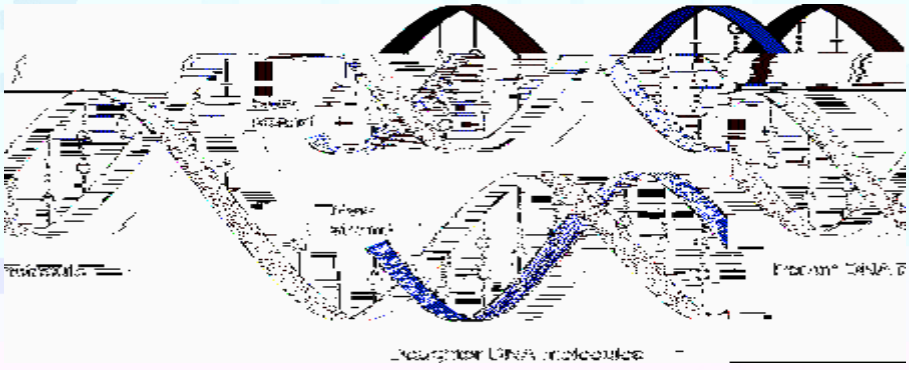
Synthesis of DNA is called replication. DNA replication starts at the early stage of cell division. It is the way in which the genetic information can pass from parental cell to daughter cell. As stated before, the double helical structure of DNA depends on the base complementarity. Also this complementarity represents the fundamental basis for the formation of new DNA strands from the parent DNA strand in a semi conservative manner. In this process, two daughter DNA's are produced, each has one parent strand (conserved) and newly synthesized strand.

Steps of Replication

Origin of DNA - Replication starts at particular DNA sequence called origin. Origin is rich in T-A-T sequence. In prokaryotic cells origin is at one site.In eukaryotic cells origin is at many sites.

To start DNA replication origin is recognized by special protein called DNA a. Gyrase is a protein which recognizes DNA - origin with the help of DNA b protein. The main function of gyrase is to put the negative super twist on double helix of DNA. But the two strands of DNA can be separated by special protein Helicase. Helicase melts the hydrogen bond of the two strands of DNA. To prevent the recoiling back to the double helix single strand binding protein (SSB) plays the role. SSB binds to the single stranded DNA and thus protects the single strand from rejoining.

Primer synthesis: After the two strands of DNA are separated at origin, short complementary



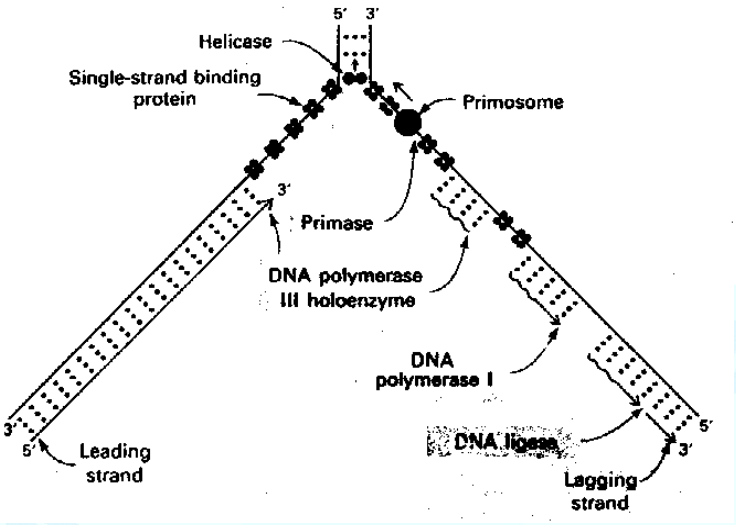


Fig 8.12 Replication fork of DNA

DNA repair

DNA is the only macromolecule that can be repaired, which is important in biological systems. Unless there is a DNA repair there may occur a mutation in DNA.

Fig 8.13 DNA repair mechanism

Certain lesions cause distortions in the helical structure of DNA. It is repaired by ABC exinuclease. It has 3 subunits, coded by Urv A, Urv B, Urv C genes. The type of lesions the system repairs include adducts 6.4-photoproducts and pyrimidines dimers.

The enzyme binds to lesion, makes two nicks on the damaged strand , cleaves at 7^{th} and fourth phophodiester bond .The segment is excised and gap is filled by DNA poly-1 and DNA ligase. Thymine dimers are also repaired the same way.

Urv A protein recognizes lesion, unwinds DNA, causes conformational changes in Urv B .When Urv B binds to the site if lesion, nicks on the 3' side of the lesion. Urv A leaves the site. Now Urv C binds, which makes another nick on 5' side. Then oligonucleotide chain is removed and the gap is filled.

Xeroderma pigmentosum

It is an autosomal recessive condition. It is caused by failure of DNA repair .The pyrimidine dimers due to exposure to UV rays, are not removed. Patient lacks the UV-endonuclease. Symptoms include high sensitivities to UV rays. There are blisters on the skin, hyperpigmentation and finally atrophy of the effected skin. Patient dies of complications like squamous cell carcinoma, skin tumors. Protective ointments like sun blockers from UV rays are beneficial.

RNA Synthesis

Transcription is the process of RNA Synthesis directed by a DNA template. It occurs in three phases:-

1. Initiation
2. Elongation
3. Termination

1. Initiation

Initiation includes promoter. Promoter is a specific sequence in DNA template which is responsible for directing RNA polymerase to initiate transcription at particular point. The left side of that particular point is called upstream. The promoter is found at this upstream. The promotes exceeds about 200 base pairs.

Here is found TATA box which is close to the initiation or starting point.It is within -10 base pairs. About -35 base pairs away is found the consensus base sequences. In between the consensus and TATA box the base sequences are highly variable and it is this sequence that

can be recognized by (sigma) subunit or RNA polymerase in prokaryotic cell and by transcription factor in eukaryotic cell. The RNA polymerase is made up of - (sigma) subunit 2 -and 2 -subunits.

To initiate transcription the sigma subunit of RNA polymerase recognizes the promoter site on DNA and separates the two strands of DNA. After the two strands of DNA are separated the RNA Polymerase with the sigma subunit starts the pairing of purine nucleotide i.e. ATP or GTP to the template of DNA.

2. Elongation

Once RNA – synthesis is started, there occurs the step by step addition of ribonucleotides i.e. ATP, GTP, CTP and UTP at 3' – OH end of RNA. The phosphodiester linkage takes place after P – Pi is removed. The template or the DNA base sequences determine the RNA base sequences. The forward movement of RNA – polymerase continuous until it reaches the termination site.

The area of DNA transcribed by polymerase rewinds back to the double helix.

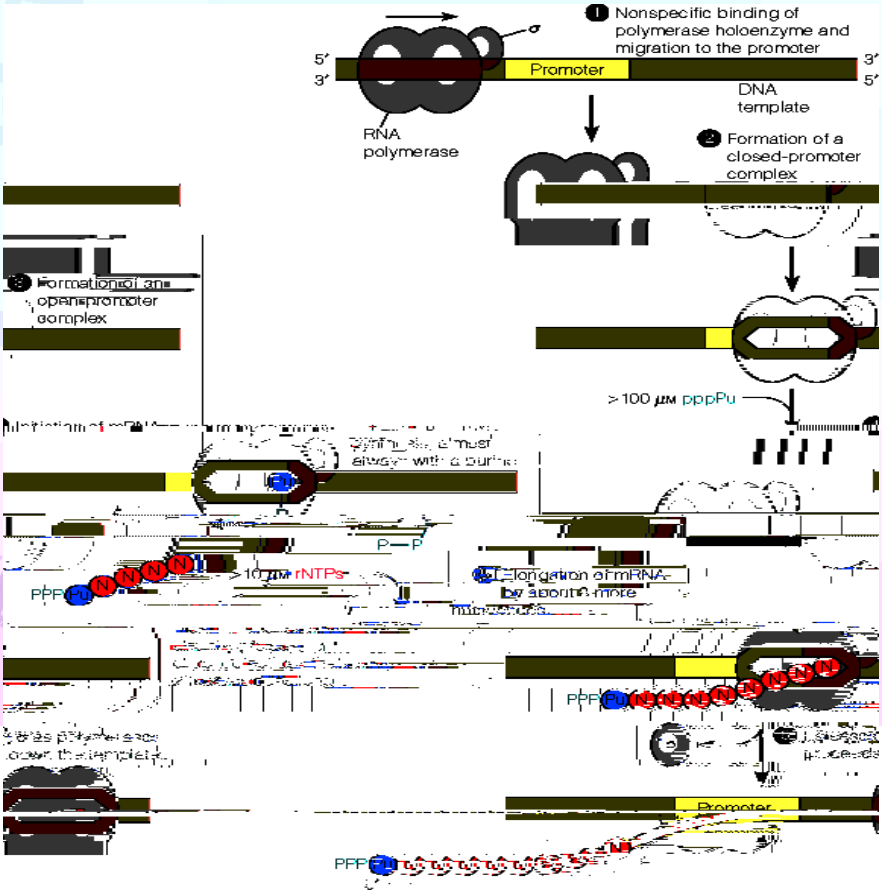


Fig 8.14. Transcription mechanism

3. Termination

After the RNA is synthesized adequately the termination process is carried out by two ways

a. By hair pin like structure of the new synthesized RNA itself. This hair-pin like structure disturbes the RNA – polymerase not to continue its synthesis. This is called rho. Independent termination.

b. A special protein called -(rho-protein) prevents the RNA - polymerase from synthesizing RNA.

These two termination mechanisms are carried out in prokaryotic cells. But in eukaryotic cells termination mapi of the1s5.4(d)(transcrippi off12.2295 But in euf-p)-235

Post - transcriptional process

Nascent RNA undergoes chemical modifications before it participates in translation. They are called post-transcriptional modifications .In prokaryotes, m RNA requires little modification but t-RNA, r-RNA are synthesized as large precursors, do require modifications.

The post-transcriptional changes of rRNA is base modification mainly methylation and the cleavage of larger precursor by some ribonucleases.

The post-transcriptional changes of tRNA includes the cleavage of larger precursor by endonucleases, addition of CCA sequence of 3' - end of the cleaved tRNA, base modification, i.e. addition of H-atoms in DHU (dihydrouracil) arm, methylation and formation of pseudouridylic acid in the T C arm.

The post - transcriptional changes of mRNA includes addition of cap at 5'-end of the chain. This occurs by addition of 7 - methyl Guanine to the 5' end and may be associated by further methylation of the adjacent sugar moiety of the next nucleotides. This capping is important for the proper translation and stability. The post-transcriptional process of mRNA at 3' end is polyadenylation or formation of poly A tails. It is template independent process that occurs after capping but before splicing. It is catalyzed by poly A polymerase enzyme. The change also includes the splicing process. The precursor molecule of mRNA (heterogenous nuclear RNA; hnRNA) is formed of extra sequences.

Some of the sequences are functional called as Exons. The remaining are intervening sequences which are non-functional called Introns.

The removal of interons to form a completely functional mRNA is catalyzed by large ribonucleoprotein complex called spliceosome. It is made of 5 snurps small nuclear RNA (SnRNA) which are important for selecting the perfect alignment. Splicing involves removal of introns and joining of exons,so that mRNA becomes functional.

The above mentioned post transcriptional processing of mRNA is a character of eukaryotes. The prokaryotic mRNA is functional without any further processing.

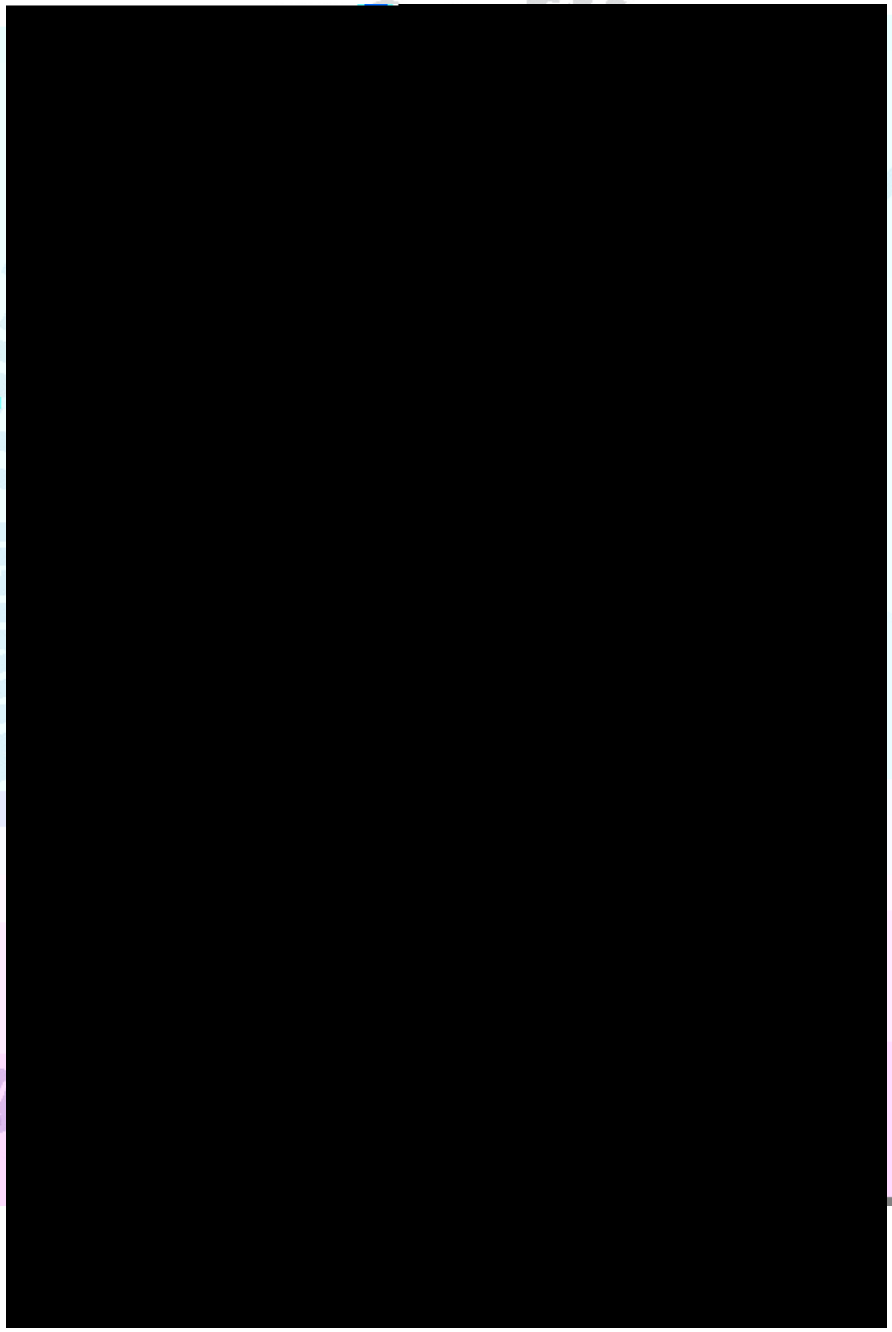
Translation or protein synthesis

The genetic information which flows from DNA to mRNA will be translated to the universal language called protein. During transcription the base sequence of DNA determines the base sequence of mRNA. Under translation the base sequence of mRNA determines the amino acid sequences in protein.

Translation has 4 – stages:

1. Activation
2. Initiation
3. Elongation
4. Termination

1



3. Elongation of protein chain

Under this stage the activated aminoacyl - tRNA enters the A - site with the help of elongation factor (EF-TU) at the expense of one GTP. The process requires 70S initiation complex, aminoacyl t RNA, complimentary to the next codon (A site) on m RNA.

Three soluble proteins or elongation factors, EF-Tu, EF-Ts, EF-G.

Elongation cycle takes place in 3 steps:

a. Aminoacyl t RNA is delivered to A site, by EF-Tu, a molecule of GTP is hydrolyzed. EF-Tu ensures, correct codon –anticodon base pairing. GDP remains attached to EF-Tu. Now EF-Ts binds to EF-Tu-GDP complex. Another GTP binds to EF-Tu and releases GDP. Thus EF-Tu-GTP is ready to start another cycle of elongation.

b. Formation of peptide bond: Both A and P sites on ribosome are occupied. N-formyl methionine from P site is transferred to amino group of aminoacyl t RNA at A site, to form dipeptidyl t RNAThe reaction is catalyzed by peptidyltransferase, located on 23S rRNA of 50 S subunit. At this stage there is an uncharged t RNA at P site and dipeptidyl t RNA at A site.

c. Translocation: Ribosome moves along m RNA by three bases .Codon-anticodon pairing remains uninterrupted during translocation. The step requires GTP binding, elongation factor (EF-G). Dipeptidyl t RNA is present at A site, t RNA from P site is released. Since ribosome has moved by 3 bases, A site is relocated at P site .A newly created A site is waiting for the next aminoacyl t RNA. This way elongation cycle is repeated until termination codon reaches to A site.

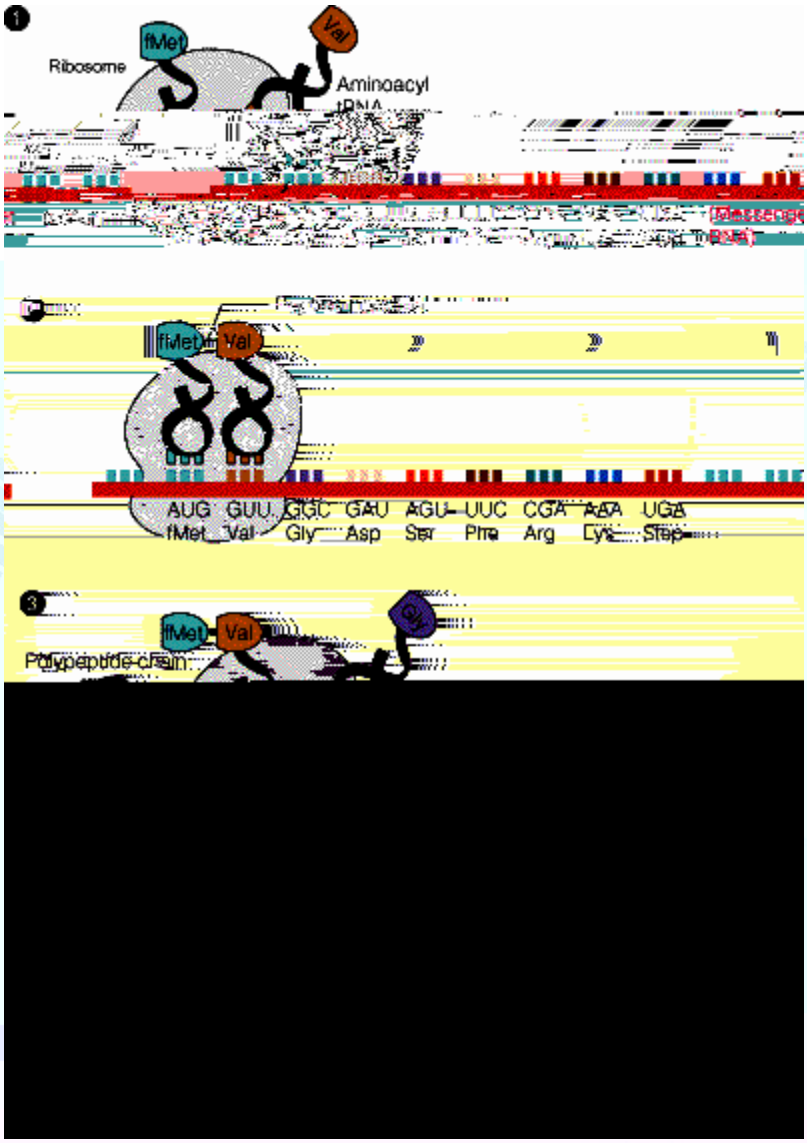


Fig 8.17. Elongation of protein chain

4. Termination stage

The movement of the ribosome consequently reaches to the termination site of mRNA. The termination site contains UAG, or UAA, or UGA. These codons are called stop codons. No tRNA has anticodons that pairs with the stop codons. In addition to that the release factors (RF) bind to stop codons and hydrolyzes the aminoacyl esters liberating the peptide chain or protein,

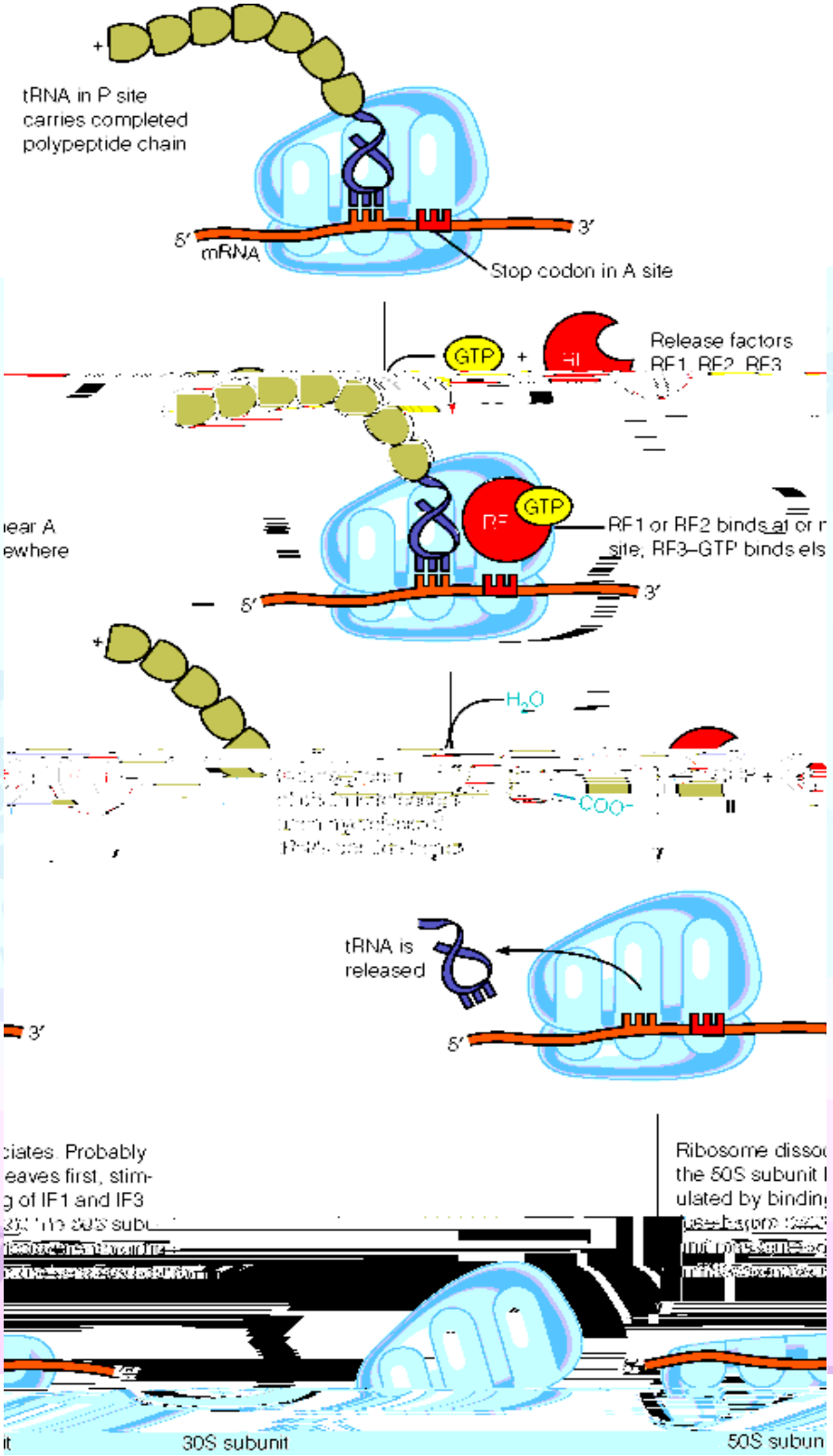


Fig:8.18 Termination of protein synthesis


Ethiopia Public Health Training Initiative
EPHTI

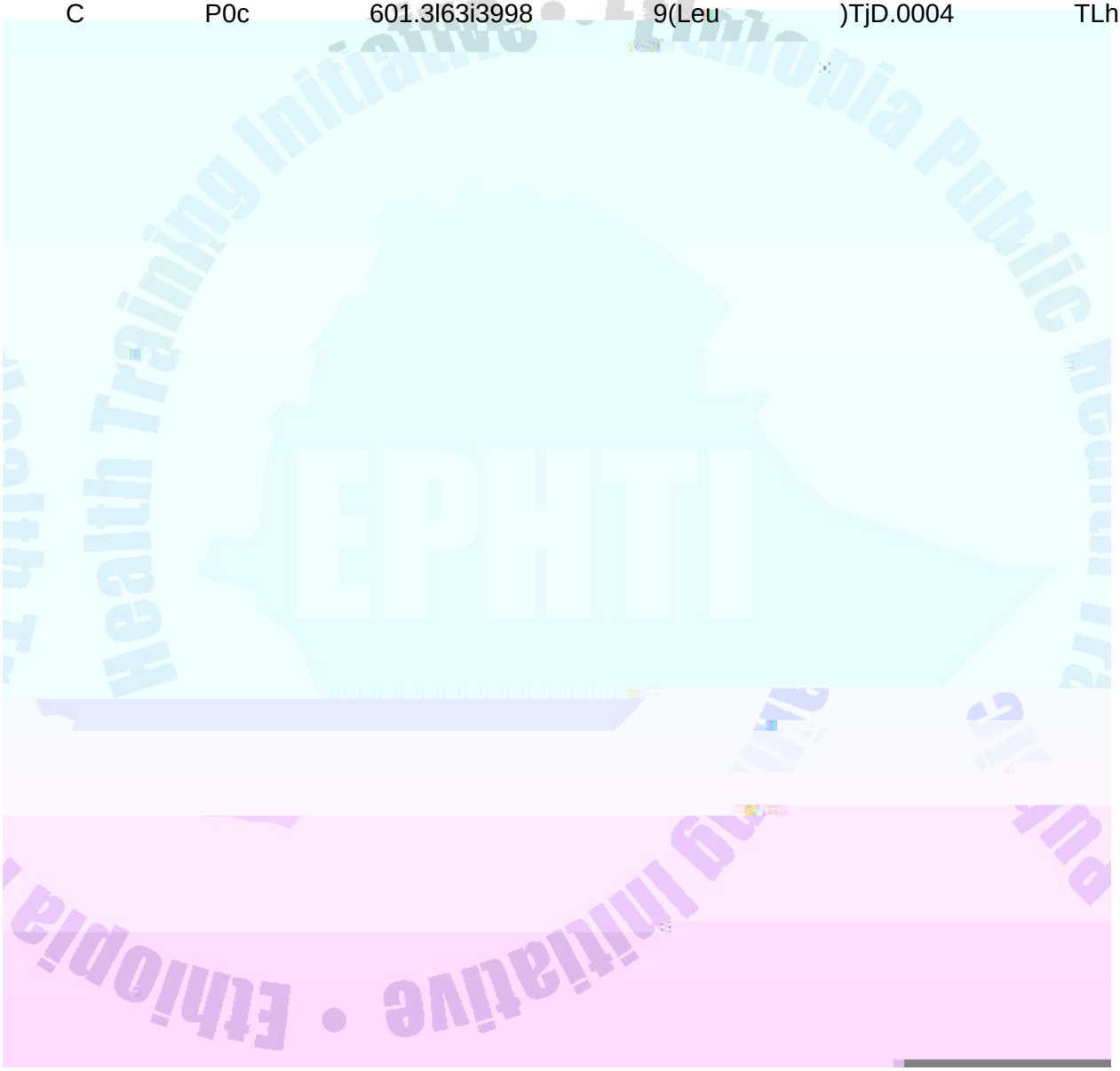
The Genetic Code

Genetic information is coded in form of base sequences. A sequence of 3 bases in m-RNA codes for a single aminoacid .Code is a triplet.

A. Genetic code is degenerate /redundant. A given aminoacid may be coded by more than one codon. Eg. Isoleucine coded by AUU, AUC, AUA. For 20 amino acids there are 61 codons.
B. Genetic code is universal. A specific codon codes for the same aminoacid in any species .Exception is AUG which codes for methionine in eukaryotes, and N-formyl methionine in prokaryotes .In case of mitochondrial DNA, UGA codes for tryptophane, rather than act as stop codon.
C. It is non-overlapping .Successive codons occur one after other .They do not share any common nucleotide.
D. It is comma less. There is no punctuation in genetic code and there are no empty spaces in between two codons.
E. It is collinear. The sequence of amino acids in the polypeptide chain, from the amino terminus to carboxyl end corresponds to the base sequence of a gene (from 5' to 3'end).
F. There are three stop codons, UAA, UAG, UGA to stop protein synthesis .They do not code for any amino acids, they act as releasing factors.
G. AUG is the initiation codon.

B a s e 2

Base 1	U	C	A	G	**Base 3**
U	Phe	Ser	Tyr	Cyst	U
	Phe	Ser	Tyr	Cyst	C
	Leu	Ser	Stop	Stop	A
	leu	ser	stop	trp	G
C	P0c	601.3l63i3998	9(Leu	)TjD.0004	TLhe



SUMMARY

The genetic information is stored in DNA. At the early stage of cell division DNA is replicated,

GLOSSARY

-helix A coiled secondary structure of a polypeptide chain formed by hydrogen bonding between amino acids separated by four residues

Actin An abundant 43-Kd protein that polymerizes to form cytoskeleton filaments

Activation energy The energy required to raise a molecule to its transition state to undergo a

Base excision repair A mechanism of DNA repair in which single damaged bases are removed from a DNA molecule.

Benign tumor A tumor that remains confined to its site of origin.

cAMP phosphodiesterase An enzyme that degrades cyclic AMP.

cAMP-dependent protein kinase see protein kinase A

Carbohydrate A molecule with the formula $(CH_2O)n$. Carbohydrates include both simple sugars and polysaccharides.

Carcinogen A cancer-inducing agent

Carcinoma A cancer of epithelial cells

Cardiolipin A phospholipids containing four hydrocarbon chains

Carrier proteins proteins that selectively bind and transport small molecules across a membrane

Catalase An enzyme that decomposes hydrogen peroxide

Cellulose The principal structural component of the plant cell wall ,a linear polymer of glucose residues linked by β-(1,4)glycosidic bonds

Cellulase An enzyme which degrades cellulose

cGMPphosphodiesterase An enzyme that degrades cGMP.

Chemiosmotic coupling The generation of ATP from energy stored in a proton gradient across a memrane.

Chitin a polymer of N-acetylglucosamine residue that is the principal component of fungal cell walls and exoskeleton of insects.

Cholesterol A sterol consisting of four hydrocarbon rings .cholesterol is a major constituent of animal cell plasma membranes and the precursor of steroid hormones

Chromosomes The carrier of genes, consisting of long DNA molecules and associated proteins.

Codon The basic unit of genetic code; one of the 64 nucleotide triplets that code for an amino acid or stop sequence.

CoenzymeA (CoA) A coenzyme that function as a carrier of acyl groups in metabolic reactions.

Coenzyme Q. A small lipid –soluble molecule that carries electrons between protein complexes in the mitochondrial electron transport chain.

Coenzymes. Low molecular-weight organic molecules that work together with enzymes to catalyze biological reactions

Collagen The major structural protein of the extracellular matrix.

Corticosteroids are steroids produced by the adrenal gland.

Cyclic AMP (cAMP) Adenosine monophosphate in which the phosphate group is covalently bound to both the 3' and 5' carbon atoms, forming a cyclic structure; an important second messenger in the response of cells to a variety of hormones.

Cytochrome oxidase A protein complex in the electron transport chain that accepts electrons from cytochrome c and transfer them to O_2.

Deoxyribonucleic acid (DNA) The genetic material of the cell.

Diacyl glycerol (DAG) A secondary messenger formed from the hydrolysis of PIP_2 that activates protein kinaseC

DNA glycosylase A DNA repair enzyme that cleaves the bond between purine or pyrimidine linked to deoxyribose of DNA

DNA ligase An enzyme that seals the breaks in the DNA strands

DNA polymerase An enzyme catalyzes the synthesis of DNA from deoxy ribonucleotides

Eicosanoids A class of lipids including prostglandins, prostacyclins, thromboxanes and leukotrienes that act in autocrine and paracrine signaling

Electron transport chain A series of carriers through which electrons are transported from a higher to lower energy state.

Endocytosis The uptake of extra cellular material in vesicles formed from the plasma membrane

Endoplasmic reticulum (ER) An excessive network of membrane-enclosed tubules and sacs involved in protein sorting and processing as well as in lipid synthesis

Enhancer transcriptional regulatory sequence that can be located at a site distant from the promoter

Enzymes Proteins or RNAs that catalyze biological reactions

Epidermal cells Cells forming a protective layer on the surface of plants and animals

Epithelial cells Cells forming sheets that cover the surface of the body and line internal organs

Exon A segment of a gene that contains a coding sequence

Exonuclease An enzyme that hydrolyzes DNA molecule at either 5' or 3' direction

Fats See triacyl glycerol

Fatty acids Long hydrocarbon chains usually linked to a carboxylic group

Feed back inhibition A type of Allosteric regulation in which the product of a metabolic pathway inhibits the activity of an enzyme involved in its synthesis

Flavin adenine dinucleotide (FAD) A co enzyme that functions as an electron carrier in oxidation/reduction reactions

G-proteins A family of cell signaling proteins regulated by guanine nucleotide binding.

Genetic code The correspondence between nucleotide triplet and amino acids in proteins

Gibbs free energy (G) The thermodynamic function that combines the effects of enthalpy and entropy to predict the energetically favorable direction of a chemical reaction

Gluconeogenesis The synthesis of glucose from non carbohydrate substrates

Glycerophospholipids Pospholipids consisting of one or two fatty acids bound to a glycerol molecule

Glycogen A polymer of glucose residues that is the principal storage form of carbohydrates in animals

Glycolipid A lipid consisting of carbohydrates

Glycolysis The aerobic breakdown of glucose

Glycoprotein A protein linked to oligosaccharides

Glycosaminoglycans (GAG) A gel forming polysaccharide of the extra cellular matrix

Glycosidic bond A bond formed between sugar residues

Glycosylation The addition of carbohydrate to proteins

Golgi apparatus A cytoplasmic organelle involved in the processing and sorting of proteins and lipids

Guanine a purine that base pairs cytosine

Heat shock proteins A highly conserved group of chaperone proteins expressed in cells exposed to stress.

Helicase an enzyme that catalyzes the unwinding of DNA

High energy bonds Chemical bond that release a large amount of free energy when they are hydrolyzed

Hormones Signaling molecules produced by endocrine glands and tissues

Hydrophilic soluble in water

Hydrophobic not soluble in water

Inositol 1, 4, 5 –tri phosphate (IP_3) A second messenger that signals the release of calcium ions from the endoplasmic reticulum.

Integral membrane proteins proteins embedded within the lipid bilayer of cell membranes

Intracellular signal transduction A chain of reactions that permits chemical signals from the cell surface to their intracellular targets

Intron The non-coding sequence that interrupts exons in a gene

Ion pump A protein that couples ATP hydrolysis to the transport of ions across a membrane

Keratin a type of intermediate protein of epithelial cells

Krebs cycle; see citric acid cycle

Lagging strand The strand of DNA synthesized opposite to the direction of movement of the replication fork by formation of Okazaki fragments

Leading strand The strand of DNA synthesized continuously in the direction of movment of the replication fork

Lipids Hydrophobic molecules, soluble in organic solvents, that function as energy storage molecules, signaling molecules and the major components of cell membranes

Lysosome A cytoplasmic organelle containing enzymes that break down biological polymers

5' methyl guanosine cap A structure consisting of GTP and methylated sugars that is added to the 5' end of eukaryotic mRNA

Mitochondria Cytoplasmic organelle responsible for synthesis of ATP in eukaryotic cells by oxidative Phosphorylation

Monosaccharides simple sugars that cannot be further hydrolyzed

Mutation A genetic alteration

Na^+- K^+ pump An ion pump that transport Na^+ out of the cell and K^+ in to the cell.

Nicotinamide-Adenine dinucleotide (NAD^+) a coenzyme that functions as an electron carrier in oxidation/reduction reactions

Nucleoside A purine or pyrimidine base linked to a sugar(Ribose or deoxy ribose)

Nucleotide A phosphorylated nucleoside

Nuclease The most prominent organelle of eukaryotic cells, contains the genetic material

Oligosaccharide A short polymer of only a few sugars

Oxidative Phosphorylation The synthesis of ATP from ADP coupled to the energetically favorable transfer of electrons to molecular oxygen as the final acceptor in an electron transport chain.

Peptide bond The bond joining amino acids in a polypeptide

Phagocytosis The uptake of large particles such as bacteria by a cell.

Phospholipids The principal components of cell membranes, consisting of two hydrocarbon chains (fatty acids) joined to glycerol and phosphate

Phosphorylation The addition of phosphate group to a molecule

Pinocytosis0 TD.r7-377(pt)5.7(f la)]TJmolecmembrbup toysTT10 1 Tf-5.3279 0 TD29 TD.001 Tc0 Tw1 Tw(Pe

OlighoPenteogl(ner5e6(t).5(c(y)19(an/TTT4 1 Tf 6.5137 623300009 Tc 0 Tw horteente(f link the eg)tionlg ofycharacidro

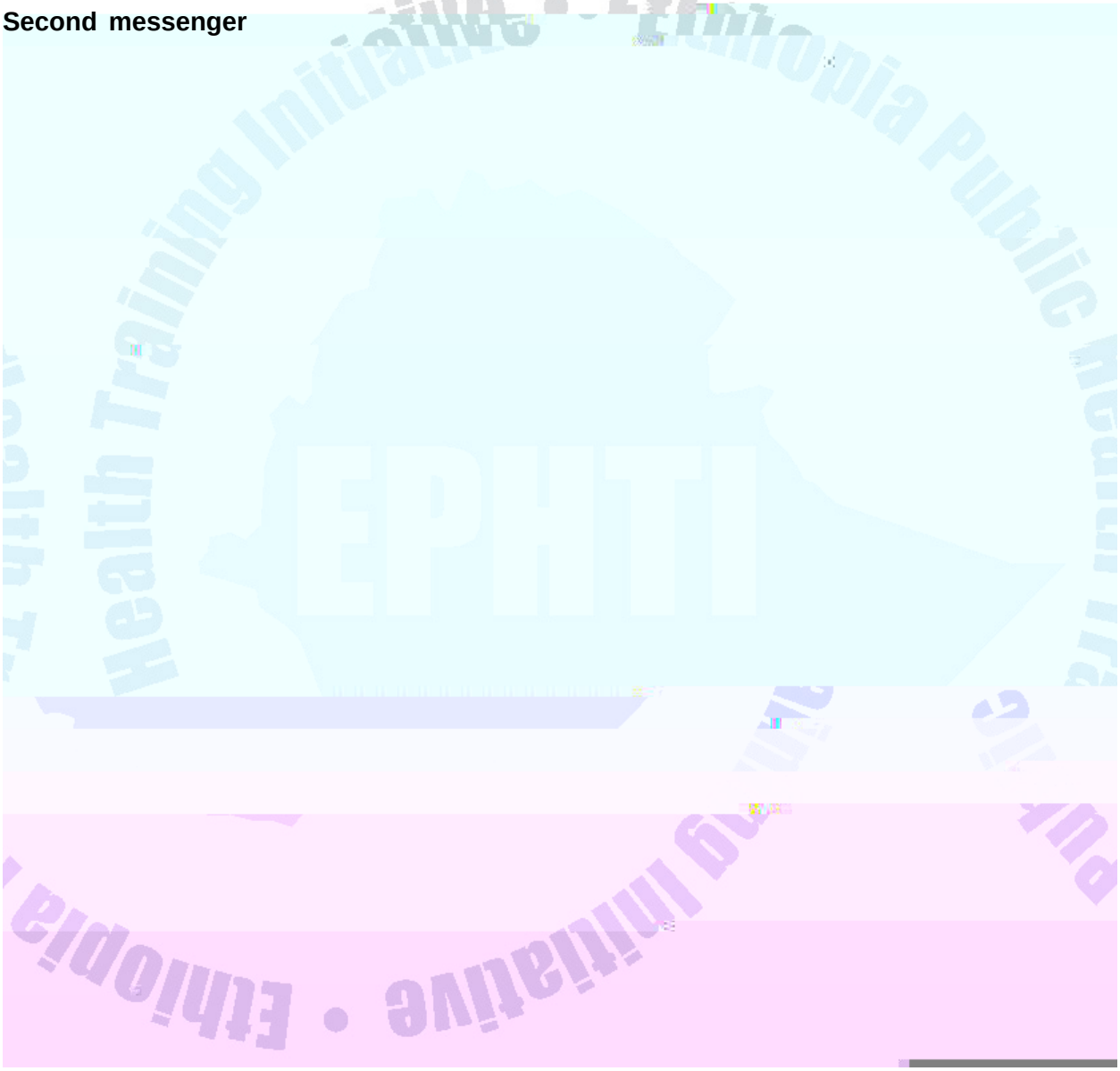
Rhodopsin A G protein- coupled photoreceptor in retinal rod cells that activate transducin in response to light absorption

Ribonucleic acid A polymer of ribonucleotides

rRNA The RNA component of Ribosomes

Ribosomes Particles composed of RNA and protein that are required for protein synthesis

Second messenger



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