BIOCHEMISTRY AND HUMAN NUTRITION

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BIOCHEMISTRY AND HUMAN NUTRITION

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Index

Module 1. Bio-molecules

Lesson 1
AMINO ACIDS

Introduction

An amino acid is a molecule containing both amino and carboxyl functional groups. General formula of Alpha-amino acids is H2NCHRCOOH, where R is an organic substituent. The amino and carboxylate groups are attached to the same carbon atom, which is called the α–carbon. Amino acids are the building blocks of proteins. Due to this central role in biochemistry, amino acids are very important in nutrition. For all animals, some amino acids are essential (an animal cannot produce them internally) and some are non-essential (the animal can produce them from other nitrogen-containing compounds). About twenty amino acids are found in the human body, and about eight of these are essential and, therefore, must be included in the diet (HITFMWLKV). A diet that contains adequate amounts of amino acids (especially those that are essential) is particularly important in some situations: during early development and maturation, pregnancy, lactation, or injury (a burn, for instance). A complete protein source contains all the essential amino acids; an incomplete protein source lacks one or more of the essential amino acids.

1.2 Optical Property

Proteins are made of twenty types of amino acids. Both one- and three-letter abbreviations for each amino acid can be used to represent the amino acids in peptides.

Except glycine, all amino acids have asymmetric (chiral) carbon so they are optically active. Some amino acids are dextrorotatory and some levorotatory depending upon the rotation of plane polarized light towards right or left direction respectively. L-amino acids represent the vast majority of amino acids found in proteins. D-amino acids are found in some proteins produced by exotic sea-dwelling organisms, components of the peptidoglycan cell walls of bacteria. The L and D convention for amino acid configuration refers not to the optical activity of the amino acid itself, but rather to the optical activity of the isomer of glyceraldehyde from which that amino acid can theoretically be synthesized (D-glyceraldehyde is dextrorotary; L-glyceraldehyde is levorotary).

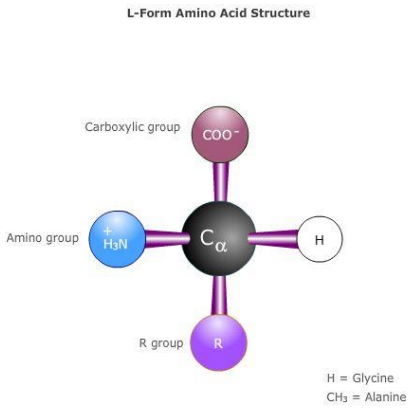

Fig 1.1 General structure of amino acid

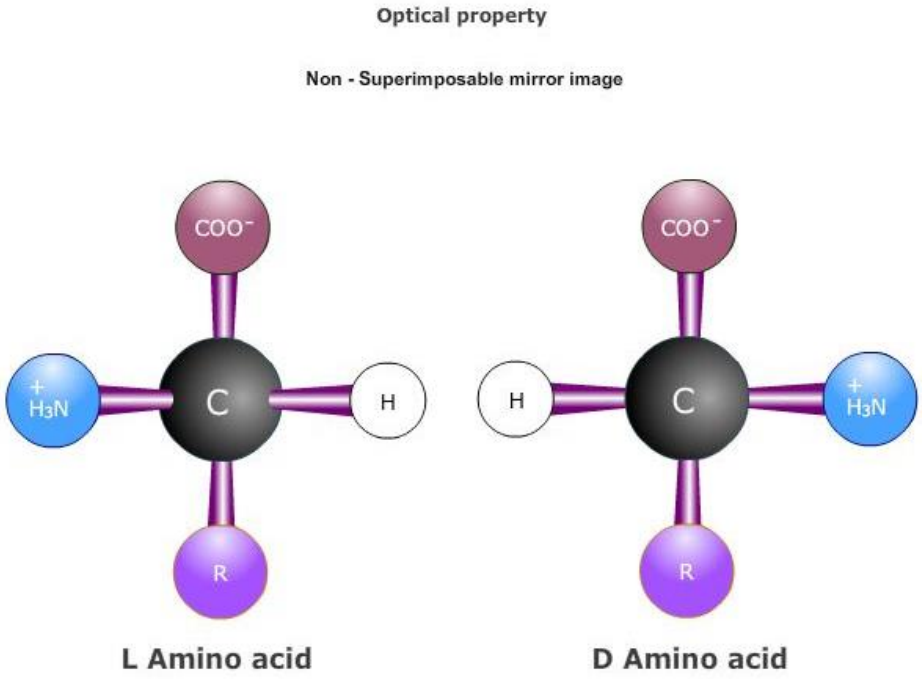

Fig 1.2 Non superimposable Mirror images of Amino Acids

1.3 Zwitterions

At a certain pH known as the isoelectric point, the number of protonated ammonium groups having positive charge and deprotonated carboxylate groups having negative charge are equal, resulting in a net neutral charge. These ions are known as a zwitterion. Thus zwitterion act as base (proton acceptor) as well as acid (proton donor).

$$\mathrm{pI} = \frac{1}{2}(\mathrm{p}K_1 + \mathrm{p}K_2) = \frac{1}{2}(2.34 + 9.60) = 5.97$$

For glycine, which has no ionizable group in its side chain, the isoelectric point is simply the arithmetic mean of the two pKa values:

Thus, glycine has a net negative charge at any pH above its pI and will thus move toward the positive electrode (the anode) when placed in an electric field. At any pH below its pI, glycine has a net positive charge and will move toward the negative electrode (the cathode).

1.4 Classification of Amino acids

Amino acids are classified as basic, acidic, aromatic, aliphatic, or sulfur-containing based on the properties of their R groups.

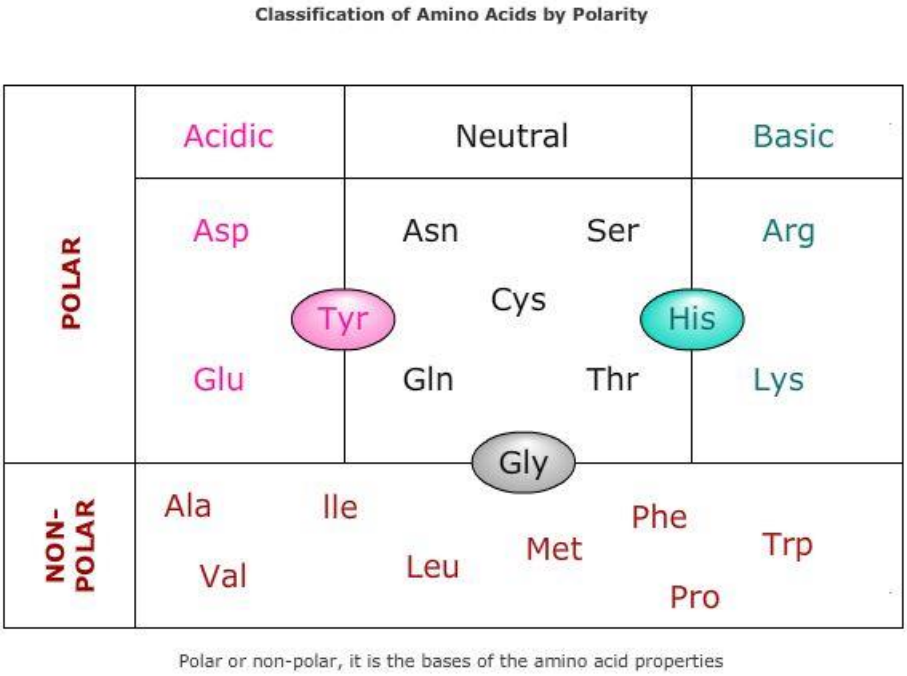

Fig 1.3 Classification of amino acid by polarity

1.4.1 Amino acids with aliphatic side chains

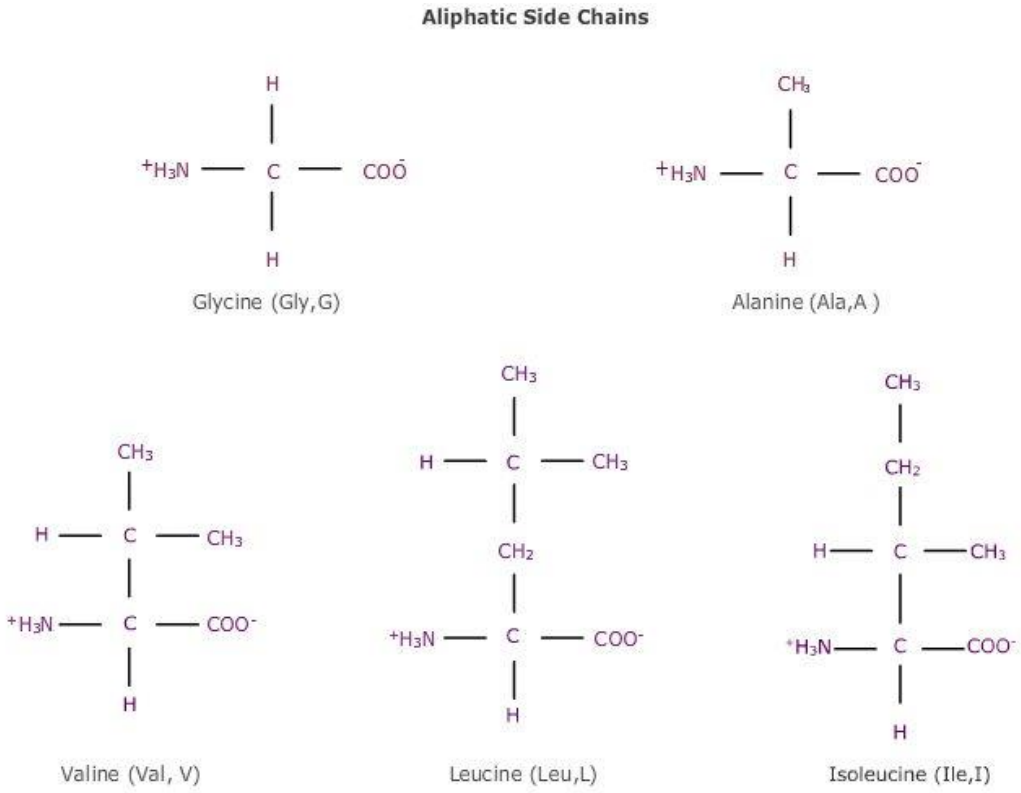

Fig 1.4 Amino acids with aliphatic side chains

1.4.2 Amino acids side chains with sulfur atoms

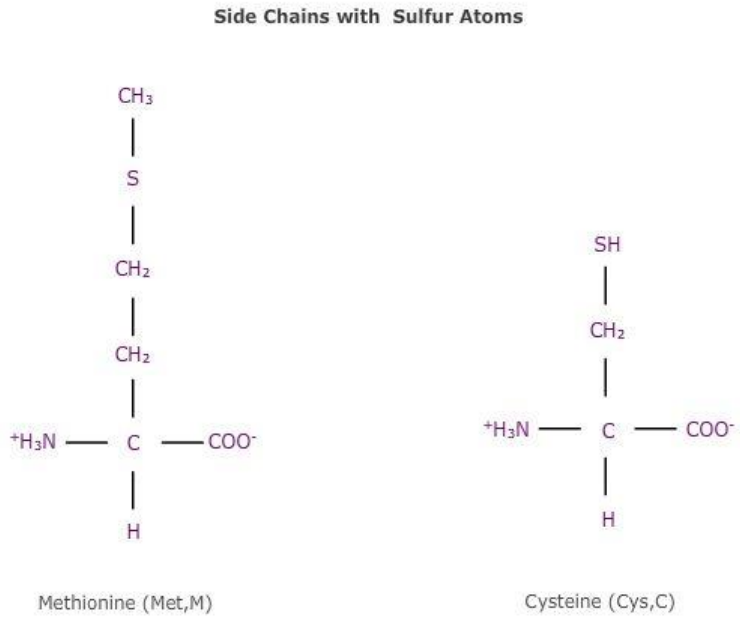

Fig 1.5 Amino acids side chains with sulfur atoms

1.4.3 Amino acids side chains with hydroxylic (oh) groups

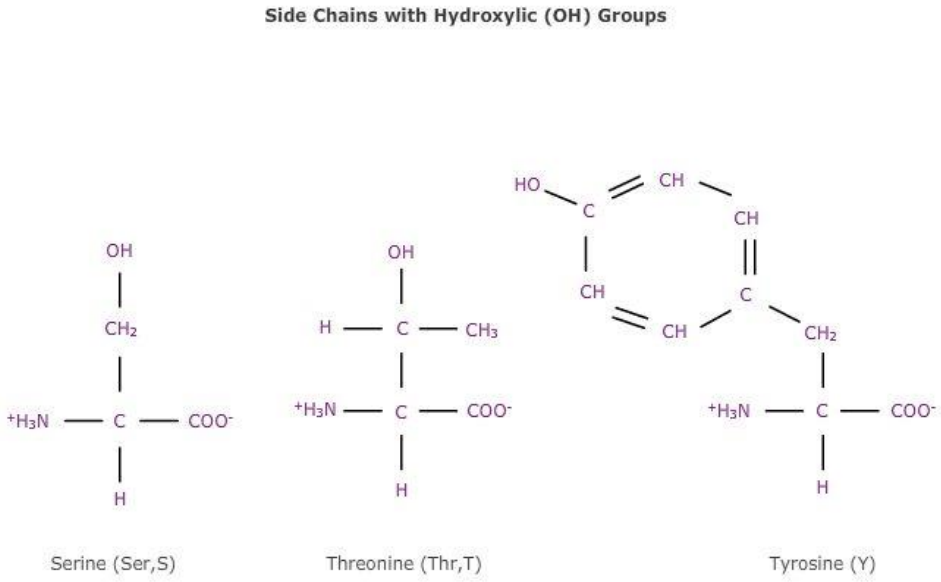

Fig 1.6 Amino acids side chains with hydroxylic (OH) groups

1.4.4 Amino acids with aromatic rings

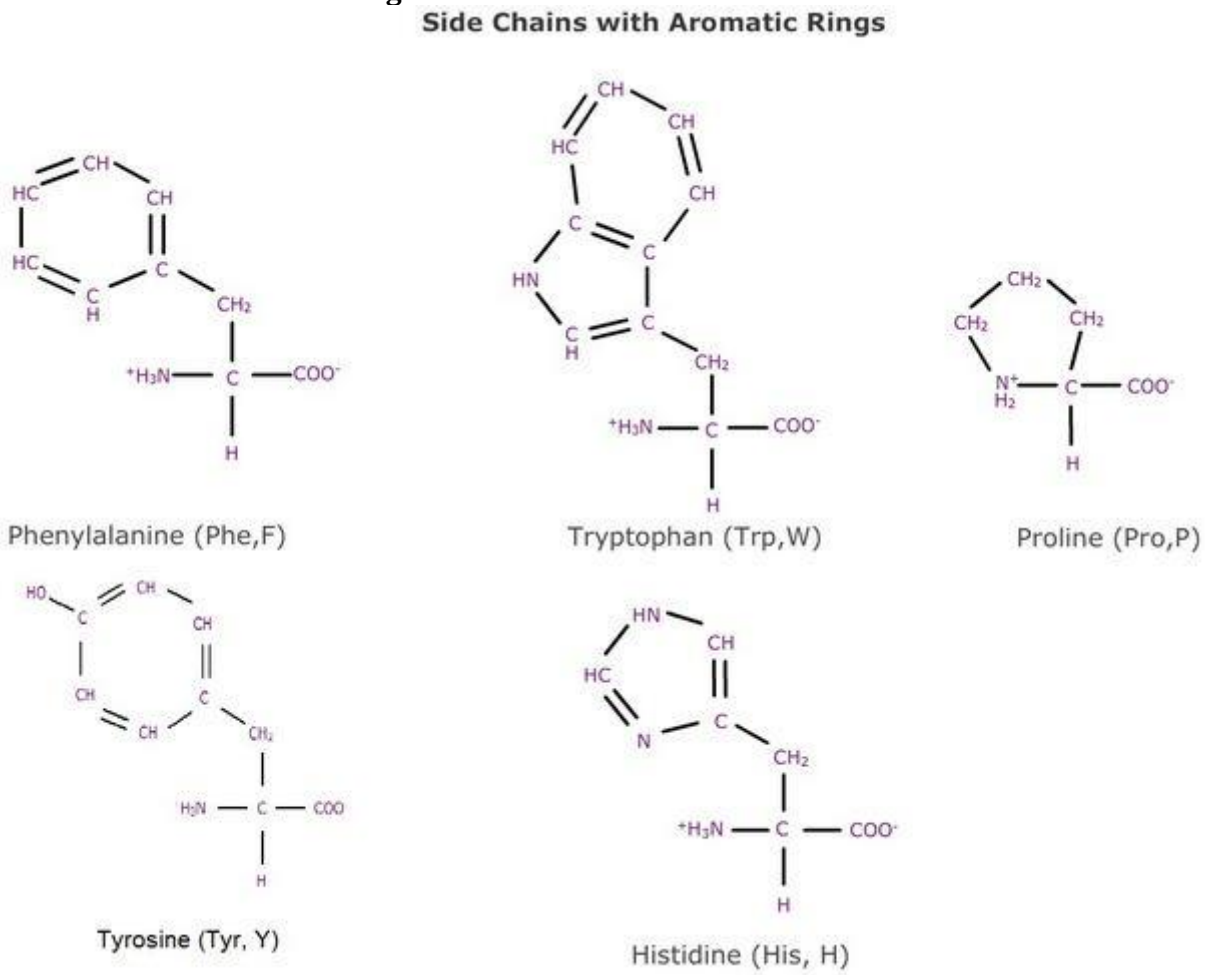

Fig 1.7 Amino acid with Aromatic Rings

1.4.5 Amino acid side chain with basic group

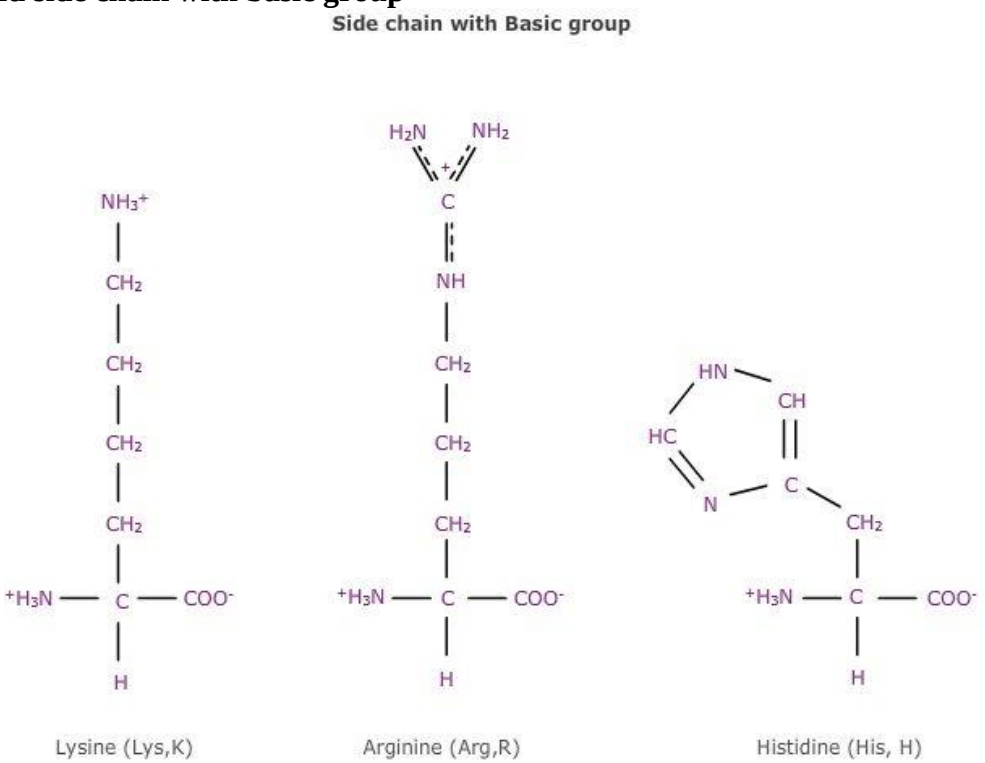

Fig 1.8 Amino acid side chain with basic group

1.4.6 Amino acids side chains with acidic groups or their amides

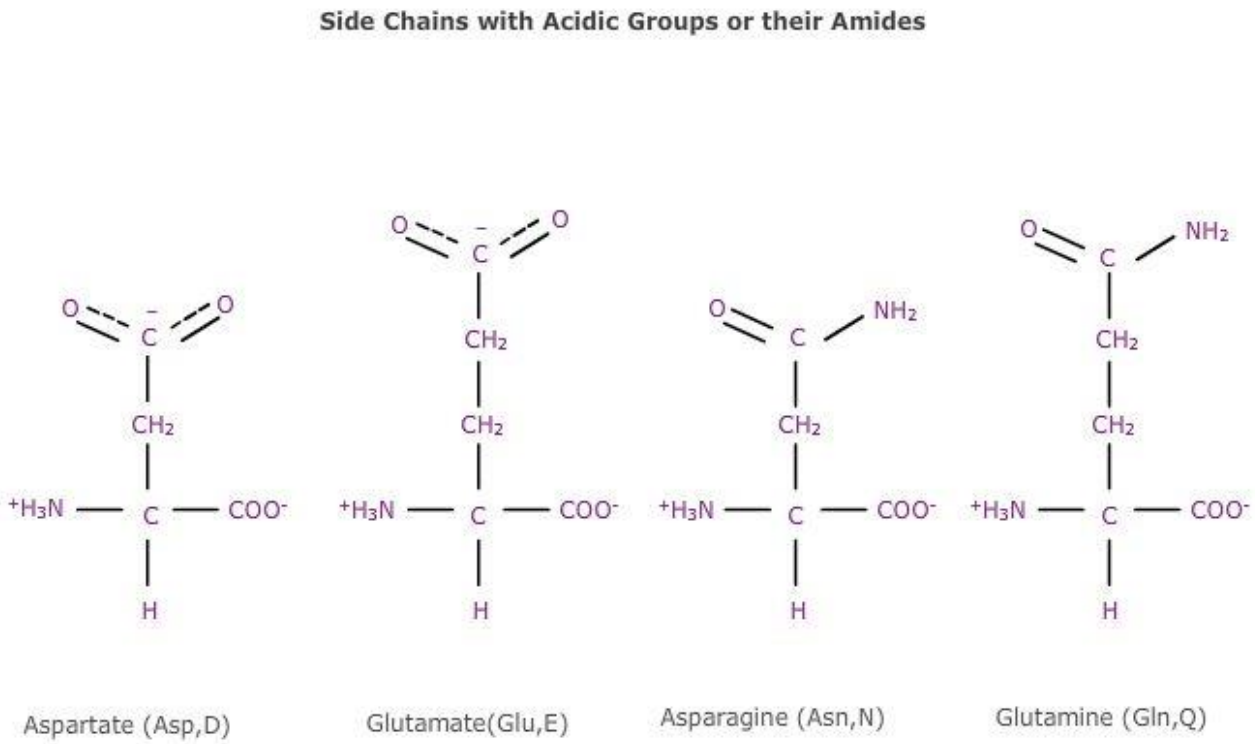

Fig 1.9 Amino acids side chains with acidic groups or their amides

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Lesson 2
PROTEIN STRUCTURE

2.1 Introduction

Proteins are linear sequences of amino acids linked together by peptide bonds. The amino acids are linked head to tail.

- The peptide bond is a covalent bond formed between the α-carboxyl group of one amino acid and the α-amino group of another. Once two amino acids are joined together via a peptide bond to form a dipeptide, there is still a free amino group at one end and a free carboxyl group at the other, each of which can in turn be linked to further amino acids.
- A long, unbranched chain of amino acids (upto 25 amino acid residues), linked together by peptide bonds is called oligopeptide.
- A peptide chain having >25 amino acids residues is called polypeptide.
- Peptide chains are written down with free α-amino (N-terminal) on the left, the free α-carboxyl group (C-terminal) on the right and a hyphen between the amino acids to indicate peptide bonds. Example of a tetrapeptide $^{+}H_3N$-serine- tyrosine-phenylalanine-leucine-COO^{-} would be written simply Ser-Tyr-Phe-Leu or S-Y-F-L.

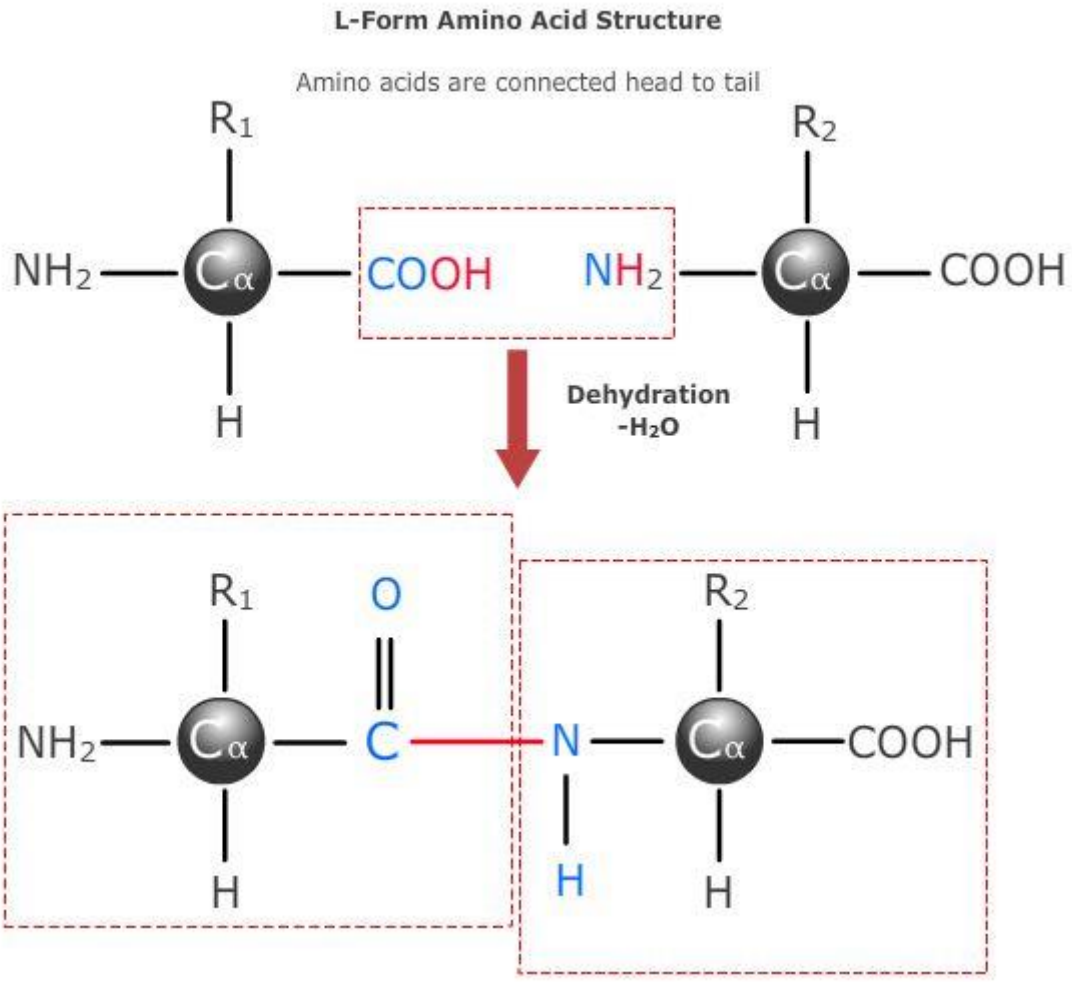

Fig. 2.1 Formation of peptide bond by dehydration

2.2 Peptide Bond

- The peptide bond between carbon and nitrogen exhibits partial double bond character due to closeness of carbonyl carbon –oxygen double bond and electron withdrawing property of oxygen and nitrogen atoms allowing the resonance structures.
- C-N bond length is also shorter than normal C-N single bond and >C=O bond is larger than normal >C=O bond. The peptide unit which is made up of CO-NH atoms is thus relatively rigid and planner, although free rotation takes place about Cα-N and Cα-C bonds (the bonds either side of the peptide bond), permitting adjacent peptide units to be at different angles.
- The H of the amino group is nearly always Trans (opposite) to oxygen of carbonyl group, rather than cis (adjacent).

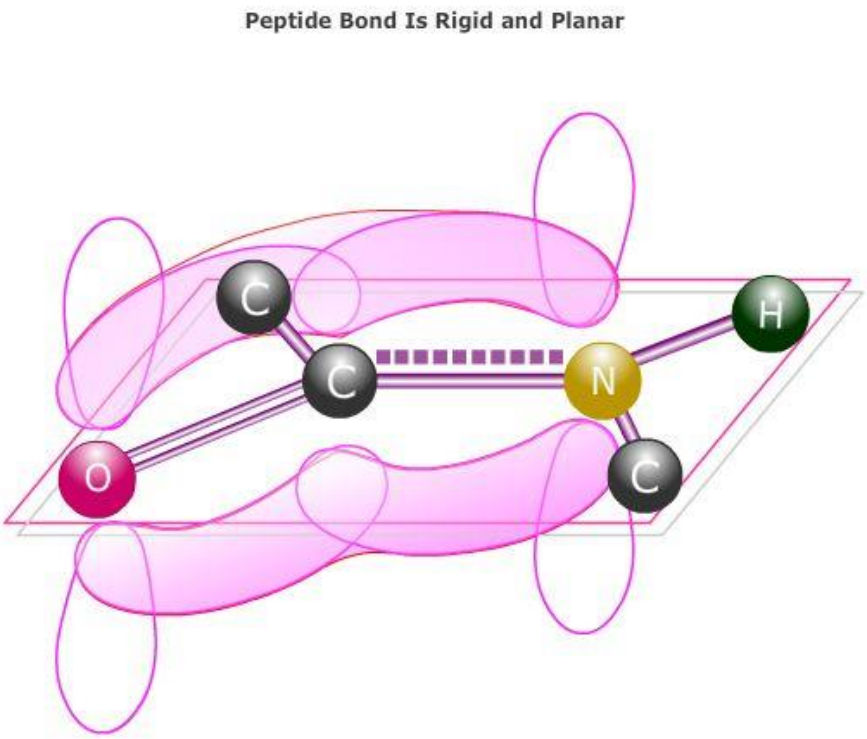

Fig. 2.2 Peptide bond

2.3 Protein Structure

The peptide chain folds up in the protein to form a specific shape (conformation). The conformation is the three dimensional arrangement of atoms in structure and is determined by the amino acid sequence. There are four levels of structure in proteins : primary, secondary, tertiary and, sometimes not always quaternary.

2.3.1 Primary structure

- The primary structure in a protein is the linear sequence of amino acids as joined together by peptide bonds.
- This also include disulfide bonds between cysteine residues that are adjacent in space but not in the linear amino acid sequence. These covalent cross-links are formed by the oxidation of SH groups on cysteine residues that are juxtaposed in space between separate polypeptide chains or between different parts of same chain. The resulting disulfide is called a cystine residues.
- Disulfide bonds are often present in extracellular proteins, but are rarely found in intracellular proteins.

2.3.2 Secondary structure

The secondary level of structure in a protein is the regular folding of regions of the polypeptide chain. The most common types of protein fold are the α-helix and the β-

2.3.2.1 Pleated sheet

In α-helix, the amino acids arrange themselves in a regular helical conformation in a rod shape. The carbonyl oxygen of each peptide bond is hydrogen bonded to the hydrogen on the amino group of the fourth amino acid away. In an α-helix there are 3.6 amino acids per turn of the helix covering a distance of 0.54 nm and each amino acid residue represents an advance of 0.15 nm along the axis of the helix. The side chain of the amino acids are all positioned along the outside of the cylindrical helix. Certain amino acids are often found in α-helix than others. In particular, Pro is rarely found in α-helical regions as it cannot form the correct pattern of hydrogen bonds due to lack of a hydrogen atom on its nitrogen atom. For this reason, Pro is often found at the end of an α-helix where it alters the direction of polypeptide chain and terminates the helix.

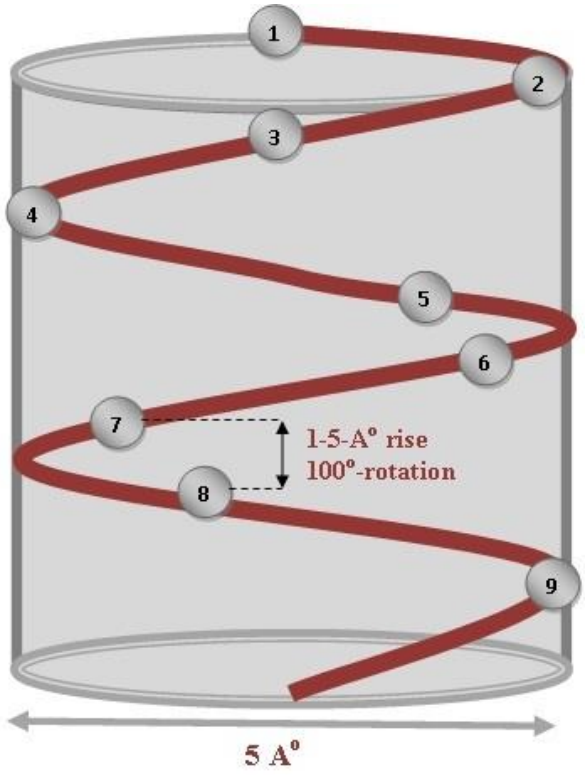

Fig. 2.3 Secondary Structure of Protein with α-helix

In the β-pleated sheet hydrogen bonds form between the peptide bonds either in different polypeptide chains or in different sections of the same polypeptide chain. The planarity of the peptide bond forces the polypeptide to be pleated with the side chains of the amino acids protruding above and below the sheet. Adjacent polypeptide chains in β-pleated sheet can be either parallel or antiparallel depending on whether they run in the same direction or in the opposite directions, respectively. The polypeptide chain within a β-pleated sheet is fully extended, such that there is a distance of 0.35nm from Cα atom to next. β-pleated sheets are always slightly curved and, if several polypeptides are involved, the sheet can close up to form a β-barrel. Multiple β-pleated sheets provide

strength and rigidity in many structural proteins, such as silk fibroin, which consists almost entirely of stacks of antiparallel β-pleated sheets.

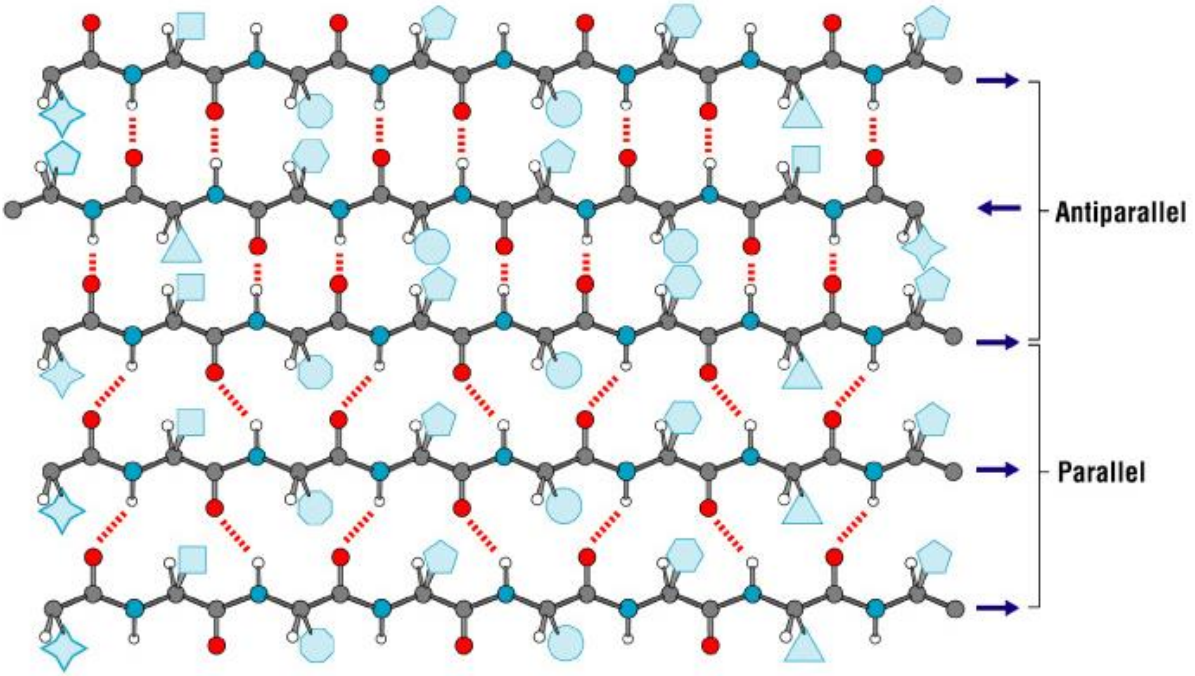

Fig. 2.4 Secondary Structure of Protein with β-sheet

2.3.3 Tertiary structure

The tertiary structure means the spatial arrangement of amino acids that are far apart in the linear sequence as well as those residues that are adjacent. The term "tertiary structure" refers to the entire three dimensional conformation of a polypeptide. It indicates, in three-dimensional space, how secondary structural features—helices, sheets, bends, turns, and loops— assemble to form domains and how these domains relate spatially to one another. A domain is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate or other ligand. For example in myoglobin, a globular protein, the polypeptide chain folds spontaneously so that the majority of its hydrophobic side chains are buried in the interior, and the majority of its polar , charged side chains are on the surface. Once folded , the three dimensional , biologically active (native) conformation of protein is maintained not only by hydrophobic interactions, but also by electrostatic forces (including salt bridges, Vander Waals interactions), hydrogen bonding and if present the covalent disulfide bonds.

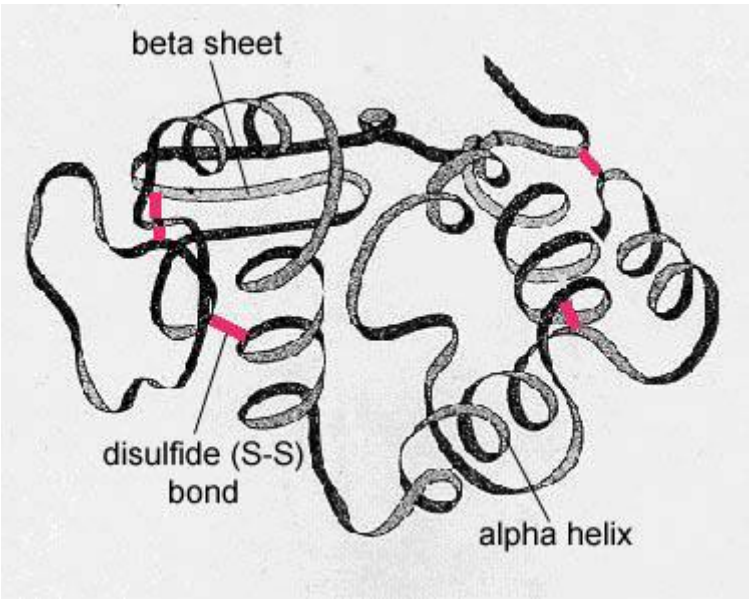

Fig 2.5 Tertiary Structure of Protein

2.3.4 Quaternary structure

Proteins containing more than one polypeptide chains, such as haemoglobin exhibit a fourth level of protein structure called quaternary structure. This level of structure refers to the spatial arrangement of the polypeptide subunits and the nature of the interactions between them. These interactions may be covalent links or noncovalent interactions (electrostatic forces, hydrophobic interactions and hydrogen bonding.

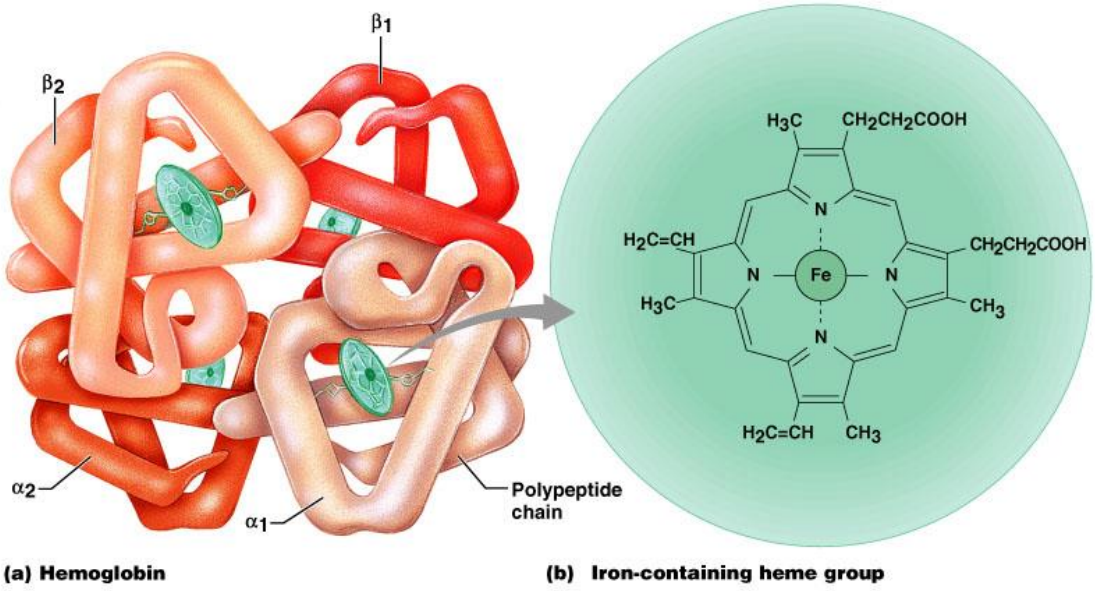

Fig 2.6 Quaternary structure of Protein

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Lesson 3
CARBOHYDRATES

3.1 Introduction

- Carbohydrates are polyhydroxy aldehydes or ketones
- Classified into three categories

1. Monosaccharides- a single polyhydroxy aldehyde or ketone unit cannot be hydrolysed further into monomers. Example – Glucose (Dextrose),
2. Oligosaccharides- made up of 2-6 monosaccharides linked together by glycosidic bonds. Example - Sucrose
3. Polysaccharides – made up of more than six monosaccharides Example- Starch, Glycogen, Cellulose

3.2 Major Functions

- They form major organic matter on earth because of their extensive roles in all forms of life.
- They serve as energy stores (starch and glycogen), fuels, and metabolic intermediates.
- Ribose and deoxyribose sugars are component of RNA and DNA
- Polysaccharides are structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to many proteins and lipids, where they play key roles in mediating interactions among cells and interactions between cells and other elements in the cellular environment.

3.3 Structure of Important Carbohydrates

3.3.1 Monosaccharides

- Monosaccharides with three, four, five, six, and seven carbon atoms in their backbones are called, triose tetroses, pentoses, hexoses, and heptoses respectively.
- The carbons of a sugar are numbered beginning at the end of the chain nearest the carbonyl group.
- All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms

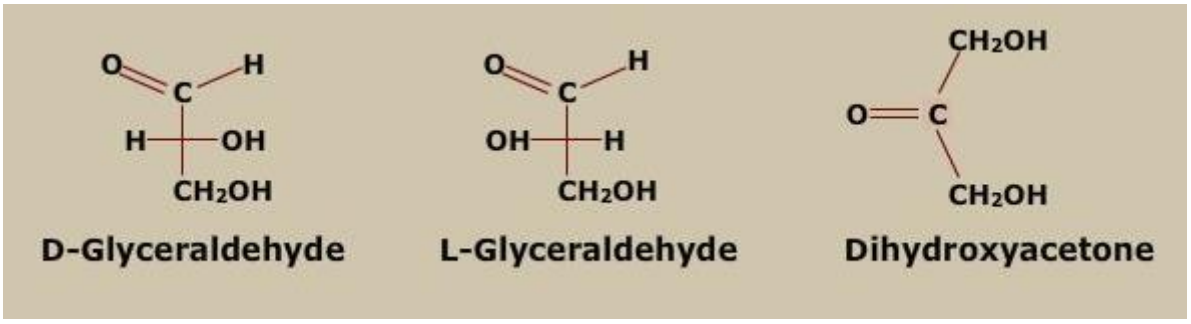


Fig 3.1 Basic Structure of Monosaccharides

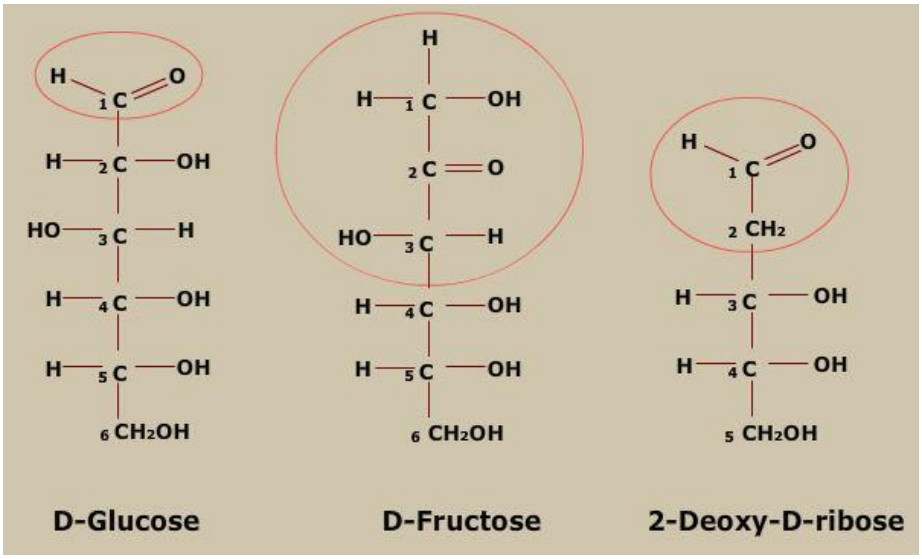


Fig. 3.2 Representative Monosaccharides

- In aqueous solution, aldotetroses and all monosaccharides with five or more carbon atoms occur predominantly as cyclic (ring) structures in which the carbonyl group has formed a covalent bond with the oxygen of a hydroxyl group along the chain.
- The formation of these ring structures is the result of a general reaction between alcohols and aldehydes or ketones to form derivatives called hemiacetals or hemiketals which contain an additional asymmetric carbon atom and thus can exist in two stereoisomeric forms. For example, D-glucose exists in solution as an intramolecular hemiacetal in which the free hydroxyl group at C-5 has reacted with the aldehydic C-1, rendering the latter carbon asymmetric and producing two stereoisomers, designated α and β.
- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called anomers. The hemiacetal (or carbonyl) carbon atom is called the anomeric carbon. The α and β anomers of D-glucose interconvert in aqueous solution by a process called mutarotation.

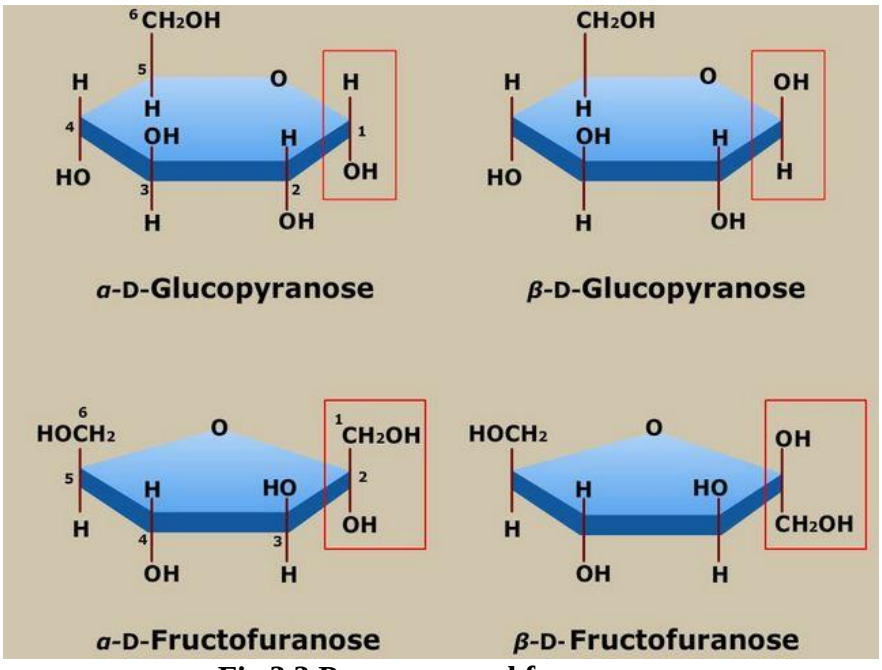


Fig 3.3 Pyranoses and furanoses

- Monosaccharides can be oxidized by relatively mild oxidizing agents such as ferric (Fe^{3+}) or cupric (Cu^{2+}) ion. The carbonyl carbon is oxidized to a carboxyl group. Glucose and other sugars capable of reducing ferric or cupric ion are called reducing sugars. This property is the basis of Fehling's reaction, a qualitative test for the presence of reducing sugar. By measuring the amount of oxidizing agent reduced by a solution of a sugar, it is also possible to estimate the concentration of that sugar.
- Two sugars that differ only in the configuration around one carbon atom are called epimers. D-Mannose differs from D-glucose only in its configuration around carbon 2. D-Galactose differs from D-glucose only in its configuration around carbon 4 . D-Galactose and D-Mannose are not epimers.

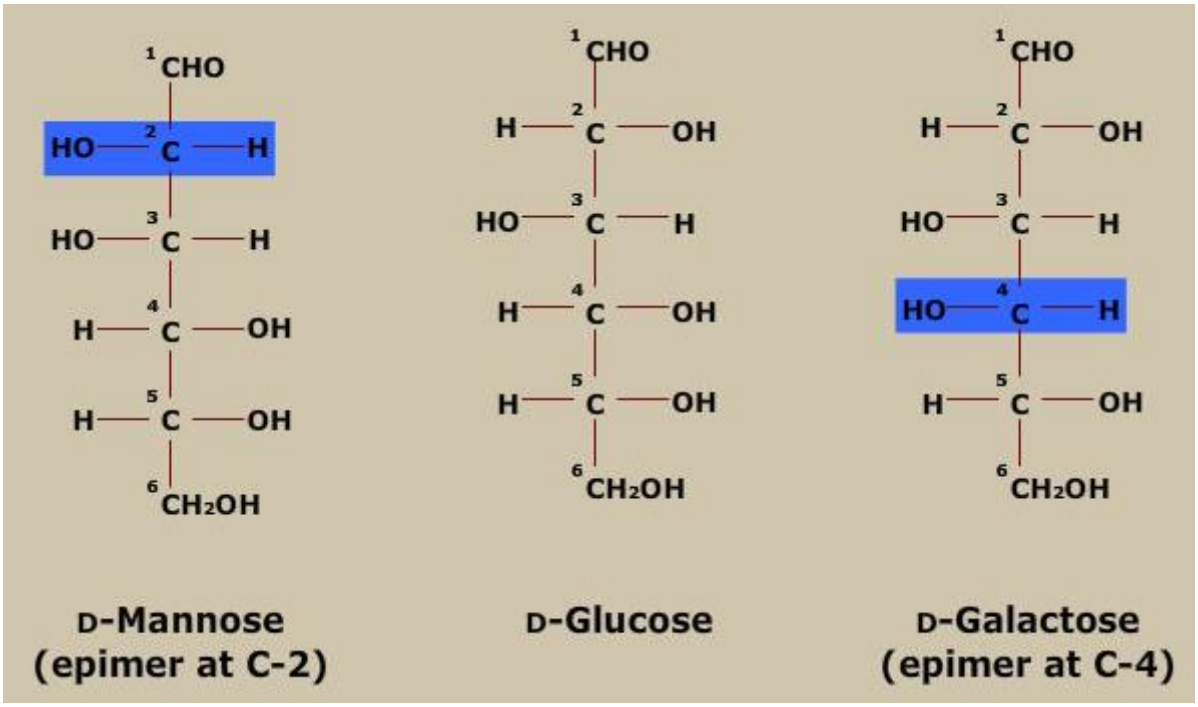


Fig 3.4 Epimers

3.3.2 Disaccharides

- Disaccharides (such as maltose, lactose, and sucrose) consist of two monosaccharides joined covalently by an O-glycosidic bond, which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other.
- The oxidation of a sugar's anomeric carbon by cupric or ferric ion (the reaction that defines a reducing sugar) occurs only with the linear form, which exists in equilibrium with the cyclic form(s). When the anomeric carbon is involved in a glycosidic bond, that sugar residue cannot take the linear form and therefore becomes a nonreducing sugar. In describing disaccharides or polysaccharides, the end of a chain with a free anomeric carbon (one not involved in a glycosidic bond) is commonly called the reducing end.

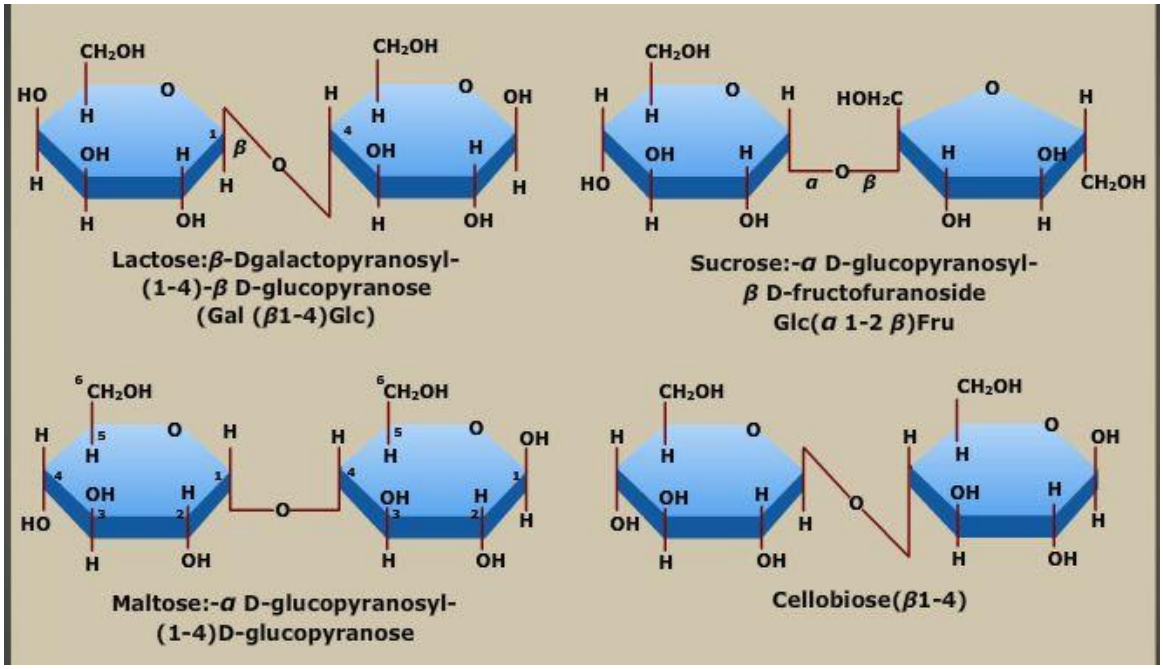


Fig. 3.5 Disaccharides

3.3.3 Polysaccharides

- Homopolysaccharides contain only a single type of monomer; heteropolysaccharides contain two or more different kinds
- Some homopolysaccharides serve as storage forms of monosaccharides that are used as fuels; starch and glycogen are homopolysaccharides of this type.
- Other homopolysaccharides (cellulose and chitin for example) serve as structural elements in plant cell walls and animal exoskeletons. Heteropolysaccharides provide extracellular support for organisms of all kingdoms. For example, the rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units. In animal tissues, the extracellular space is occupied by several types of heteropolysaccharides, which form a matrix that holds individual cells together and provides protection, shape, and support to cells, tissues, and organs.

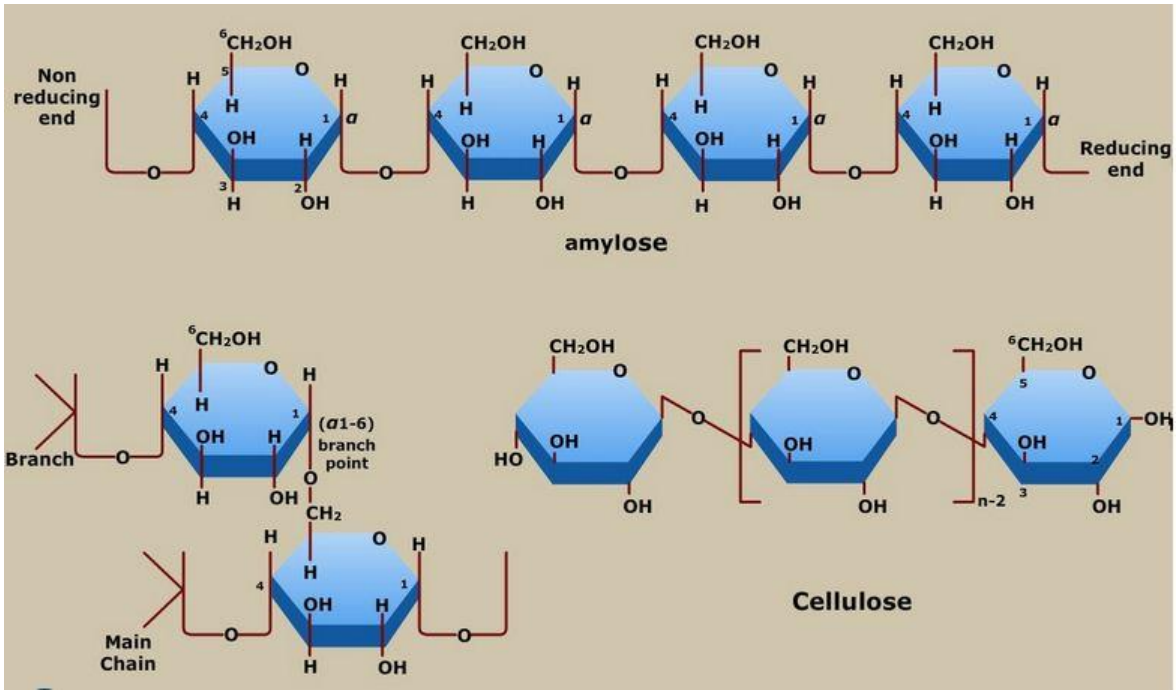


Fig 3.6 Polysaccharides

Lesson 4
LIPIDS

4.1 Introduction

- Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoacylglycerols, di acylglycerols, phospholipids, and others.
- Lipids consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water.
- The main biological functions of lipids include energy storage, as structural components of cell membranes, vitamins, hormones and as important signaling molecules.
- Lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called tri acylglycerols.
- Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoacylglycerols and phospholipids), as well as other sterol-containing metabolites such as cholesterol.
- Humans and other mammals use various biosynthetic pathways to both degrade and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

4.2 Lipid Classification

- Fatty Acids
- Acylglycerols
- Glycerophospholipids
- Sphingolipids
- Sterols
- Prenol lipids
- Saccharolipids

4.2.1 Fatty acids

- A fatty acid consists of a hydrocarbon chain and a terminal carboxylic acid group.
- This arrangement confers the molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water.
- The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building block of more structurally complex lipids.
- The carbon chain, typically between 4 to 24 carbons long, may be saturated or unsaturated. A saturated fatty acid has all of the carbon atoms in its chain saturated with hydrogen atoms with general formula $CH_3(CH_2)nCOOH$ where n is an even number.
- Mono-unsaturated fatty acids have one double bond in their structure while polyunsaturated fatty acids have two or more double bonds.
- The double bonds in polyunsaturated fatty acids are generally separated by at least one methylene group.
- Where a double bond exists, there is the possibility of either a cis or trans geometric isomerism, which significantly affects the molecule's molecular configuration.
- Cis-double bonds cause the fatty acid chain to bend, an effect that is more pronounced when more double bonds are there in a chain. This in turn plays an important role in the structure and function of cell membranes.

- Most naturally occurring fatty acids are of the cis configuration, although the trans form does exist in some natural and partially hydrogenated fats and oils.
- Shorter the chain of fatty acids lower is the melting temperature than those with longer chains.
- Unsaturated fatty acids have lower melting temperatures than saturated fatty acids of same chain length.

4.2.2 Glycerolipids

- Glycerolipids are composed mainly of mono-, di- and tri-substituted glycerols, the most well-known being the fatty acid esters of glycerol (triacylglycerols), also known as triglycerides or fats. In these compounds, all three hydroxyl groups of glycerol are esterified, usually by different fatty acids (Mixed Lipids).
- They function as a food store, these lipids comprise the bulk of storage fat in animal tissue and oil seeds.
- Triglycerides or fats may be either solid or liquid at room temperature, depending on their structure and composition.
- Oils" is usually used to refer to fats that are liquids at normal room temperature, while "fats" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats, along with other related substances.

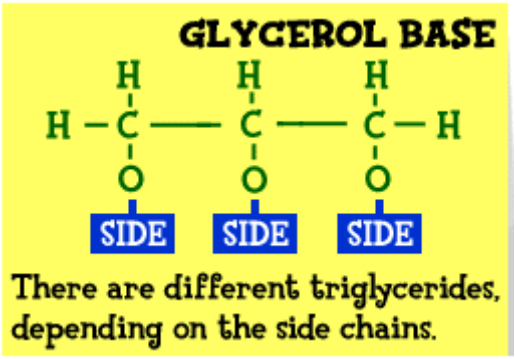


Fig 4.2 Triacylglycerols

4.2.3 Glycerophospholipids

- Glycerophospholipids, also referred to as phospholipids, are key components of the lipid bilayer of cells, as well as being involved in metabolism and cell signaling.
- Neural tissue (including the brain) contains relatively high amounts of glycerophospholipids, and alterations in their composition has been implicated in various neurological disorders.
- Examples of glycerophospholipids found in biological membranes are phosphatidylcholine (also known as PC, or lecithin), phosphatidylethanolamine (PE) and phosphatidylserine (PS).
- Plasmalogens are also a type of glycerolipids that contain a fatty alcohol at C-1 of Sn glycerol with double bond instead of a fatty acid.

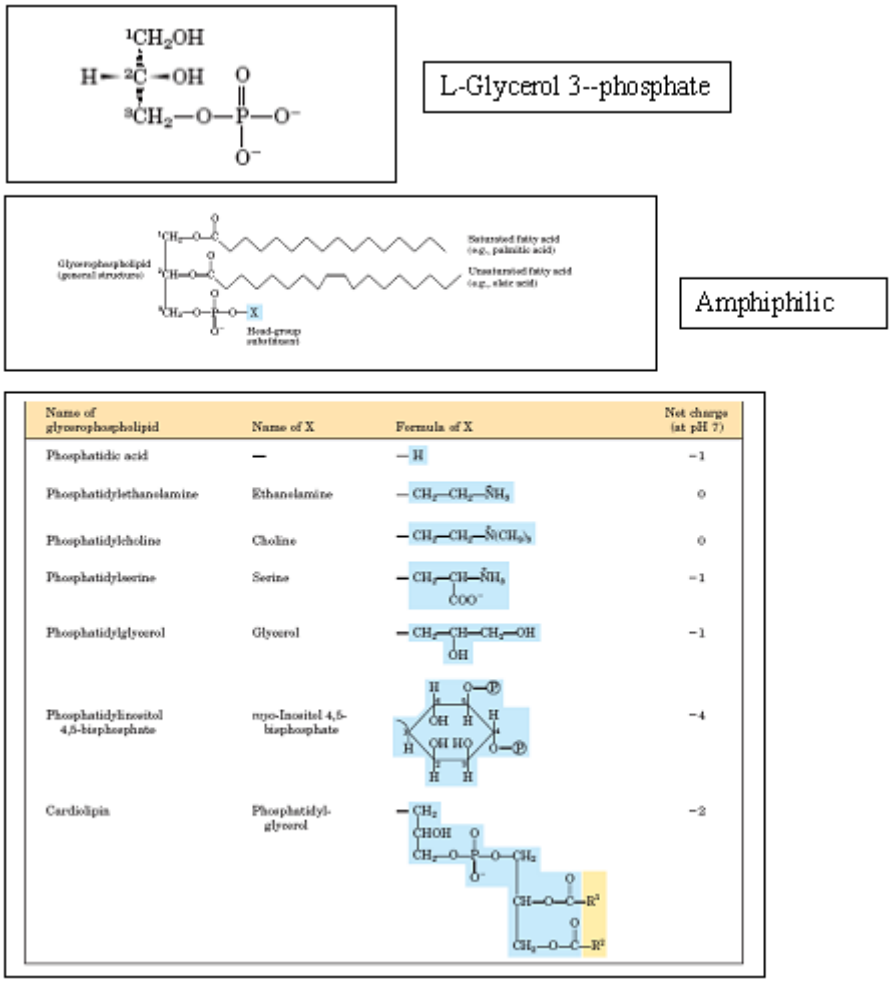
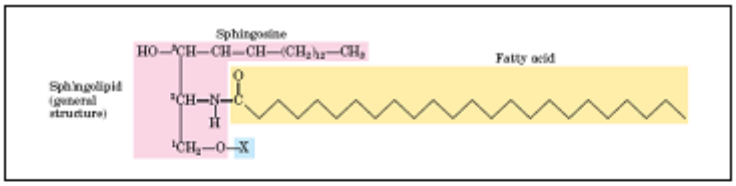


Fig 4.3 Glycerophospholipids

4.2.4 Sphingolipids

- Sphingolipids are a complex family of compounds that share a common structural feature, a sphingoid base backbone that is synthesized de novo from the amino acid serine and a long-chain fatty acyl CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids and other compounds.
- The major sphingoid base of mammals is commonly referred to as sphingosine. Ceramides (N-acyl-sphingoid bases) are a major subclass of sphingoid base derivatives with an amide-linked fatty acid. The fatty acids are typically saturated or mono-unsaturated with chain lengths from 16 to 26 carbon atoms.



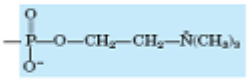
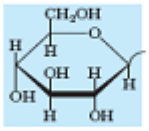
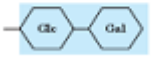
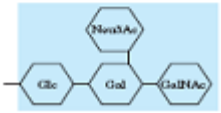
Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Fig 4.4 Sphingolipids

4.2.5 Sterols

- Sterol lipids, such as cholesterol and its derivatives, are an important component of membrane lipids, along with the glycerophospholipids and sphingomyelins.
- The steroids, all derived from the same fused four-ring core structure, have different biological roles as hormones and signaling molecules. The eighteen-carbon (C18) steroids include the estrogen family whereas the C19 steroids comprise the androgens such as testosterone and androsterone. The C21 subclass includes the progestogens as well as the glucocorticoids and mineralocorticoids. The secosteroids, comprising various forms of vitamin D, are characterized by cleavage of the B ring of the core structure.
- Other examples of sterols are the bile acids and their conjugates, which in mammals are oxidized derivatives of cholesterol and are synthesized in the liver.

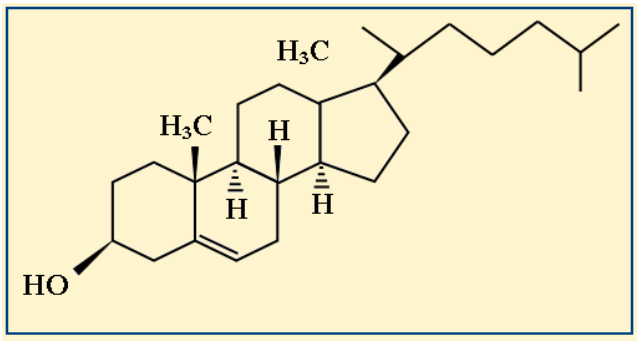


Fig 4.5 Cholesterol

4.2.6 Prenol lipids

- Prenol, or 3-methyl-2-buten-1-ol, is a natural alcohol. It is one of the most simple terpenes.
- Prenol lipids are synthesized from the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate that are produced mainly via the mevalonic acid (MVA) pathway.

- The simple isoprenoids (linear alcohols, diphosphates, etc.) are formed by the successive addition of C5 units, and are classified according to number of these terpene units.
- Structures containing greater than 40 carbons are known as polyterpenes.
- Carotenoids are important simple isoprenoids that function as antioxidants and as precursors of vitamin A.
- Another biologically important class of molecules is exemplified by the quinones and hydroquinones, which contain an isoprenoid tail attached to a quinonoid core of non-isoprenoid origin.
- Vitamin E and vitamin K, as well as the ubiquinones, are examples of this class.

4.2.7 Saccharolipids

- Saccharolipids describe compounds in which fatty acids are linked directly to a sugar backbone, forming structures that are compatible with membrane bilayers.
- In the saccharolipids, a monosaccharide substitutes for the glycerol backbone present in glycerolipids and glycerophospholipids.
- The most familiar saccharolipids are the acylated glucosamine precursors of the Lipid A component of the lipopolysaccharides in Gram-negative bacteria

Table 1.1 Common Fatty Acids

Chemical Names and Descriptions of some Common Fatty Acids				
Common Name	Carbon Atoms	Double Bonds	Scientific Name	Sources
Butyric acid	4	0	butanoic acid	butterfat
Caproic Acid	6	0	hexanoic acid	butterfat
Caprylic Acid	8	0	octanoic acid	coconut oil
Capric Acid	10	0	decanoic acid	coconut oil
Lauric Acid	12	0	dodecanoic acid	coconut oil
Myristic Acid	14	0	tetradecanoic acid	palm kernel oil
Palmitic Acid	16	0	hexadecanoic acid	palm oil
Palmitoleic Acid	16	1	9-hexadecenoic acid	animal fats
Stearic Acid	18	0	octadecanoic acid	animal fats
Oleic Acid	18	1	9-octadecenoic acid	olive oil
Vaccenic Acid	18	1	11-octadecenoic acid	butterfat
Linoleic Acid	18	2	9,12-octadecadienoic acid	grape seed oil
Alpha-Linolenic Acid (ALA)	18	3	9,12,15-octadecatrienoic acid	flaxseed (linseed) oil
Gamma-Linolenic Acid (GLA)	18	3	6,9,12-octadecatrienoic acid	borage oil
Arachidic Acid	20	0	eicosanoic acid	peanut oil, fish oil
Arachidonic Acid (AA)	20	4	5,8,11,14-eicosatetraenoic acid	liver fats
EPA	20	5	5,8,11,14,17-eicosapentaenoic acid	fish oil

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Lesson 5
NUCLEIC ACIDS (DNA & RNA)

5.1 Introduction

1. Nucleotides are building blocks of nucleic acids as the proteins are made of amino acids.
2. They are the energy currency in metabolic transactions
3. Nucleotides are the essential chemical links in the response of cells to hormones and other extracellular stimuli.
4. They are structural components of an array of enzyme cofactors and metabolic intermediates.

5.2 Structure of Nucleotides

5.2.1 Nucleotides have three characteristic components

- A nitrogenous (nitrogen-containing) base
- a pentose sugar
- A phosphate

The nitrogenous bases in nucleotides are derivatives of two parent compounds, pyrimidine and purine.

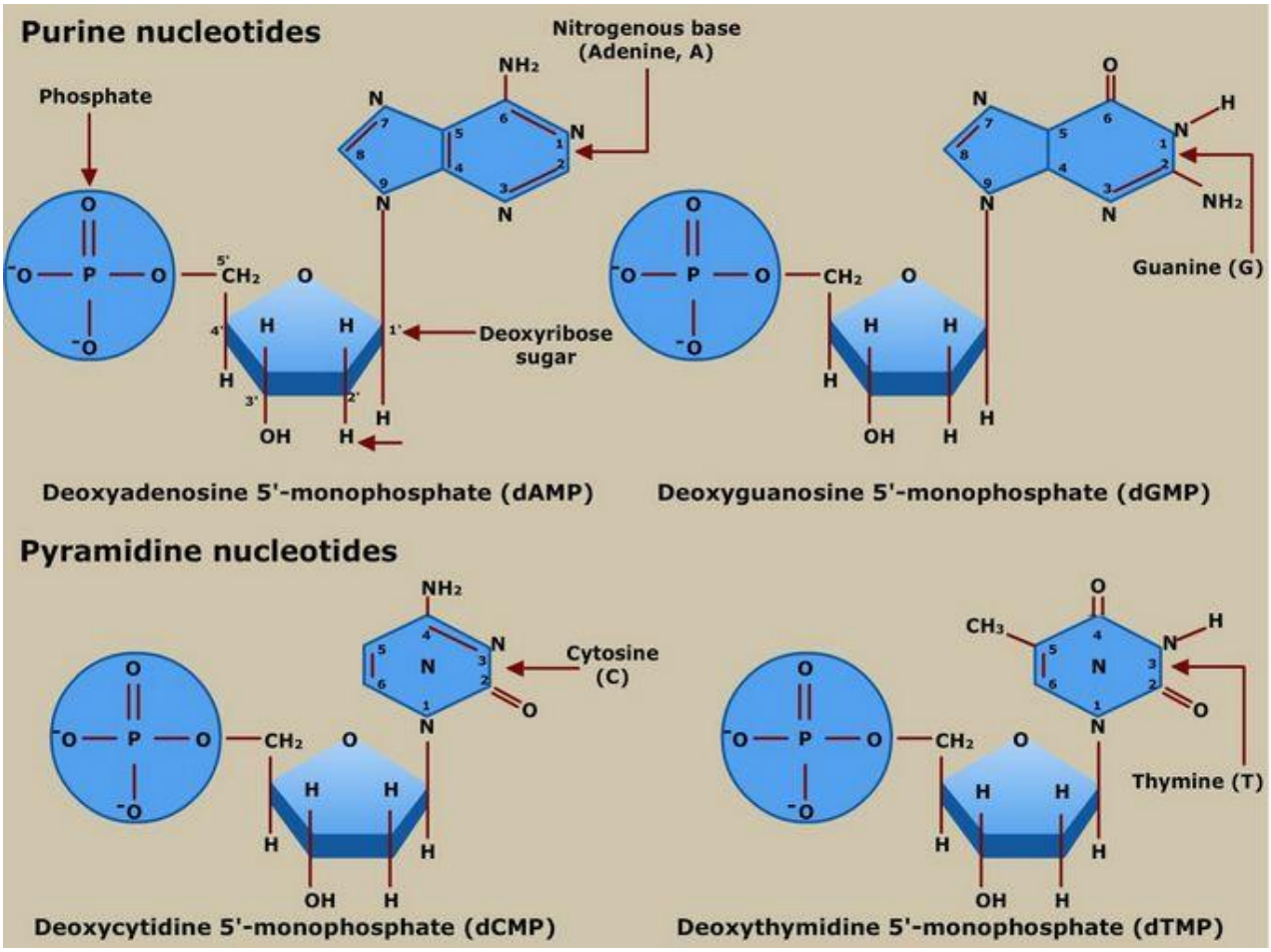

Fig. 5.1 Structure of nucleotides

- Both DNA and RNA contain two major purine bases, Adenine (A) and guanine (G),
- Pyrimidines in DNA are cytosine (C) and thymine (T).
- Pyrimidines in RNA are cytosine (C) and uracil

Nucleic acids have two kinds of pentoses

- DNA contains 2'-deoxy-D-ribose,
- RNA contains D-ribose.

The names of the four major deoxyribonucleotides (deoxyribonucleoside 5'-monophosphates)

- Deoxyadenylate (deoxyadenosine 5'-monophosphate) Symbols : A, dA, dAMP
- Deoxyguanylate (deoxyguanosine 5'-monophosphate) Symbols : G, dG, dGMP
- Deoxythymidylate (deoxythymidine 5'-monophosphate) Symbols: T,dT,dTMP
- Deoxycytidylate (deoxycytidine 5'-monophosphate) Symbols : C.dC,dCMP

The names of four major ribonucleotides (ribonucleoside 5'- monophosphates),

- Adenylate (adenosine 5'-monophosphate) Symbols : A, AMP
- Guanylate (guanosine 5'-monophosphate) Symbols : G, GMP
- Uridylate (uridine 5'-monophosphate) Symbols : U,UMP
- Cytidylate (cytidine 5'-monophosphate) Symbols : C,CMP

5.3 Nucleoside

The molecule without the phosphate group is called a nucleoside
DNA RNA

- Deoxyadenosine Adenosine
- Deoxyguanosine Guanosine
- Deoxythymidine Uridine
- Deoxycytidine Cytosine

5.4 Phosphate "Bridges"

The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group "bridges,"

- The 5'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide, creating a phosphodiester linkage.
- Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals.
- Each linear nucleic acid strand has a specific polarity and distinct 5' and 3' ends.

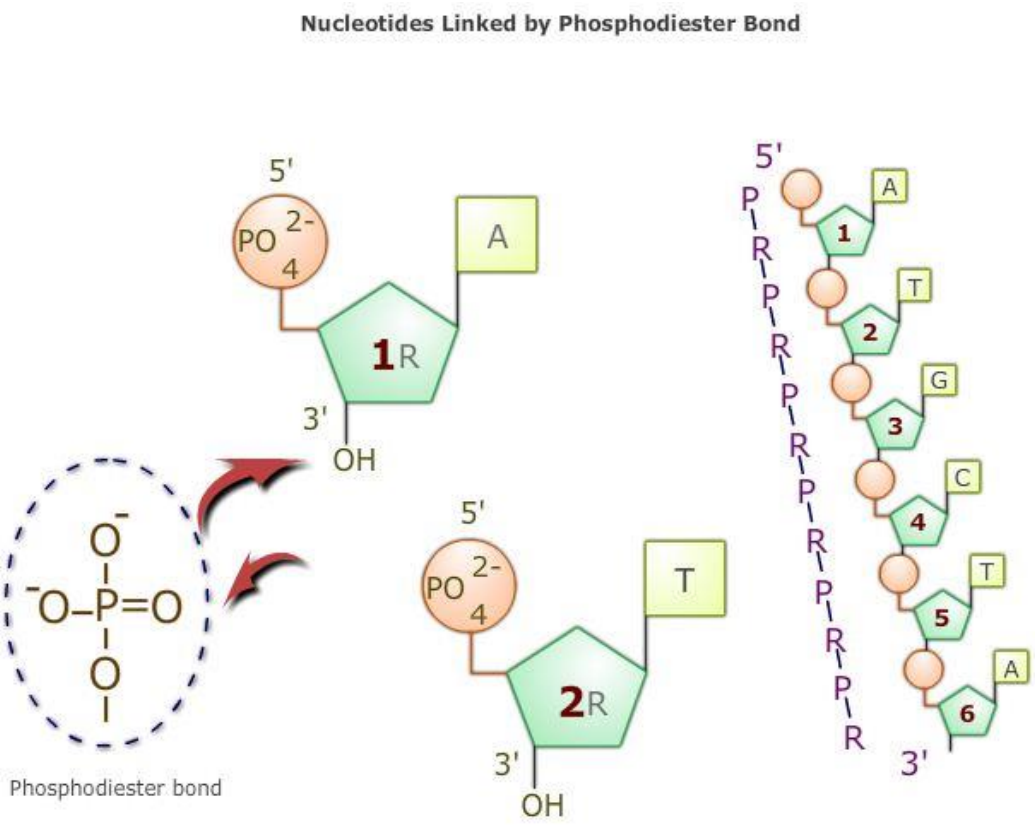

Fig. 5.2 Nucleotides linked by phosphodiester bond

5.5 Structure of DNA

- In 1953 Watson and Crick postulated a three dimensional model of DNA structure.
- In a DNA molecule, the different nucleotides are covalently joined to form a long polymer chain by covalent bonding between phosphates and sugars.
- The phosphate attached to the hydroxyl group at the 5'postion of the sugar is attached to hydroxyl group at the on the 3' carbon of the sugar of the next nucleotide.
- Thus the linkage between the phosphate and hydroxyl bond is an ester linkage and is called 3'-5'posphodiester bond.
- The DNA chain has the polarity having 5'end and 3'end because first nucleotide has a 5' phosphate not bounded to any other nucleotide and last nucleotide has a free 3' hydroxyl .
- DNA consists of two helical chains of nucleotides wound around the same axis to form double helix. The two DNA strands are organized in an anti-parallel arrangement i.e. one strand is oriented 5'-3' and other is oriented 3'-5'.
- The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water.
- The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis.
- Each nucleotide base of one strand is paired in the same plane with a base of the other strand. G with C and A with T, are those that fit best within the structure. This is called complementary base pairing. Three hydrogen bonds can form between G and C, but only two can form between A and T

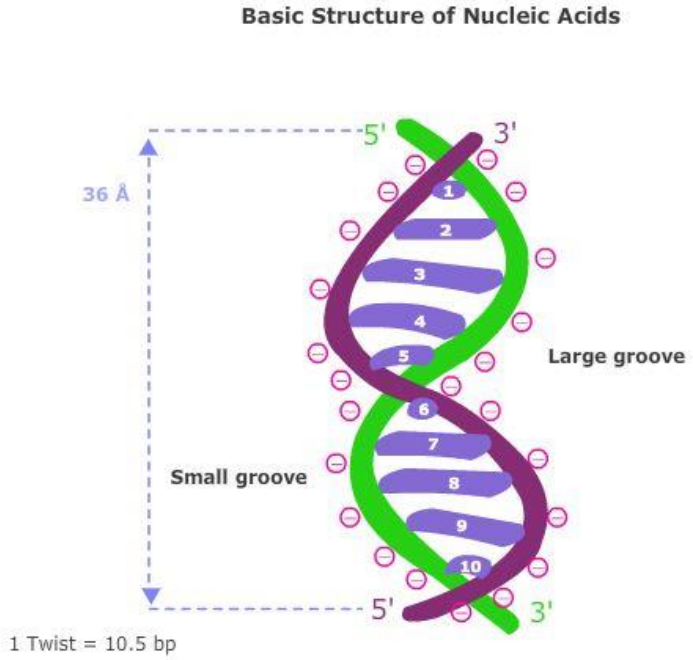

Fig. 5.3 Basic structure of nucleic acids

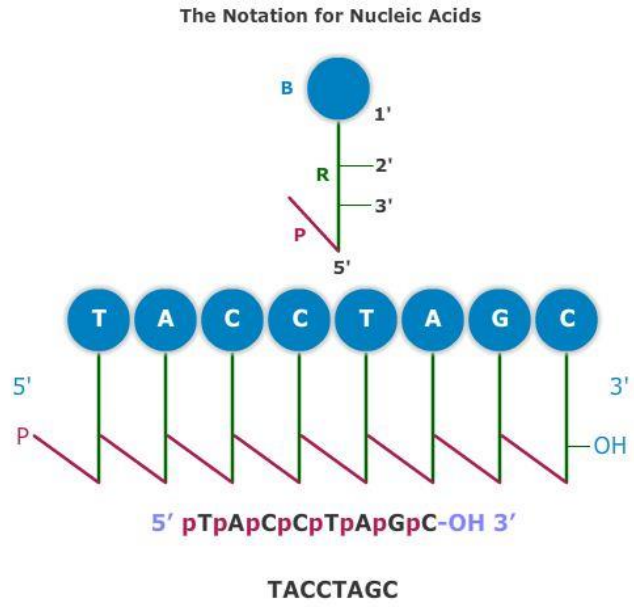

Fig. 5.4 The notation for nucleic acids

5.6 Structure of RNA

Most RNA molecules are single stranded but an RNA molecule may contain regions which can form complementary base pairing where the RNA strand loops back on it. If so RNA will have some double – stranded regions. RNA molecules are of three types.

- m RNA (messenger RNA) – carries message in the form of codons from DNA
- rRNA (ribosomal RNA) – creates site where protein synthesis takes place
- t RNA (transfer RNA) - specific t RNA with specific anticodon carries amino acid

Lesson 6
SPECTROPHOTOMETRIC ASSAYS OF BIO-MOLECULES

6.1 Introduction

- Spectroscopy is a technique that measures the interaction of molecules with electromagnetic radiation. Light in the near-ultraviolet (UV) and visible (vis) range of the electromagnetic spectrum has an energy of about 150– 400 kJ /mol.
- The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength.
- Molecules with electrons in delocalized aromatic systems often absorb light in the near-UV (150–400 nm) or the visible (400–800 nm) region.
- Absorption spectroscopy is usually performed with molecules dissolved in a transparent solvent, such as in aqueous buffers.
- The absorbance of a solute depends linearly on its concentration and therefore absorption spectroscopy is ideally suited for quantitative measurements.
- The wavelength of absorption and the strength of absorbance of a molecule depend not only on the chemical nature but also on the molecular environment of its chromophores.
- Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids. Spectroscopic measurements are very sensitive and nondestructive, and require only small amounts of material for analysi

6.2 Lambert–Beer law

- In this technique, the amount of light that a sample absorbs at a particular wave length is measured and used to determine the concentration of the sample by comparison with appropriate standards or reference data.
- The most useful measure of light absorption is the absorbance (A), also commonly called the optical density (OD). The absorbance is defined as A = log I0 / I where I0 is the intensity of light that is incident on the sample and I is the intensity of light that is transmitted by the sample.
- The absorbance of a sample can be related to the concentration of the absorbing species through Beer's law:

$A = \varepsilon cl$
Where c is concentration, usually measured in moles per liter;
l is the length of the light path, usually 1 cm;
ε is a proportionality constant known as the molar extinction coefficient, with the units of liters per mole per centimeter.
The value of ε is a function of both the particular compound being measured and the wavelength.

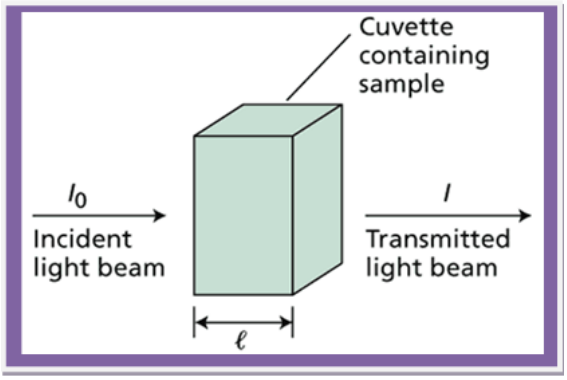

Fig 6.1 Beer's law Optical Density

- Chlorophylls typically have an ε value of about 100,000 L mol^{-1} $cm^{-1.}$ When more than one component of a complex mixture absorbs at a given wavelength, the absorbances due to the individual components are generally additive.

6.3 The Spectrophotometer

The absorbance is measured by an instrument called a spectrophotometer. The essential parts of a spectrophotometer include a light source, a wavelength selection device such as a monochromator that contains a wavelength selection device such as a prism or filter, a sample chamber, a light detector such as a photomultiplier tube or silicon diode, and a readout device, usually also include a computer, which is used for storage and analysis of the spectra. The most useful machines scan the wavelength of the light that is incident on the sample and produce, as output, spectra of absorbance versus wavelength.

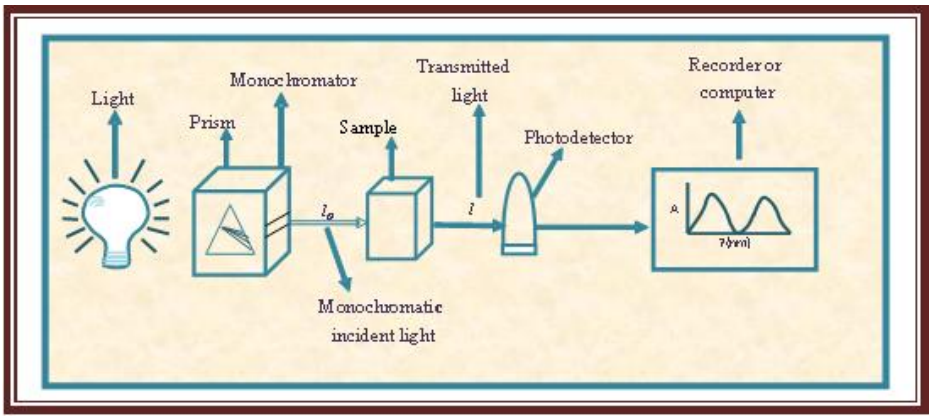

Fig. 6.2 Components of Spectrophotometer

6.4 Estimation of DNA

DNA (Deoxyribonucleic Acid) is present in the nucleus of all cells. It is a double stranded molecule, made up of a chain of units called nucleotides and is a repository of genetic information.

6.4.1 Principle

Based on the reaction of deoxyribose sugar with diphenylamine reagent. Under extreme acidic conditions, the deoxyribose moiety of DNA is dehydrated and forms an aldehyde product ω-hydroxylevulinic acid, which condenses in acidic medium with diphenykamine to produce deep-blue coloured products, having absorption maxima at 595 nm. The colour produced is stable for several hours.

6.4.2 Notes

- This method is commonly employed for samples of 50-500 µg DNA.
- In the original method by Dische, acetaldehyde is not added. In Burton's modified method, acetaldehyde is added, as it potentiates colour development and makes the method 3.5 times more sensitive than the original method.
- In DNA, only the deoxyribose of the purine nucleotides reacts, so that the value obtained represents half of the total deoxyribose present.
- Standard DNA solution is prepared in 0.1N NaOH, because this helps in dissolving the DNA, resulting in a clear solution.
- Glacial acetic acid is added to increase the rate of colour development.

6.5 Estimation of RNA

RNA (ribonucleic acid) is found in the cytoplasm of cells. There are three major classes of RNA (messenger RNA), tRNA (transfer RNA) and rRNA (ribosomal RNA). Ribosomal RNA constitutes the larger percentage of total RNA.

6.5.1 Principle

Estimation of RNA is carried out by the reaction of ribose in RNA with orcinol (Bial's test). The method depends upon the conversion of the pentose sugar, Ribose to furfural, in the presence of hot acid. Furfural then reacts with orcinol, in the presence of ferric ions ($Fe^{+++)}$ to yield a green colour. The colour formed depends largely upon the concentration of hydrochloric acid, ferric chloride, orcinol and the time for which the solution is heated to 1000C.

6.5.2 Notes

1. Apart from orcinol, other reagents e.g. phloroglucinol, aniline etc. are also used for RNA estimation. However orcinol method (Bial's test) is widely used, because in this method interference by DNA is only 0.85 % compared to 12% by other methods as given in literature.
2. Evaporation may be minimized by employing glass-stoppered tubes or by covering mouths of tubes with carefully cleaned marbles.
3. In the determination of RNA by Bial reaction, only the purine bound sugars react significantly.
4. The green color developed, if clear, is read at 660 nm, against blank. If turbid, extract with 5 ml of isoamyl alcohol and then read.
5. Xylose or adenylic acid can also be used as standard.
6. Dilute using n-butanol if the concentration of sample is high.
7. The yield and purity of RNA preparation can be assessed by measuring the absorbance of UV light by a solution of RNA. A pure solution should give a 260 nm: 280 nm of 2; one unit of A260 measured in 1 cm path length is equivalent to 40 µg/ml.

6.6 Estimation of Proteins

Proteins can be estimated by a number of methods e.g. Kjeldahl method, Nessler reaction, Biuret method, Ninhydrin reaction, Lowry method, UV absorbtion etc. Each method has its advantages and disadvantages. The choice of method eventually depends upon nature of sample, number of samples and ease of performance of assay. The Lowry's method is the most commonly used method of protein estimation. It is easy to perform and is very sensitive (useful range is 0.005-0.02 mg protein). However it has got two major disadvantages- the intensity of colour varies with different proteins and the colour developed is not always proportional to concentration at higher values. Estimation of protein content of enzyme extracts is usually does by this method.

6.6.1 Estimation – Lowry (Folin-Ciocalteau) method

- The first step in the reaction involves the formation of a copper-protein complex in alkaline solution. This complex then reduces a phosphomolybdic-phosphotungstate reagent (Folin's reagent) to yield an intense blue colour.
- Phenols are capable of reducing molybdenum in a complex of phosphomolybdotungstic acid. The tyrosine and tryptophan residues of proteins provide phenolic groups and cupric ions enhance the sensitivity. Thus, when treated with Folin-Ciocalteau's reagent, proteins produce blue colour in varying degrees depending upon their tyrosine content. Hence, different proteins give different colour values.
- The blue colour produced by reduction of phosphomolybdotungstic acid by phenolic groups of the amino acids, tryptophan present in proteins plus the colour developed by the biuret reaction of the proteins with alkaline cupric tartarate are measured in Lowry's method.
- The precaution to be observed when performing the assay concerns the addition of Folin's reagent. This reagent is stable only at acidic pH; however the reduction reaction mentioned above occurs only at pH 10. Therefore, when Folin's reagent is added to the alkaline copper-protein solution, mixing must occur immediately so that the reduction can proceed before the phosphomolybdic-phosphotungstate reagent breaks down.

6.6.2 Biuret method

Compounds with two or more peptide bonds give a violet colour with alkaline copper sulphate solution with absorbtion maxima at 540-560 nm. There is no interference from free amino acids and there is little dependence on amino acid composition as the copper reagent reacts with peptide chain itself rather than with side groups. The main disadvantage of this method is its low sensitivity – 1-6mg protein/ml, which severely limits its applicability.

6.6.3 Note

1. A linear relationship between amount of protein and colour intensity following treatment with the Folin-Ciocalteau reagent is observed only over a relatively limited range of protein concentration. A sample of unknown protein concentration should therefore be diluted above the upper limit of calibration curve.
2. A suggested range for establishing the curve is 0.01-0.20 mg (10-200 µg) of protein/tube.
3. For complete enzyme extraction, sometimes chemicals like EDTA, magnesium salts and mercaptoethanol are included, which interfere with the estimation. Hence, when these are present, the proteins should be precipitated by adding 10% TCA (trichloroacetic acid), centrifuged and the precipitate dissolved in 1N NaOH, before proceeding for protein estimation.
4. Rapid mixing as the Folin-Ciocalteau reagent is added is important for reproducibility.
5. Folin-Ciocalteau reagent must be stored refrigerated, in amber coloured bottles (to protect it from light). A good quality reagent is straw yellow in colour. The reagent should not be used if it has a greenish tint. To such reagent add some bromine water and mix. The colour turns straw yellow and becomes usable.

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Lesson7
ENZYME CATALYSIS AND CLASSIFICATION

7.1 Introduction

Enzymes are biologic polymers that catalyze the chemical reactions. With the exception of a few catalytic RNA molecules, or ribozymes, the vast majority of enzymes are proteins.

- The enzymes catalyze the conversion of one or more compounds (substrates) into one or more different compounds (products).
- They enhance the rates of the corresponding non-catalyzed reaction. Catalysts do not affect reaction equilibria.
- Like all catalysts, enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.
- Their catalytic activity depends on the integrity of their native protein conformation. If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost. If an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed.
- The primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.

7.2 Enzymes are Highly Specific Catalysts

Enzymes are also extremely selective catalysts. Unlike most catalysts used in synthetic chemistry, enzymes are specific both for the type of reaction catalyzed and for a single substrate or a small set of closely related substrates.

- Enzymes are substrate specific or group specific Conformation of complex proteins and uniqueness of active site of enzymes make them substrate specific or absolute group specific. For example glucokinase recognize glucose as absolute substrate while hexokinase recognizes aldohexose (Glucose or mannose etc) as substrate. Similarly, trypsin, chymotrypsin and elastase cleaves proteins or polypeptides on carboxyl side of positively charged (Lysine, Arginine), aromatic amino acids (Tyrosine, Phenylalanine) and small group side chains (alanine, glyscine) amino acids respectively.

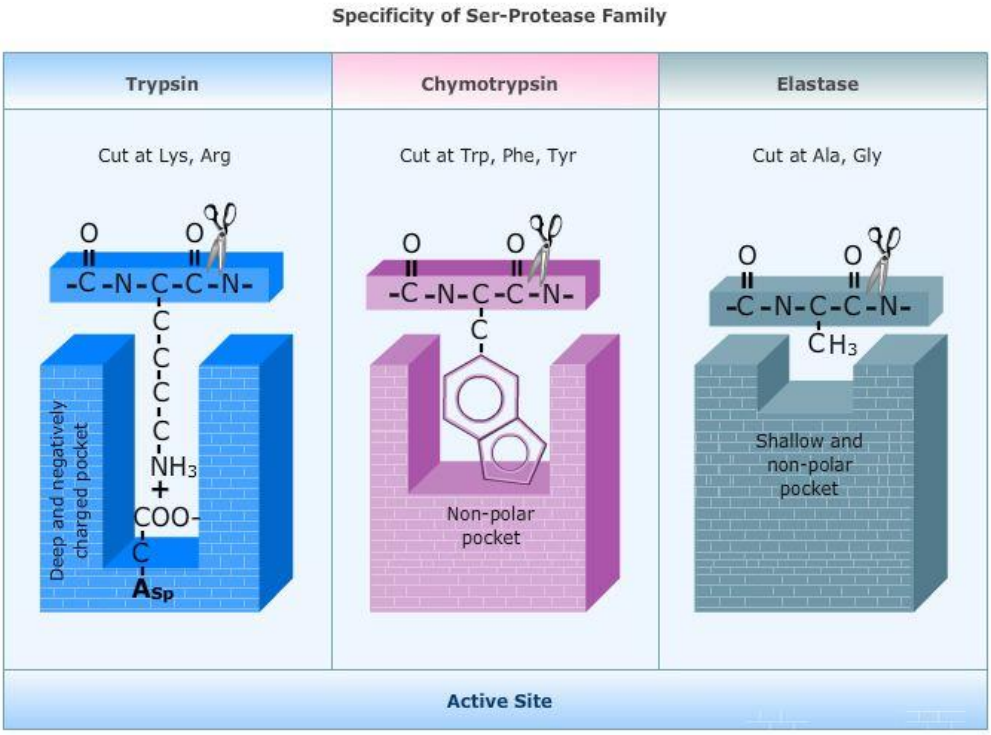
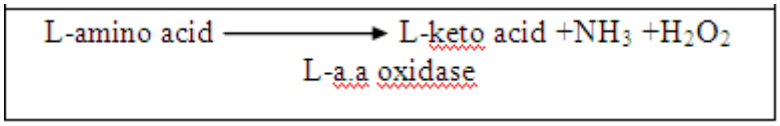


Fig. 7.1 Substrate Specificity of Enzymes

Enzymes are also stereospecific catalysts and typically catalyze reactions only of specific stereoisomers of a given compound—for example, D- but not L-sugars, L- but not D-amino acids.



- Enzymes show geometric specificity

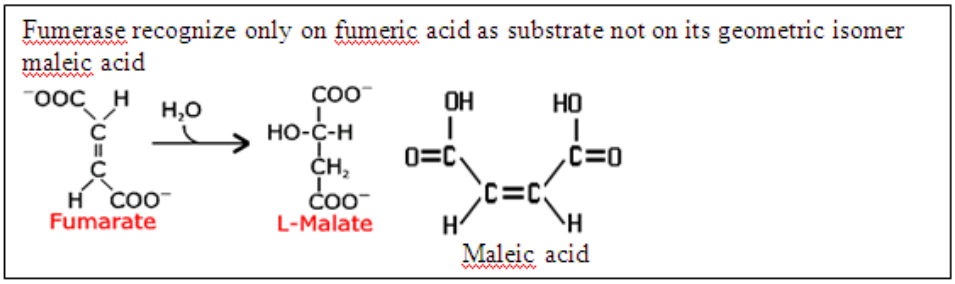


Fig 7.2 Geometric Specificity of Enzymes

- Enzyme Since they bind substrates through at least “three points of attachment,” enzymes can even convert nonchiral substrates to chiral products.

7.3 Enzyme Active Site

ACTIVE SITE
The active site is a specialized region of the protein where the enzyme interacts with the substrate. It is the special place, cavity, crevice, chasm, cleft, or hole that binds and then magically transforms the substrate to the product. The kinetic behavior of enzymes is a direct consequence of the protein's having a limited number (often 1) of specific active sites.

7.3.1 Common features of enzyme active site

- The active site of an enzyme is generally a pocket or cleft that is specialized to recognize specific substrates and catalyze chemical transformations. Hence, the enzyme and substrate should have complementary shapes.
- The active site takes up a relatively small part of the total volume of an enzyme.
- It is formed in the three-dimensional structure by a collection of different amino acids (active-site residues) that may or may not be adjacent in the primary sequence.
- The interactions between the active site and the substrate occur via the same forces that stabilize protein structure: hydrophobic interactions, electrostatic interactions (charge–charge), hydrogen bonding, and Vander Waals interactions.
- Enzyme active sites do not simply bind substrates; they also provide catalytic groups to facilitate the chemistry and provide specific interactions that stabilize the formation of the transition state for the chemical reaction.

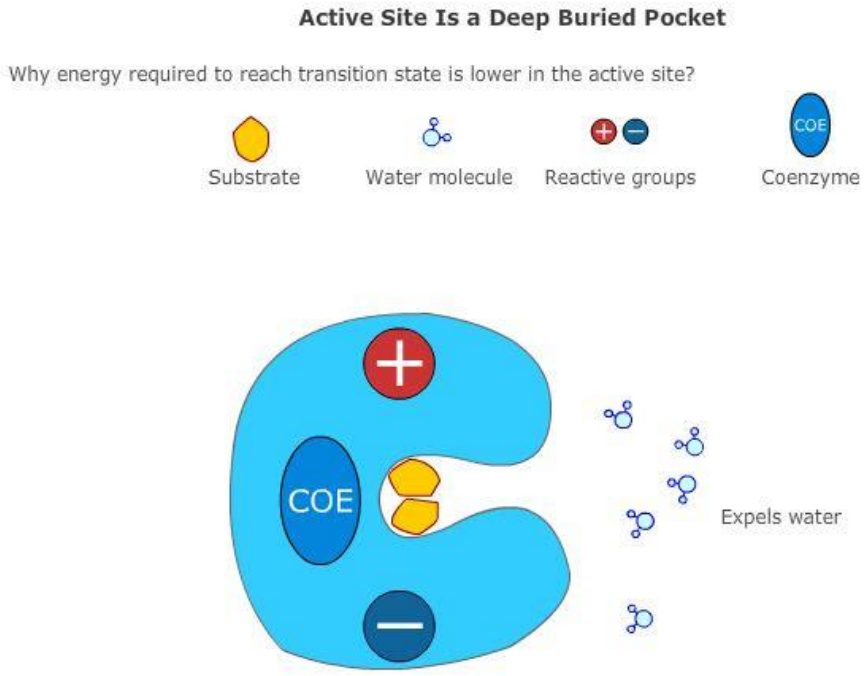


Fig.7.3 Enzyme active site

TRANSITION STATE
The transition state is the highest-energy arrangement of atoms during a chemical reaction.

During a chemical reaction, the structure of the substrate changes into the structure of the product. Somewhere in between, some bonds are partly broken; others are partly formed. The transition state is the highest- energy arrangement of atoms that is intermediate in structure between the structure of the reactants and the structure of the products. The diagram shows the free energy of the reactants, transition state, and product. The free-energy difference between the product and reactant is the free-energy change for the overall reaction. The free-energy change between the products and reactants tells you how favorable the reaction is thermodynamically. It does not tell you anything about how fast it is. Reactions don't all occur with the same rate. Some energy must be put into the reactants before they can be converted to products. This activation energy provides a barrier to the reaction—the higher the barrier, the slower the reaction. The difference in free energy between the transition state and the reactant(s) is called the free energy of activation.

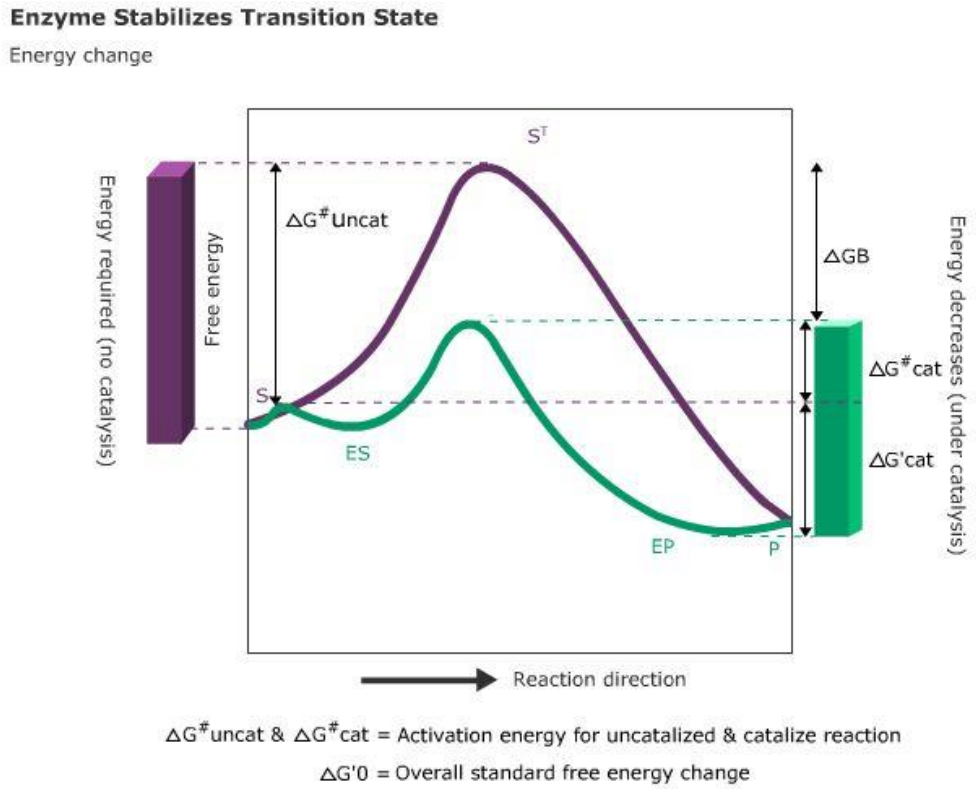


Fig. 7.4 Free-Energy Changes Occur during a Chemical Reaction

Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier. Alternatively, the activation energy can be lowered by adding a catalyst (Fig.). Catalysts enhance reaction rates by lowering activation energies. The role of enzymes is to accelerate the interconversion of S and P. The enzyme is not used up in the process, and the equilibrium point is unaffected. However, the reaction reaches equilibrium much faster when the appropriate enzyme is present, because the rate of the reaction is increased.

7.4 Enzyme Classification

Many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity. Thus urease catalyzes hydrolysis of urea, and DNA polymerase catalyzes the polymerization of nucleotides to form DNA. Some enzymes were named by their discoverers for a broad function, before the specific reaction catalyzed was known. For example, lysozyme was named for its ability to lyse bacterial cell walls. Sometimes the same enzyme has two or more names, or two different enzymes

have the same name. Because of such ambiguities, and the ever increasing number of newly discovered enzymes, biochemists, by international agreement, have adopted a system for naming and classifying enzymes. This system divides enzymes into six classes, each with subclasses, based on the type of reaction catalyzed . Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes. As an example, the formal systematic name of the enzyme catalyzing the reaction

is ATP:glucose phosphotransferase, which indicates that it catalyzes the transfer of a phosphoryl group from ATP to glucose. Its Enzyme Commission number (E.C.number) is 2.7.1.1. The first number (2) denotes the major class name (transferase); the second number (7), the subclass (phosphotransferase); the third number denotes sub-subclass (1), a phosphotransferase with a hydroxyl group as acceptor; and the fourth number (1) denotes the enzyme number in the sub-subclass.

7.4.1 International classification of enzymes

Table 7.1 International classification of enzymes

No.	Class	Type of Reaction	Example
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)	Lactate dehydrogenase
2	Transferases	Group transfer reactions	NMP kinase
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups	Fumarase
5	Isomerases	Transfer of groups within molecules to yield isomeric forms	Triose phosphate isomerase
6	Ligases	Formation of COC, COS, COO, and CON bonds by condensation reactions coupled to ATP cleavage	Aminoacyl-tRNA synthetase

7.5 Cofactors

Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a cofactor—either one or more inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}, or a complex organic or metalloorganic molecule called a coenzyme. Some enzymes require both a coenzyme and one or more metal ions for activity. A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group. A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme. The protein part of such

an enzyme is called the apoenzyme or apoprotein. Coenzymes act as transient carriers of specific functional groups. Most are derived from vitamins, organic nutrients required in small amounts in the diet.

7.6 Some Inorganic Elements as Cofactors for Enzymes

Cu^{+2}	Cytochrome oxidase
Fe^{+2} or Fe^{+3}	Cytochrome oxidase, catalase, peroxidase
K^{+1}	Pyruvate kinase
Mg^{+2}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{+2}	Arginase, ribonucleotide reductase
Se^{+2}	Glutathione peroxidase
Zn^{+2}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

7.7 Coenzymes as Transient Carriers of Specific Atoms or Functional Group

Table 7.2 Coenzymes as transient carriers of specific atoms or functional group

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid
FAD	Electrons	Riboflavin (vitamin B 2)
Lipoate	Electrons and acyl groups	Not required in diet
NAD	Hydride ion (:H_)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B 6)
Tetrahydrofolate	One-carbon groups	Folate
TPP	Aldehydes	Thiamine (vitamin B 1)

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Lesson 8
ENZYME KINETICS

8.1 Introduction

- In the absence of enzyme, the conversion of S to P is slow and uncontrolled. In the presence of a specific enzyme, S is converted swiftly and specifically to product.
- Enzyme is specific; it will not convert A to B or X to Y.
- Enzymes also provide rate acceleration. On comparing the rate of a chemical reaction in solution with the rate of the same reaction with the reactants bound to the enzyme, the enzyme reaction will occur up to 10^{14} times faster.
- A unit is the amount of enzyme that will catalyze the conversion of 1 μmol of substrate to product in 1 min under a given set of conditions.
- Units of enzyme can be converted to milligrams of enzyme by a conversion factor called the specific activity. Specific activity is the amount of enzyme activity per milligram of protein (micromoles of product formed per minute per milligram of protein, or units per milligram).
- For a given pure enzyme under a defined set of conditions, the specific activity is a constant; however, different enzymes have different specific activities.
- An enzyme assay is the act of measuring how fast a given (or unknown) amount of enzyme will convert substrate to product—the act of measuring a velocity.
- Velocity (rate, v, activity, d[P]/dt, d[S]/dt) is how fast an enzyme converts substrate to product, the amount of substrate consumed, or product formed per unit time. Units are micromoles per minute (μmol/min) = units.

8.2 The Michaelis-Menten Equation

The primary function of enzymes is to enhance rates of reactions so that they are compatible with the needs of the organism. To understand how enzymes function, we need a kinetic description of their activity. For many enzymes, the rate of catalysis V_0, which is defined as the number of moles of product formed per second, varies with the substrate concentration [S] in a manner shown in Figure 8.1. The rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations. Consider an enzyme that catalyzes the S to P by the following pathway:

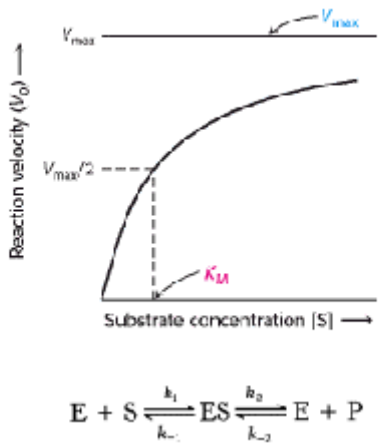


Fig 8.1 Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction

The extent of product formation is determined as a function of time for a series of substrate concentrations. As expected, in each case, the amount of product formed increases with time, although eventually a time is

reached when there is no net change in the concentration of S or P. The enzyme is still actively converting substrate into product and visa versa, but the reaction equilibrium has been attained. We define V_0 as the rate of increase in product with time when [P] is low; that is, at times close to zero (hence, V_0). Thus, for the graph in Figure, V_0 is determined for each substrate concentration by measuring the rate of product formation at early times before P accumulates .We begins our kinetic examination of enzyme activity with the graph shown in Figure. At a fixed concentration of enzyme, V_0 is almost linearly proportional to [S] when [S] is small but is nearly independent of [S] when [S] is large. In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics. The critical feature in their treatment is that a specific ES complex is a necessary intermediate in catalysis. The model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (1)$$

An enzyme E combines with substrate S to form an ES complex, with a rate constant k 1. The ES complex has two possible fates. It can dissociate to E and S, with a rate constant k -1, or it can proceed to form product P, with a rate constant k 2. Our starting point is that the catalytic rate is equal to the product of the concentration of the ES complex and k 2.

$$V_0 = k_2[ES] \qquad (2)$$

Now we need to express [ES] in terms of known quantities. The rates of formation and breakdown of ES are given by:

$$\text{Rate of formation of ES} = k_1[E][S] \qquad (3)$$

$$\text{Rate of breakdown of ES} = (k_{-1} - k_2)[ES] \qquad (4)$$

To simplify matters, we will work under the steady-state assumption. In a steady state, the concentrations of intermediates, in this case [ES], stay the same even if the concentrations of starting materials and products are changing. This occurs when the rates of formation and breakdown of the ES complex are equal. Setting the right-hand sides of equations 3 and 4 equal gives

$$k_1[E][S] = (k_{-1} + k_2)[ES] \quad (5)$$

By rearranging equation 5, we obtain

$$[E][S]/[ES] = (k_{-1} + k_2)/k_1 \quad (6)$$

Equation 6 can be simplified by defining a new constant, K M, called the *Michaelis constant:*

$$K_M = \frac{k_{-1} + k_2}{k_1} \quad (7)$$

Note that K_M has the units of concentration. K_M is an important characteristic of enzyme-substrate interactions and is independent of enzyme and substrate concentrations. Inserting equation 7 into equation 6 and solving for [ES] yields

$$[ES] = \frac{[E][S]}{K_M} \quad (8)$$

Now let us examine the numerator of equation 8. The concentration of uncombined substrate [S] is very nearly equal to the total substrate concentration, provided that the concentration of enzyme is much lower than that of substrate. The concentration of uncombined enzyme [E] is equal to the total enzyme concentration [E]T minus the concentration of the ES complex

$$[E] = [E]_T - [ES] \quad (9)$$

Substituting this expression for [E] in equation 8 gives

$$[ES] = \frac{([E]_T - [ES])[S]}{K_M} \quad (10)$$

Solving equation 10 for [ES] gives

$$[ES] = \frac{[E]_T[S]/K_M}{1 + [S]/K_M} \quad (11)$$

$$[ES] = [E]_T \frac{[S]}{[S] + K_M} \quad (12)$$

By substituting this expression for [ES] into equation 2,

$$V_0 = k_2[ES]$$

$$V_0 = k_2 (E_T) \frac{(S)}{(S)+K_m} \quad (13)$$

But

$$V_{max} = k_2[E]_T \quad (14)$$

Substituting equation 14 into equation 13 yields the *Michaelis-Menten equation:*

$$V_0 = V_{max} \frac{[S]}{[S] + K_M} \quad (15)$$

- At very low substrate concentration, when [S] is much less than K M, V_0 = (V max/K M)[S]; that is, the rate is directly proportional to the substrate concentration.
- At high substrate concentration, when [S] is much greater than K M, V_0 = V max; that is, the rate is maximal, independent of substrate concentration.
- The meaning of K M is evident from equation 15.

When V_0 = V max/2
Then [S] = Km,

- Thus, K M is equal to the substrate concentration at which the reaction rate is half its maximal value.
- K_m is an important characteristic of an enzyme-catalyzed reaction and is significant for its biological function. For most enzymes, K_m lies between 10^{-1} and 10^{-7} M.

8.3 The Significance of K_m and Vmax Values

- The K_m value for an enzyme depends on the particular substrate and on environmental conditions such as pH, temperature, and ionic strength.
- K_m is the concentration of substrate at which half the active sites are filled. Thus, K_m provides a measure of the substrate concentration required for significant catalysis to occur.
- K_m is equal to the dissociation constant of the ES complex if k_2 is much smaller than k_{-1} .
- High K_m indicates weak binding; a low K_m indicates strong binding. K_m indicates the affinity of the ES complex only when k_{-1} is much greater than k_2.
- The maximal rate, V max, reveals the turnover number of an enzyme, which is the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.

8.4 The Double-Reciprocal Plot

The Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting experimental data.

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

Separating the components of the numerator on the right side of the equation which simplifies to

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

This form of the Michaelis-Menten equation is called the Lineweaver-Burk equation. For enzymes obeying the Michaelis-Menten relationship, a plot of 1/V0 versus 1/[S] yields a straight line. This line has a slope of Km/Vmax, an intercept of 1/Vmax on the 1/V0 axis, and an intercept of -1/Km on the 1/[S] axis. The double-reciprocal presentation, also called a Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of Vmax, which can only be approximated from a simple plot of V0 versus [S]. The double-reciprocal plot of enzyme reaction rates is very useful in distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition.

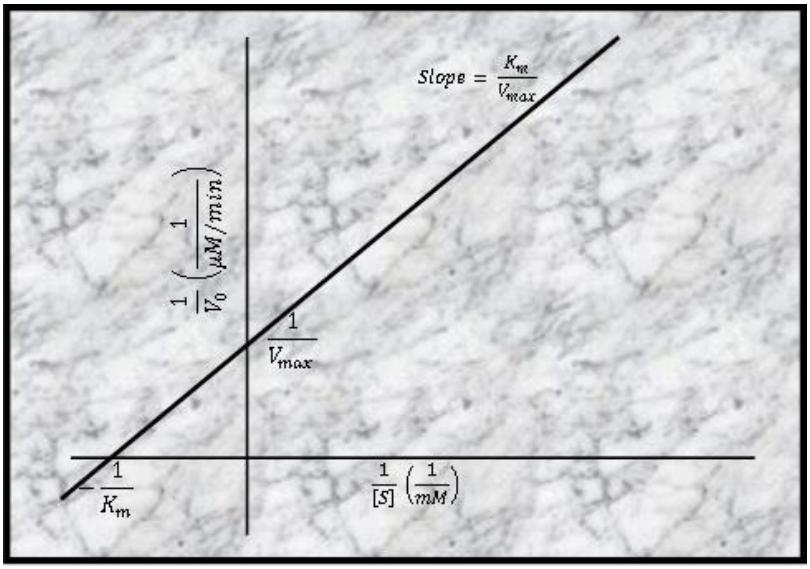


Fig 8.2 The Double-Reciprocal Plot

Lesson 9
MECHANISM OF ENZYME ACTION

9.1 Introduction

- Enzymes do two important things: they recognize very specific substrates, and they perform specific chemical reactions on them at fantastic speeds.
- Their role is to make and break specific chemical bonds of the substrates at a faster rate and to do it without being consumed in the process.
- At the end of each catalytic cycle, the enzyme is free to begin again with a new substrate molecule.
- Catalysis is simply making a reaction go faster, it follows that the activation energy of a catalyzed (faster) reaction is lower than the activation energy of an uncatalyzed reaction. Thus enzymes work by lowering the activation energy of the reaction they catalyze.
- The way they accomplish all this can be described by a number of different models, each one of which accounts for some of the behavior that enzymes exhibit. Most enzymes make use of all these different mechanisms of specificity and/or catalysis.

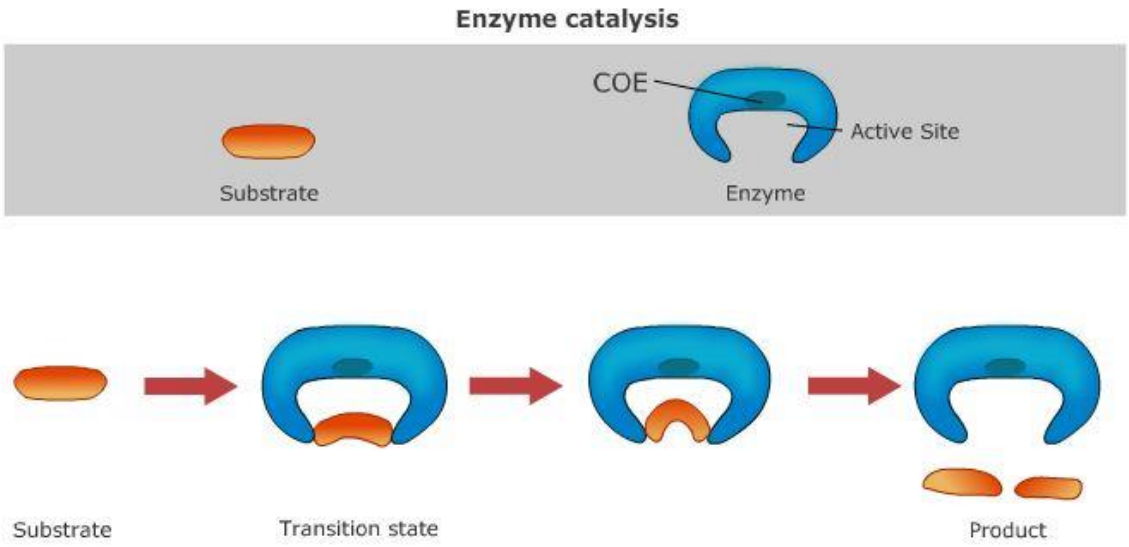


Fig. 9.1 Enzyme catalysis

9.2 Lock-and-Key Model of Enzyme-Substrate Binding

In this model, the active site of the unbound enzyme is complementary in shape to the substrate. As if the key fits in the lock will then open the lock. It accounts for why the enzyme only works on certain substrates.

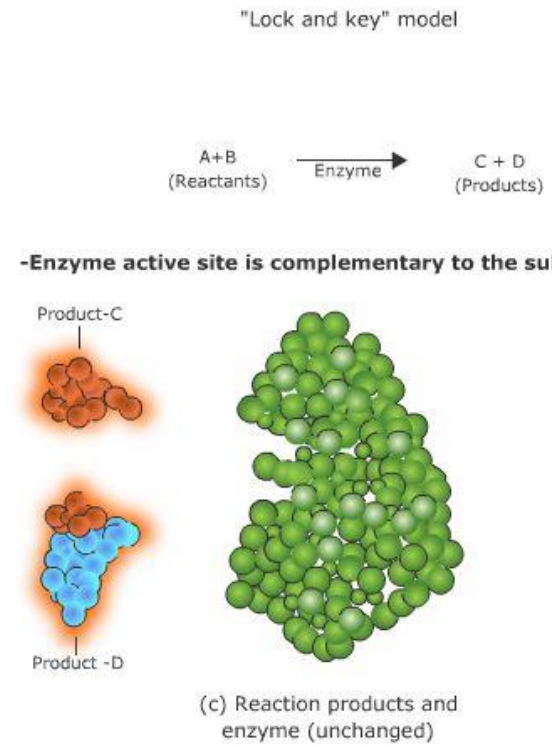


Fig 9.2 Lock-and-key model of enzyme-substrate binding

9.3 Induced-Fit Model of Enzyme-Substrate Binding

In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound. The binding of the correct substrate triggers a change in the structure of the enzyme that brings catalytic groups into exactly the right position to facilitate the reaction. In the induced-fit model, the structure of the enzyme is different depending on whether the substrate is bound or not. The enzyme changes the shape (undergoes a conformational change) on binding the substrate. This conformation change converts the enzyme into a new structure in which the substrate and catalytic groups on the enzyme are properly arranged to accelerate the reaction.

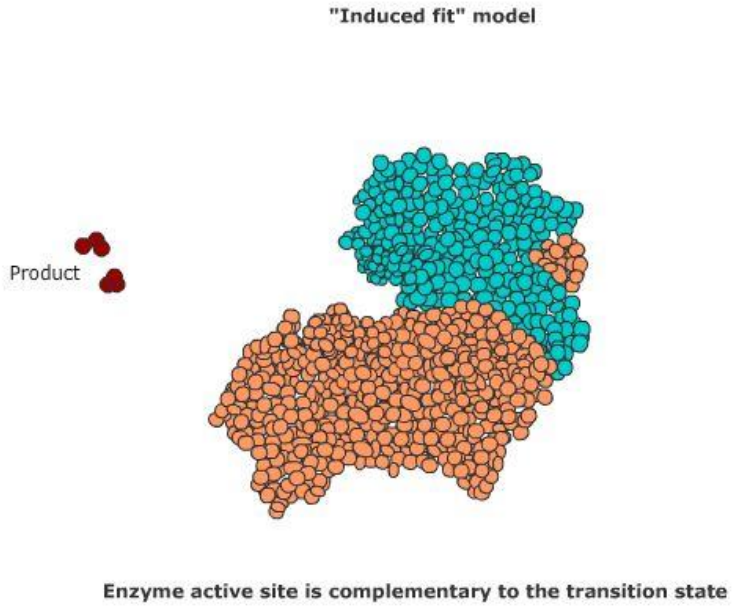


Fig 9.3 Induced-fit model of enzyme-substrate binding

9.4 Multi Substrate Reaction Mechanism

Most reactions in biological systems usually include two substrates and two products and can be represented by the bisubstrate reaction. The majority of such reactions entail the transfer of a functional group, such as a phosphoryl or a hydroxyl group, from one substrate to the other.

$$A + B \rightleftharpoons P + Q$$

There are three general mechanisms which describe multi-substrate enzyme system.

- Ordered mechanism
- Random mechanism
- Ping-Pong mechanism

9.4.1 Ordered mechanism

In this type of reaction all substrates must bind to the enzyme before any product is released. Consequently, in a bisubstrate reaction, a ternary complex of the enzyme and both substrates forms. In ordered mechanism the substrates bind the enzyme in a defined sequence. Many enzymes that have NAD^+ or NADH as a substrate exhibit the sequential ordered mechanism. Consider lactate dehydrogenase, an important enzyme in glucose metabolism. This enzyme reduces pyruvate to lactate while oxidizing NADH to NAD^+. In the ordered sequential mechanism, the coenzyme always binds first and the lactate is always released first.

This sequence is represented below fig 9.3

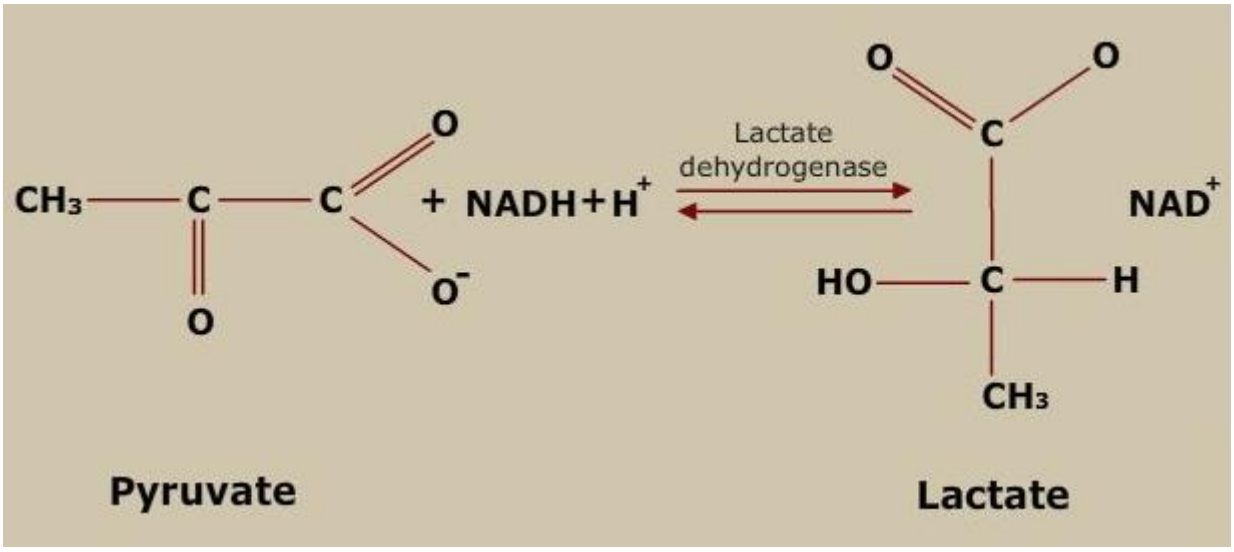


Fig. 9.4 Conversaiton to lactate

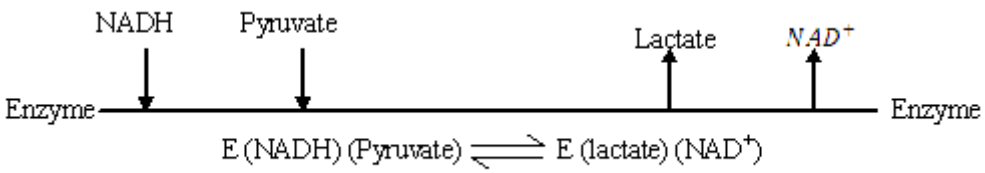


Fig 9.5 Ordered Mechanism

9.4.2 Random mechanism

In this mechanism also enzyme exists as a ternary complex: first, consisting of the enzyme and substrates and, after catalysis, the enzyme and products. In the random sequential mechanism, the order of addition of substrates and release of products is random. Sequential random reactions are illustrated by the formation of phosphocreatine and ADP from ATP and creatine, a reaction catalyzed by creatine kinase. Phosphocreatine is an important energy source in muscle. Sequential random reactions can also be depicted as below. Although the order of certain events is random, the reaction still passes through the ternary complexes including, first, substrates and, then, products.

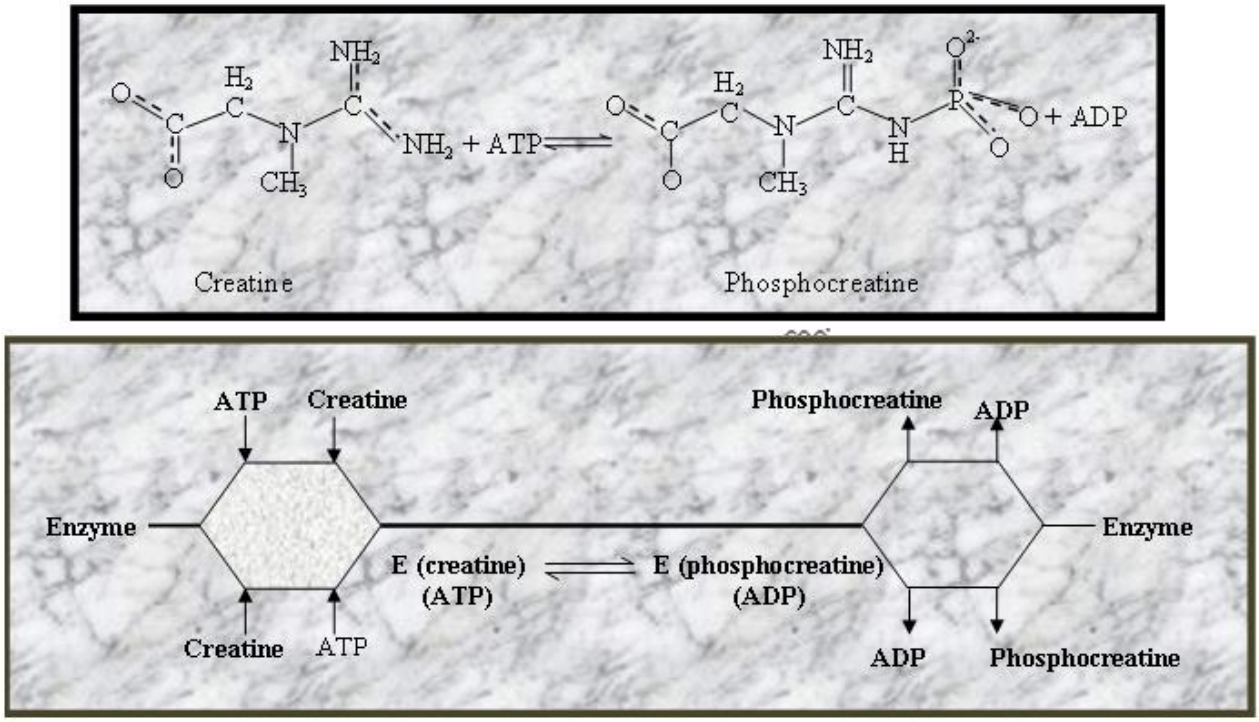
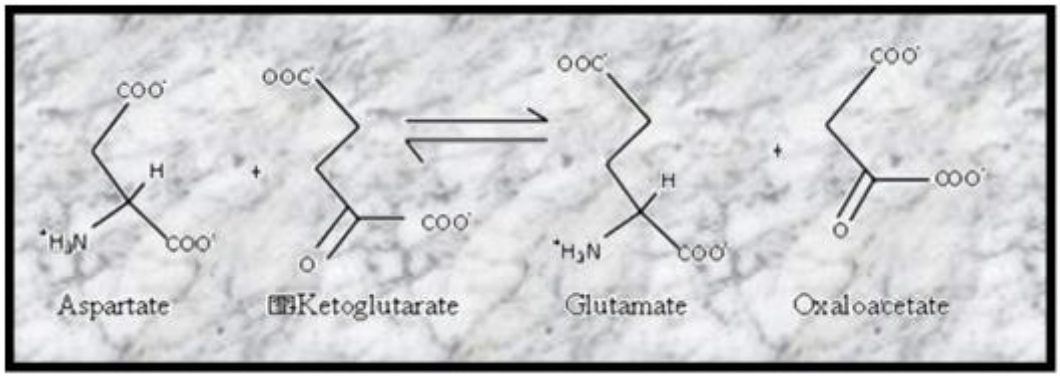


Fig 9.6 Random Mechanism

9.4.3 Double-displacement (Ping-pong) reactions.

In double-displacement, or Ping-Pong, reactions, one or more products are released before all substrates bind the enzyme. The defining feature of double-displacement reactions is the existence of a substituted enzyme intermediate, in which the enzyme is temporarily modified. Reactions that shuttle amino groups between amino acids and α-keto acids are classic examples of double-displacement mechanisms. The enzyme aspartate aminotransferase catalyzes the transfer of an amino group from aspartate to α-ketoglutarate.



The sequence of events can be portrayed as the following diagram.

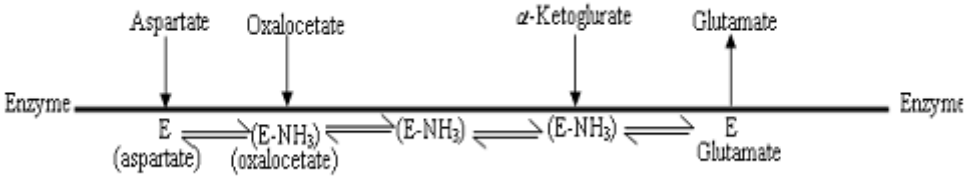


Fig 9.7 Double-displacement (Ping-pong) reactions

After aspartate binds to the enzyme, the enzyme removes aspartate's amino group to form the substituted enzyme intermediate. The first product, oxaloacetate, subsequently departs. The second substrate, α-ketoglutarate, binds to the enzyme, accepts the amino group from the modified enzyme, and is then released as the final product, glutamate. In this, the substrates appear to bounce on and off the enzyme analogously to a Ping-Pong ball bouncing on a table.

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Lesson 10
FACTORS AFFECTING ENZYME ACTIVITY

10.1 Introduction

An enzyme assay measures the conversion of substrate to product, under conditions of cofactors, pH and temperature at which enzyme is optimally active. An enzyme is most conveniently assayed by measuring the rate of appearance of product or the rate of disappearance of substrate. If the substrate absorbs light at a specific wavelength, then changes in concentration of these molecules can be measured by following the change of absorbance at this wavelength. Typically this is carried out using spectrophotometer since absorbance is proportional to the rate of enzyme activity in moles of substrate used (or product formed) per unit time.

10.2 Enzyme Velocity

The rate of an enzyme catalyzed reaction is often called its velocity. It is normally reported as values at time zero (V0 ; micomoles /min) since the rate is fastest at the point where no product is yet present. This is because the substrate concentration is greatest before any substrate has been transformed to product. A typical plot of product formed against time for an enzyme catalyzed reaction show an initial period of rapid product formation which gives the linear portion of the plot. This is followed by a slowing down of the enzyme rate as substrate is used up during the reaction.

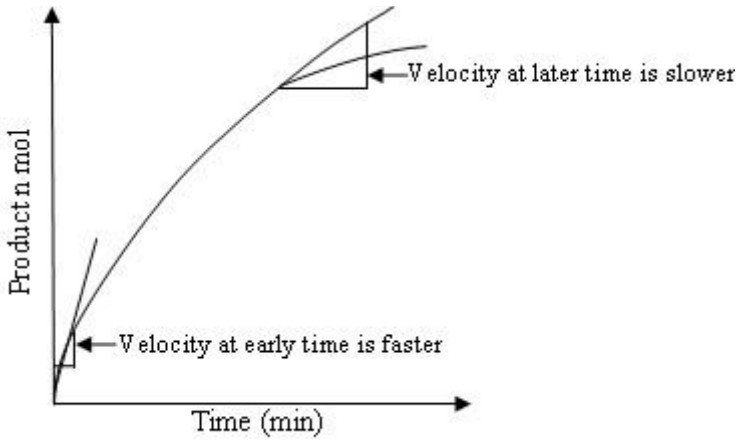

Fig. 10.1 Rate of enzyme-catalyzed reaction with time

10.3 Enzyme Unit

Enzyme activity may be expressed as µmol of substrate transformed per minute (µmol min^{-1}). The standard unit of enzyme activity are enzyme unit and katal (kat). An enzyme unit is that amount of enzyme which catalyse the transformation of 1 µmol of substrate per minute at 25ºC under optimal conditions for that enzyme. The katal is the SI unit of enzyme activity and is defined as that catalytic activity which will raise the rate of reaction by one mole per second in a specified system,. 1U=16.67 nanokatal. The term activity refers to total units of enzyme in the sample, whereas the specific activity is the number of enzyme units per milligram of protein (Units/mg).

10.4 Substrate Concentration

The normal pattern of dependence of enzyme rate on substrate concentration (S) is that at low substrate concentrations a doubling of (S) will lead to doubling of intial velocity (V_0). However, at higher substrate

concentration the enzyme becomes saturated, and further increase in (S) leads to very small changes in V_0 this is called V_{max} . This occurs because at saturating substrate concentrations effectively all of the enzyme molecules have bound to substrate. The over all rate is now dependent on the rate at which the product can dissociate from the enzyme, and adding further substrate will not effect this. The shape of the resulting graph when V_0 is plotted against (S) is called hyperbola curve (Fig 10.2).

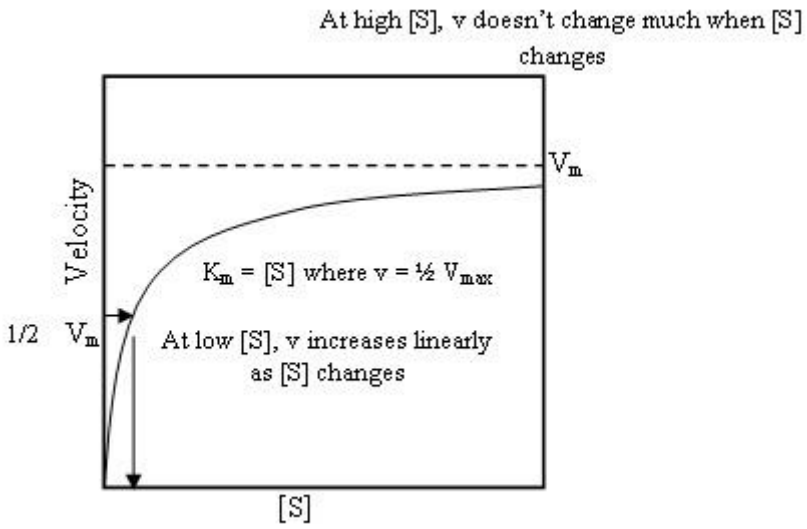

Fig. 10.2 Effect of substrate concentration on enzyme-catalyzed reaction

10.5 Effect of pH

Enzymes have an optimum pH (or pH range) at which their activity is maximal. At higher or lower pH, activity decreases. Amino acid side chains in the active site may act as weak acids and bases with critical functions that depend on their maintaining a certain state of ionization, and elsewhere in the protein ionized side chains may play an essential role in the interactions that maintain protein structure. Large deviations in pH (Fig 10.3) lead to denaturation of enzyme protein itself , due to interference with many weak noncovalent bonds maintaining the three dimensional structure . A graph of V0 plotted against pH will usually give a bell shaped curve. Many enzyme have pH optimum of around 6.8 but there is a great diversity in pH optima of enzyme due to different environments in which they are adapted to work.

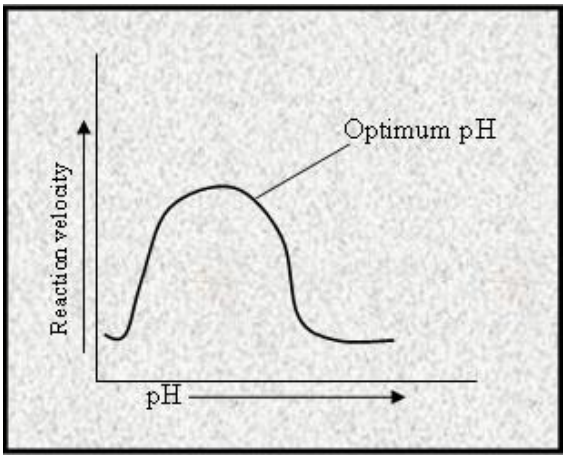

Fig 10.3 Effect of pH on enzyme-catalyzed reaction

10.6 Enzyme Concentration

In situation where the substrate concentration is saturating (i.e all the enzyme molecules are bound to substrate) , doubling the enzyme concentration will lead to doubling of V_0. This gives a straight line graph

when V_0 is plotted against enzyme concentration

10.7 Effect of Temperature

Raising the temperature increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules. However, heat energy can also increase the kinetic energy of the enzyme to a point that exceeds the energy barrier for disrupting the noncovalent interactions that maintain the enzyme's three-dimensional structure. The polypeptide chain then begins to unfold, or denature, with an accompanying rapid loss of catalytic activity. The temperature range over which an enzyme maintains a stable, catalytically competent conformation depends upon—and typically moderately exceeds—the normal temperature of the cells in which it resides. Enzymes from humans generally exhibit stability at temperatures up to 45–55 °C. By contrast, enzymes from the thermophilic microorganisms that reside in volcanic hot springs or undersea hydrothermal vents may be stable up to or above 100 °C.

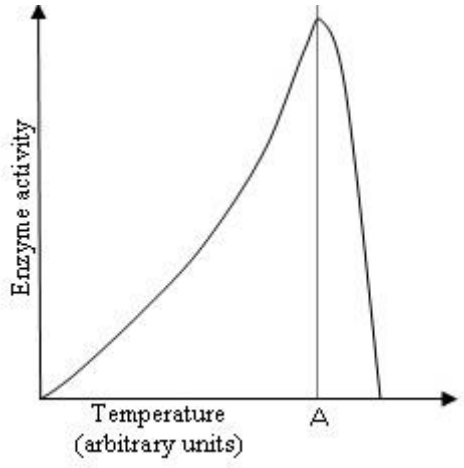

Fig 10.4 Effect of Temperature on enzyme-catalyzed reaction

10.8 Isoenzymes

Isoenzymes are different forms of an enzyme which catalyze the same reaction, but exhibit different physical or kinetic properties, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors. Different isoenzymes forms of a given enzyme are usually derived from different genes and often occur in different tissues of body. Functional lactate dehydrogenase are homo or hetero tetramers composed of M and H protein subunits encoded by the LDHA and LDHB genes respectively:

- LDH-1 (4H) - in the heart and RBCs
- LDH-2 (3H1M) - in the reticuloendothelial system
- LDH-3 (2H2M) - in the lungs
- LDH-4 (1H3M) - in the kidneys, placenta and pancreas
- LDH-5 (4M) - in the liver and striated muscle

The five isoenzymes that are usually described in the literature each contain four subunits. The major isoenzymes of skeletal muscle and liver, M4, has four muscle (M) subunits; while H4 is the main isoenzymes for heart muscle in most species, containing four heart (H) subunits. The other variants contain both types of subunits.

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Lesson 11
ENZYME INHIBITION

11.1 Introduction

- Inhibitors are molecules that often resemble the substrate(s) or product(s) and bind to the active site thus they interfere with catalysis, slowing or halting enzymatic reactions.
- Many drugs are reversible enzyme inhibitors. They have their physiological effect by decreasing the activity of a specific enzyme. For example, aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.
- The concentration of inhibitor needed to inhibit the enzyme depends on how tightly the inhibitor binds to the enzyme.
- The inhibition constant (Ki) is used to describe how tightly an inhibitor binds to an enzyme. The bigger the Ki, the weaker the binding.

11.2 Types of Inhibitors

There are two broad classes of enzyme inhibitors

- Irreversible
- Reversible

11.2.1 Irreversible

The irreversible inhibitors are those that bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or those that form a particularly stable noncovalent association. Formation of a covalent link between an irreversible inhibitor and an enzyme is common. For example reaction of chymotrypsin with diisopropylfluorophosphate (DIFP) irreversibly inhibits the enzyme by binding with Ser195 in the active-site of chymotrypsin.

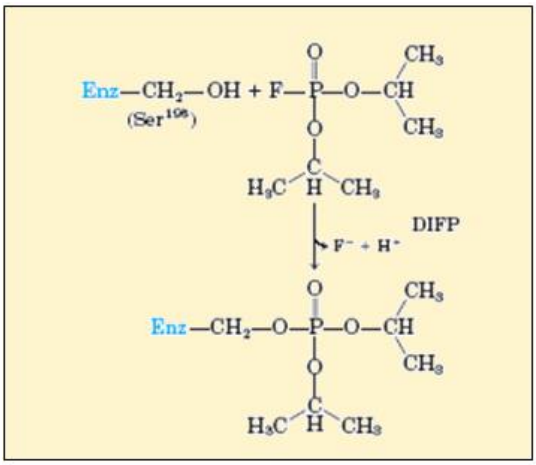


Fig 11.1 Diisopropylfluorophosphate as irreversible inhibitors of chymotrypsin

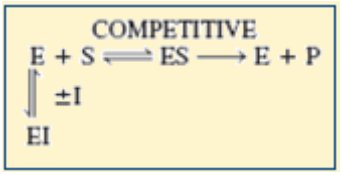
11.2.2 Reversible

This type of inhibition involves equilibrium between enzyme and the inhibitor, the equilibrium constant (ki) being the measure of affinity of the inhibitor for the enzyme. This inhibition is further classified into three categories

- Competitive
- Uncompetitive
- Noncompetitive.

11.3 Competitive Inhibition

Competitive inhibitors bind only to the free enzyme and to the same site as the substrate. Competitive inhibitors are molecules that usually look like the substrate but can't undergo the reaction. At an infinite concentration of the substrate the competitive inhibitor cannot bind to the enzyme since the substrate concentration is high enough that there is virtually no free enzyme present.



Since competitive inhibitors have no effect on the velocity at saturating (V_{max}) concentrations of the substrate, the intercepts of the doublereciprocal plots ($1/V_{max}$) at all the different inhibitor concentrations are the same. The lines at different inhibitor concentrations must all intersect on the y axis at the same $1/V_{max}$. At low concentrations of substrate ([S] << Km), the enzyme is predominantly in the E form. The competitive inhibitor can combine with E, so the presence of the inhibitor decreases the velocity when the substrate concentration is low.

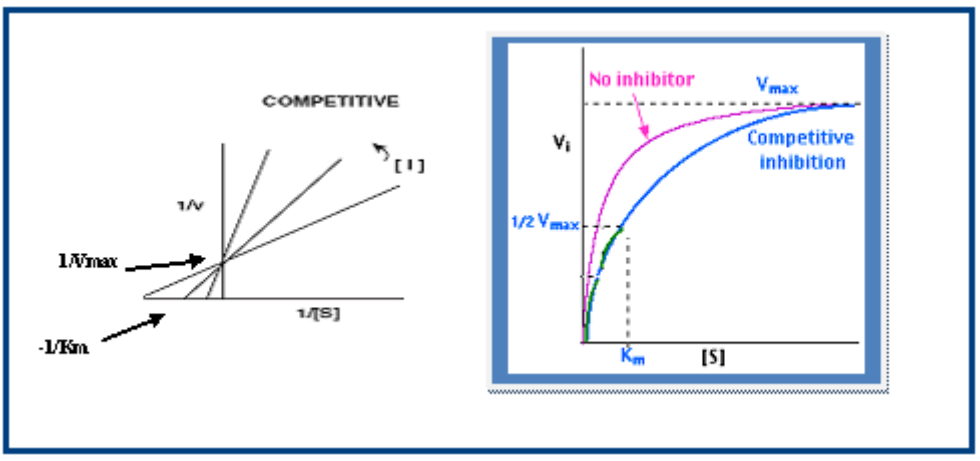
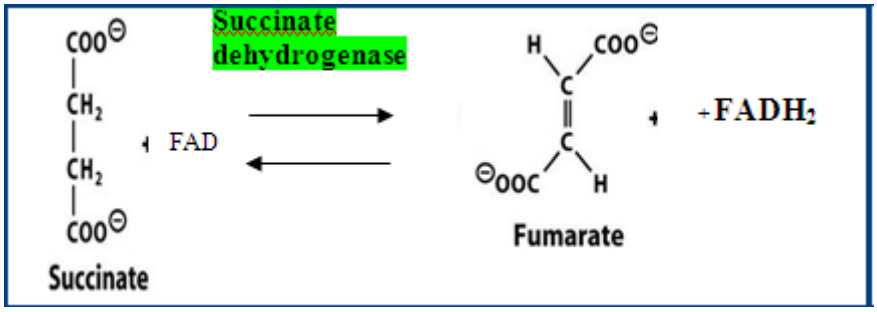


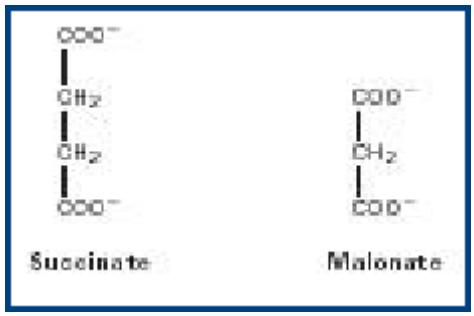
Fig. 11.2 Competitive Inhibition

Under competitive inhibition
Vmax remains unchanged ; Km increases

Example :

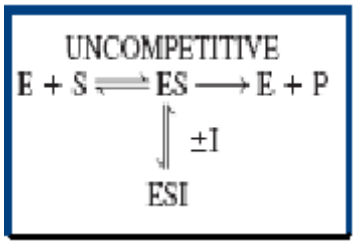


- Malonate is a competitive inhibitor of succinate dehydrogenase. The enzyme uses succinate as its substrate but inhibited by malonate which is structurally similar to succinate and differs in having one rather than two methylene groups.



11.4 Uncompetitive Inhibition

If the inhibitor combines only with ES (and not E), the inhibitor exerts its effect only at high concentrations of substrate at which there is lots of ES around. This means that the increasing substrate concentration (S) doesn't prevent the binding of the inhibitor. Interestingly Km value is consistently smaller than Km value of the uninhibited reaction, which implies that S is more effectively bound to the ezyme in the presence of the inhibitor. The sequence of this type of reaction is



This type of inhibition is often observed for enzymes that catalyze the reaction between two substrates. Often an inhibitor that is competitive against one of the substrates is found to give uncompetitive inhibition when the other substrate is varied. The inhibitor does combine at the active site but does not prevent the binding of one of the substrates (and vice versa). In this type of inhibition Vmax as well as Km both are decreased

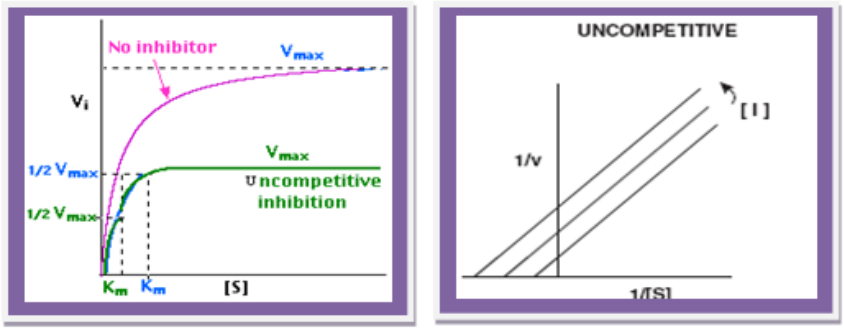


Fig. 11.3 Uncompetitive Inhibition

11.5 Noncompetitive Inhibition

Compounds that reversibly bind with either the enzyme or the enzyme substrate complex are designed as noncompetitive inhibitors and the following reaction describe these events.

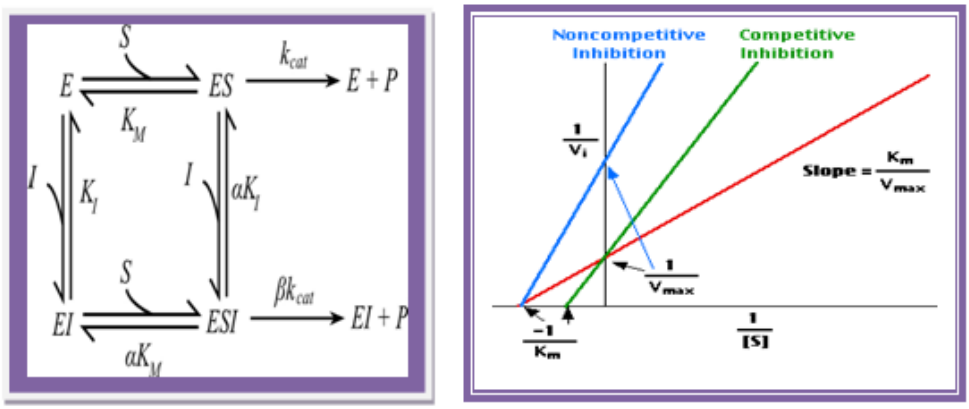
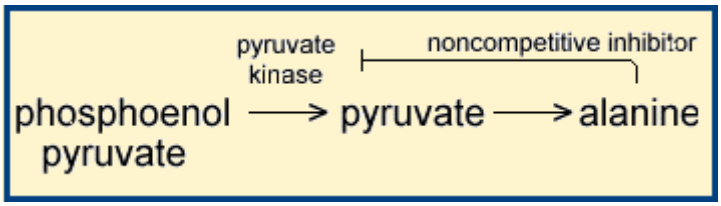


Fig. 11.4 Noncompetitive Inhibition

Noncompetative inhibition therefore differs from competitive inhibition in that the inhibitor can combine with ES, and S can combine with EI to form in both instances EIS. This type of inhibition is not completely reversed by high substrate concentration since closed sequence will occur regardless of the substrate concentration. Since inhibitor binding site is not identical to nor does it modify the active site directly, the Km is not altered but Vmax is decreased.

For example, the amino acid alanine noncompetitively inhibits the enzyme pyruvate kinase. Alanine is one product of a series of enzyme-catalyzed reactions, the first step of which is catalyzed by pyruvate kinase.



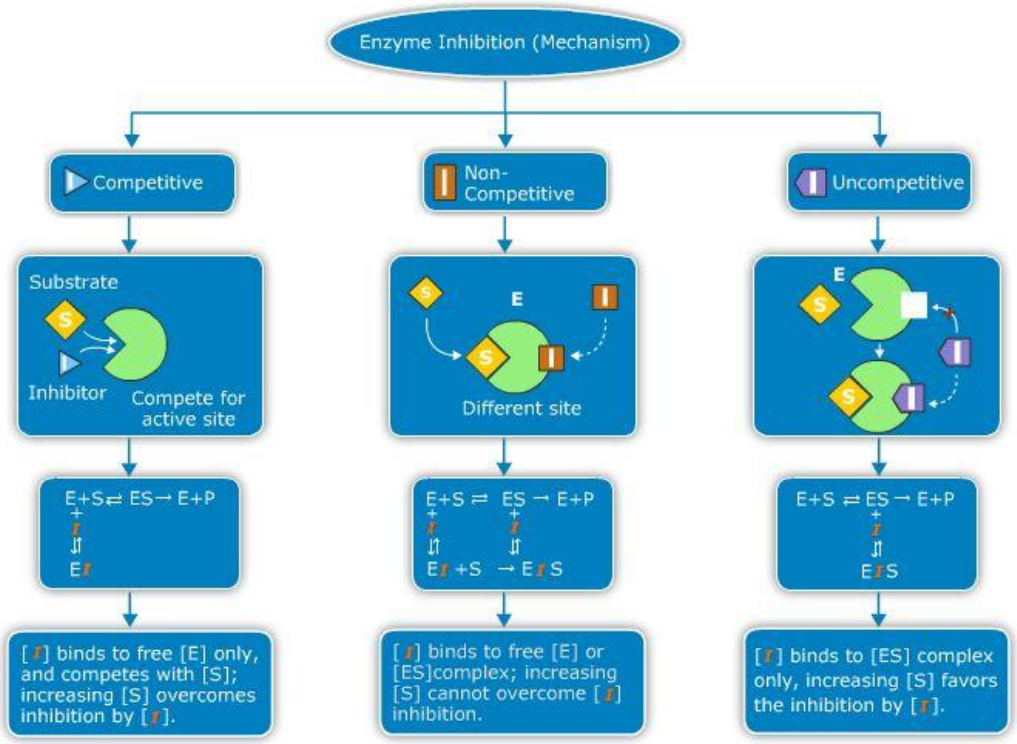


Fig. 11.5 Enzyme inhibition

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Lesson 12
REGULATORY ENZYMES

12.1 Introduction

- A regulatory enzyme is an enzyme in a biochemical pathway which, through its responses to the presence of certain other bio-molecules, regulates the pathway's activity.
- This is usually done for pathways whose products may be needed in different amounts at different times, such as hormone production.
- Regulatory enzymes are usually the enzymes that are the rate-limiting or committed step, in a pathway, meaning that after this step a particular reaction pathway will go to completion.
- Frequently, regulatory enzymes are at or near the initial steps in a pathway, or part of a branch point or cross-over point between pathways (where a metabolite can be potentially converted into several products in different pathways). In general, a cell needs to conserve energy - therefore costly (in metabolic terms) biosynthetic reaction pathways will not be operational unless a particular metabolite is required at a given time.
- Regulatory enzymes control the overall quantities of enzyme or concentration of substrates present or lead to alteration of the catalytic efficiency of the enzyme

12.2 Types of Regulatory Enzymes

Regulatory enzymes are of two types

- Allosteric enzymes
- Covalently modulated enzymes.

12.2.1 Allosteric enzymes

Allosteric means an additional space/site to active site where modulator (effector) molecule interacts with enzyme.

- So allosteric enzymes have additional site to active site where modulator interacts.
- The rates of enzyme-catalyzed reactions in biological systems are altered by activators and inhibitors, collectively known as effect or molecules or modulators.
- Interaction of modulator with enzyme is reversible and non-covalent.
- Allosteric enzymes generally have two or more polypeptides and are more complex than non regulatory enzymes.
- In allosteric enzymes, the binding of a substrate molecule to one active site affects the binding of other molecules of substrate to other active sites in the enzyme. Thus different active sites behave cooperatively. Allosteric enzymes are multi-subunit proteins, with one or more active sites on each subunit. The binding of substrate at one active site induces a conformational change in the protein that is conveyed to other active sites, altering their affinity for substrate molecules.
- A plot of V_0 against (S) for allosteric enzyme gives a sigmoidal curve rather than the hyperbolic plots predicted by the Michaelis-Menten equation for non-regulatory enzymes. The sigmoidicity is thought to result from the cooperativity of structural changes between enzyme subunits (again similar to oxygen binding to hemoglobin). NOTE: A true Km cannot be determined for allosteric enzymes, so a comparative constant like $S_{0.5}$ or $K_{0.5}$ is used.
- The curve has a steep section in the middle of the substrate concentration. So there is a rapid increase in the enzyme velocity which occurs over a narrow range of substrate concentration. This property makes the allosteric enzymes more sensitive towards substrate concentration.

- These enzymes are controlled by effectors molecules or modulators (activator/inhibitor) that bind to the enzyme at a site other than the active site(either on same subunit or on other subunit) , thereby causing a change in conformation of active site which alters the rate of enzyme activity.

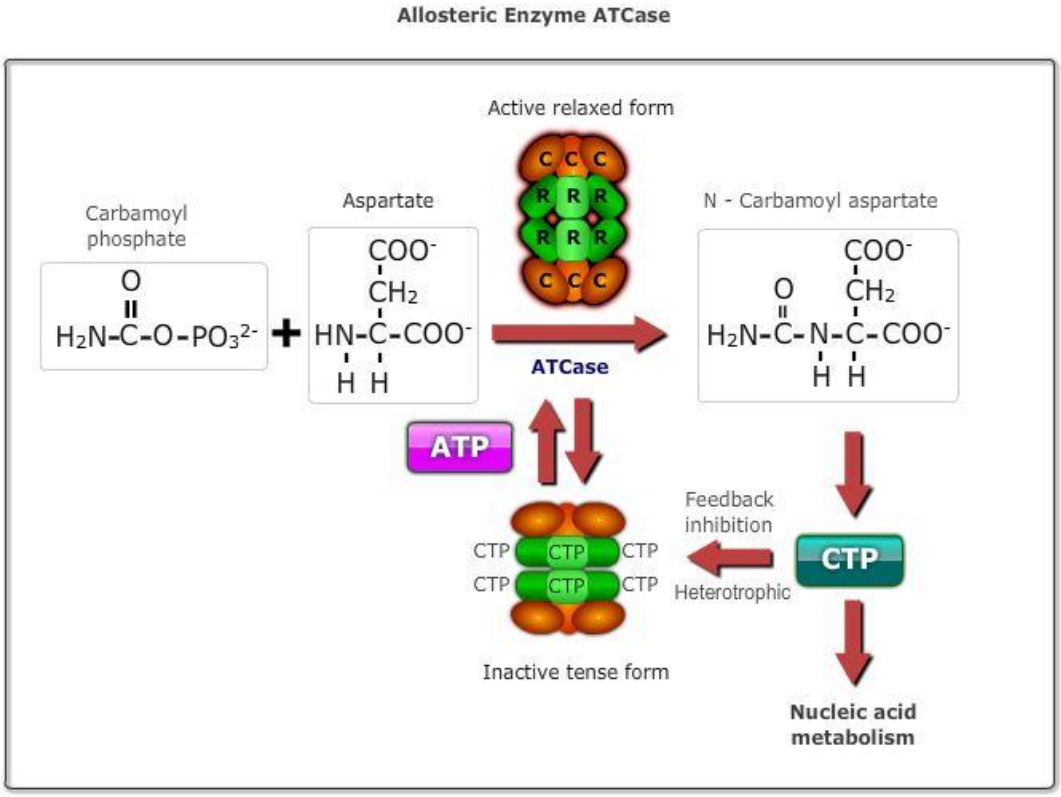


Fig. 12.1 Example of Allosteric enzyme-1

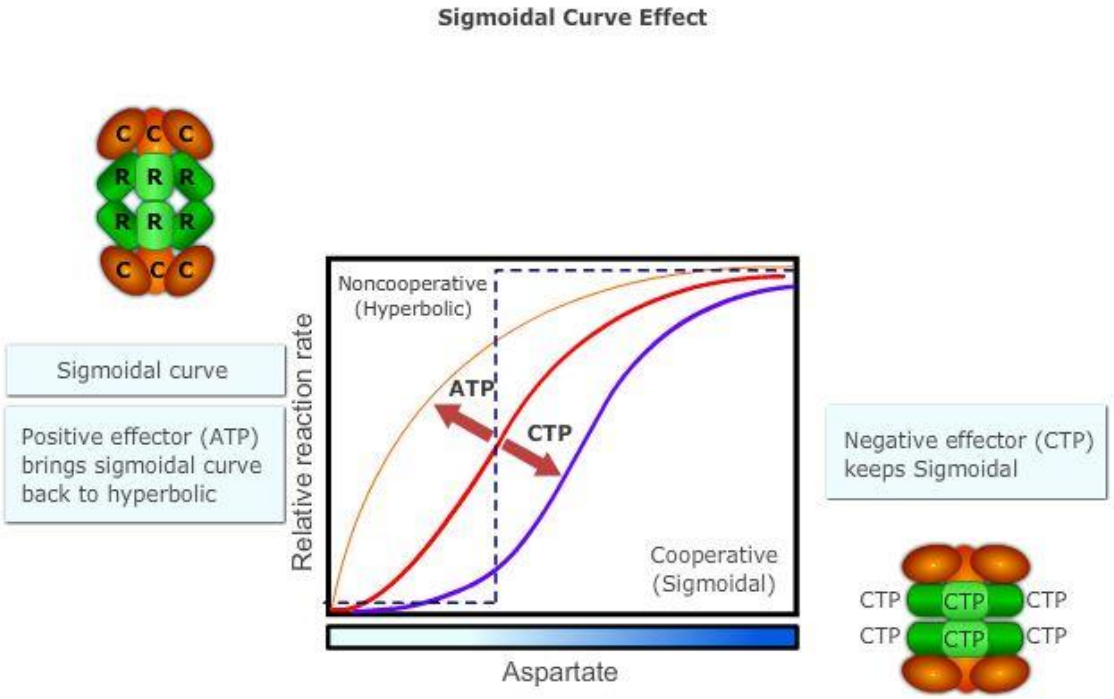


Fig 12.2 Sigmoidal curve of Allosteric enzyme-2

- Cooperativity - in relation to multiple subunit enzymes, changes in the conformation of one subunit leads to conformational changes in adjacent subunits. These changes occur at the tertiary and quaternary levels of protein organization and can be caused by an allosteric regulator.
- Homotropic regulation - when binding of one molecule to a multi-subunit enzyme causes a conformational shift that affects the binding of the same molecule to another subunit of the enzyme.
- Heterotropic regulation - when binding of one molecule to a multi-subunit enzyme affects the binding of a different molecule to this enzyme (Note: These terms are similar to those used for oxygen binding to hemoglobin)

12.2.2 Feedback Inhibitions

- In metabolic pathway the end products often inhibits the committed step earlier in the same pathway to prevent the buildup of intermediates and unnecessary use of metabolites and energy and the process is known as feedback inhibition.
- End product inhibition is negative feedback used to regulate the production of a given molecule.

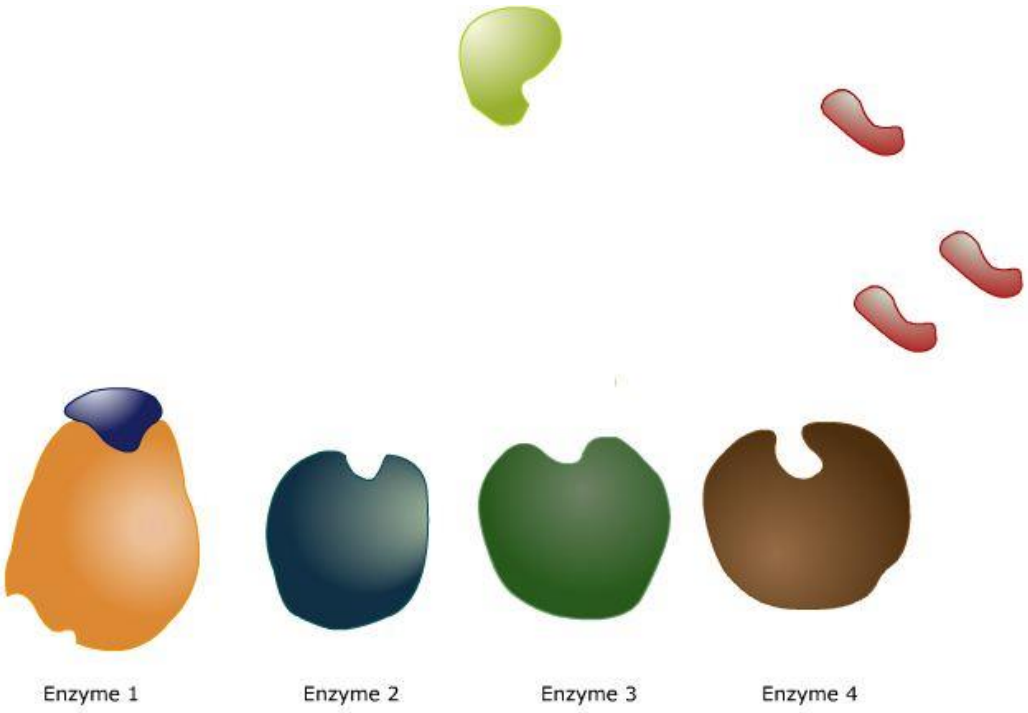


Fig.12.3 Feedback inhibition

The initial substrate is a molecule that is altered in three steps by enzymes 1,2 and 3. The end product will combine with enzyme 1 to stop the reaction so there will not be an excess production of the end product.

12.2.3 Reversible covalent modification

- It involves making or breaking of covalent bond between nonprotein group and an enzyme molecule.
- A range of nonprotein groups may be reversibly attached to enzymes which effect their activity. The most common modification is the addition and removal of a phosphate group called as phosphorylation or dephosphorylation respectively.
- Phosporylation is catalysed by protein kinases, often using ATP as the phosphate donar, and deposphorylation is catalysed by protein phosphatases. The addtion and deletion of phosphate group causes changes in the tertiary structure of the enzyme that alter its catalytic activity.
- Serine kinases transfer the phosphate groups specifically to serine residues on the target enzyme. Similarly tyrosine kinases transfer phosphate groups to tyrosine residues of target enzymes and make them active or inactive.

- A phosphorylated enzyme may be either more or less active than its dephosphorylated form. Thus phosphorylation and dephosphorylation may be used as a rapid, reversible switch to turn a metabolic pathway on or off according to the needs of cell.
- For example glycogen phosphorylase, an enzyme involved in glycogen breakdown, is active in its phosphorylated form and glycogen synthetase involved in glycogen synthesis is most active in its dephosphorylated form.

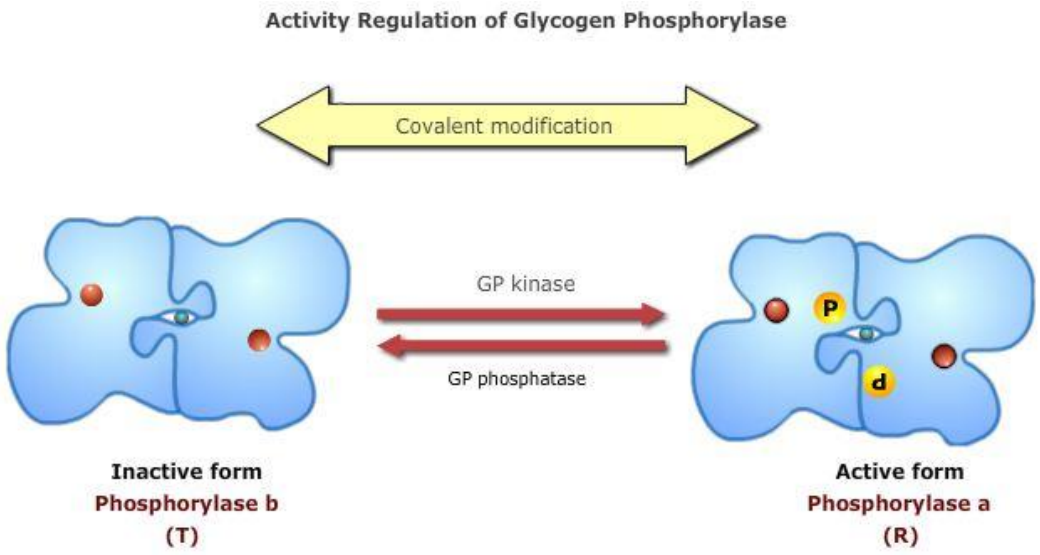


Fig. 12.4 Active regulation of glycogen phosphorylase

12.3 Isozymes

- An enzyme which has multiple molecular forms in the same organism catalyzing the same reaction is known as isozyme. Example is Lactate dehydrogenase (LDH)
- LDH occur in five possible forms in organs of most vertebrates, as observed by electrophoretic separation.
- Basically two different types of LDH occur. One type, which predominates in the Heart, is called heart LDH (H4). The second types are characteristic of Muscles (M4).
- The Heart LDH consists of four identical monomers which are called H subunits. The muscle enzyme consists of four identical M subunits. The two types of subunits H and M, have same molecular weight (35000) but different amino acid composition.
- There is genetic evidence that two subunits are produced by two separate genes.
- Combination of H and M subunits will produce three additional types of hybrid enzymes. These possible combinations are M4, M3H, M2H2, MH3, H4.
- These various combinations have different kinetic properties, depending on the physiological roles which they perform.

Fig 12.6 Isozymes of lactate dehydrogenase

Lesson 13
IMMOBILIZATION OF ENZYMES

13.1 Introduction

Enzyme immobilization may be defined as confining the enzyme molecules to a distinct phase from the one in which the substrates and the products are present. It is the process of attachment of an enzyme to a solid matrix so that it cannot escape but can still act on its substrate.

13.2 Materials

The materials used for immobilization of enzymes, called carrier matrices, are usually inert polymers or inorganic materials
The ideal carrier matrix has the following properties:

1. Low cost,
2. Inertness,
3. Physical strength
4. Stability
5. Regenerability after the useful lifetime of the immobilized enzyme,
6. Enhancement of enzyme specificity,
7. Reduction in product inhibition,
8. A shift in the ph optimum for enzyme action to the desired value for the process, and reduction in microbial contamination and nonspecific adsorption

13.3 Methods used for the Immobilization of Enzymes

13.3.1 Physical adsorption onto an inert carrier

- Adsorption of enzymes onto insoluble supports is a very simple method of wide applicability and capable of high enzyme loading (about one gram per gram of matrix).
- Simply mixing the enzyme with a suitable adsorbent, under appropriate conditions of pH and ionic strength, followed, after a sufficient incubation period, by washing off loosely bound and unbound enzyme will produce the immobilised enzyme in a directly usable form.
- The driving force causing this binding is usually due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule.
- The particular choice of adsorbent depends principally upon minimizing leakage of the enzyme during use.
- Although the physical links between the enzyme molecules and the support are often very strong, they may be reduced by many factors including the introduction of the substrate. Care must be taken that the binding forces are not weakened during use by inappropriate changes in pH or ionic strength.
- Examples of suitable adsorbents are ion-exchange matrices, porous carbon, clays, hydrous metal oxides, glasses and polymeric aromatic resins.

13.3.2 Covalent binding to a reactive insoluble support

- Only small amounts of enzymes may be immobilised by covalent binding (about 0.02 gram per gram of matrix).
- The strength of binding is very strong, however, and very little leakage of enzyme from the support occurs.

- The relative usefulness of various groups, found in enzymes, for covalent link formation depends upon their availability and reactivity (nucleophilicity), in addition to the stability of the covalent link, once formed.
- The reactivity of the protein side-chain nucleophiles is determined by their state of protonation (i.e. charged status) and roughly follows the relationship $^{-}S^{-} > ^{-}SH > ^{-}O^{-} > ^{-}NH_2 > ^{-}COO^{-} > ^{-}OH >> ^{-}NH3^{+}$where the charges may be estimated from a knowledge of the pKa values of the ionising groups and the pH of the solution.
- Lysine residues are found to be the most generally useful groups for covalent bonding of enzymes to insoluble supports due to their widespread surface exposure and high reactivity, especially in slightly alkaline solutions. They also appear to be only very rarely involved in the active sites of enzymes.

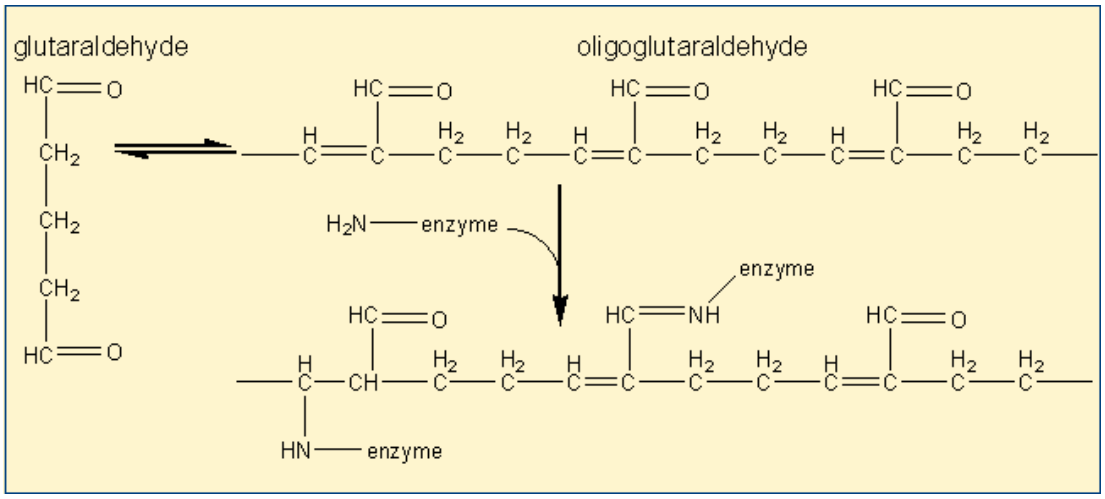
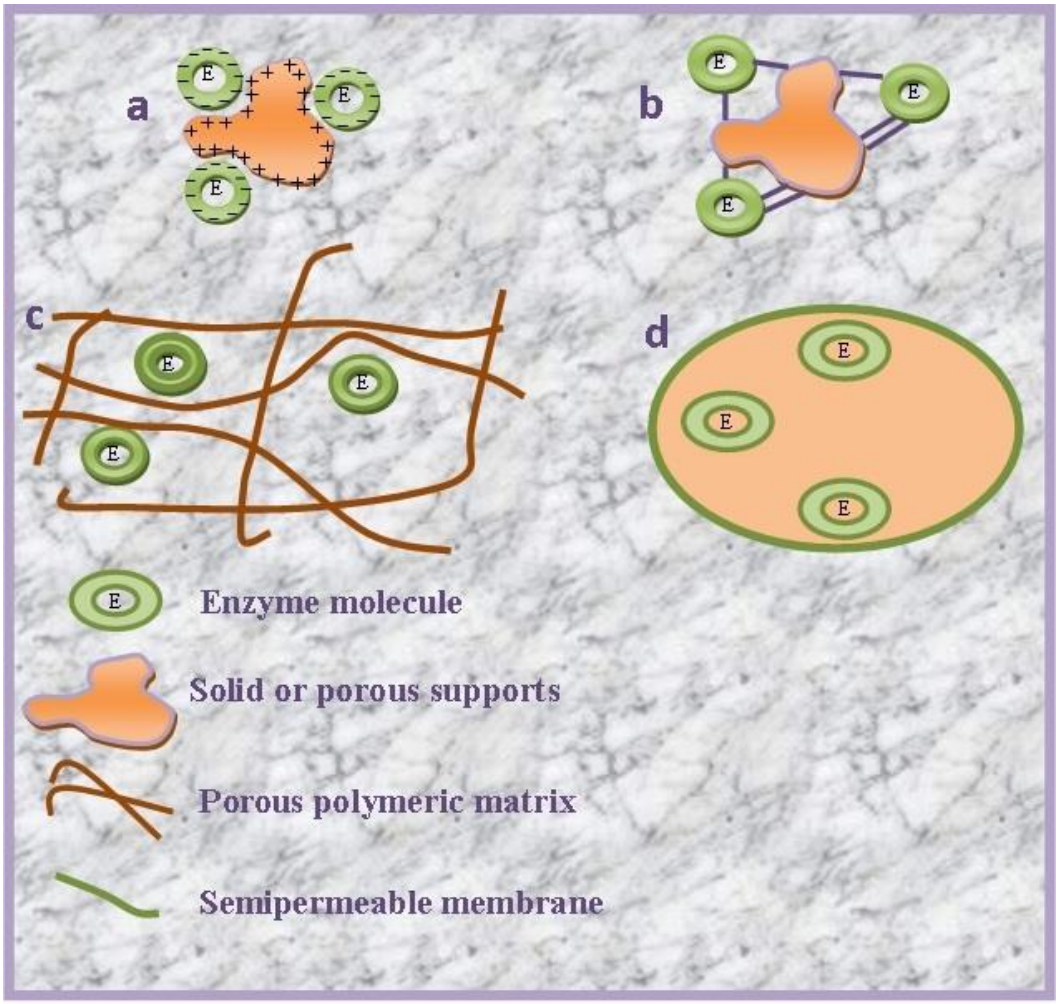


Fig 13.1 Commonly used method for the covalent immobilisation of enzymes

13.3.3 Inclusion in the lattices of a polymerized gel or entrapment or membrane confinement

- Entrapment of enzymes within gels or fibres is a convenient method for use in processes involving low molecular weight substrates and products.
- Amounts in excess of 1 g of enzyme per gram of gel or fibre may be entrapped.
- Large molecules have difficulty in approaching the catalytic sites of entrapped enzymes precludes the use of entrapped enzymes with high molecular weight substrates.
- The entrapment process may be a purely physical caging or involve covalent binding.
- As an example of this latter method, the enzymes' surface lysine residues may be derivatised by reaction with acryloyl chloride (CH_2=CH-CO-Cl) to give the acryloyl amides. This product may then be copolymerised and cross-linked with acrylamide (CH2=CH-CO-NH_2) and bisacrylamide (H_2N-CO-CH=CH-CH=CH-CO-NH_2) to form a gel.
- Enzymes may be entrapped in cellulose acetate fibres by, for example, making up an emulsion of the enzyme plus cellulose acetate in methylene chloride, followed by extrusion through a spinneret into a solution of an aqueous precipitant.
- Entrapment is the method of choice for the immobilisation of microbial, animal and plant cells, where calcium alginate is widely used.

(a) enzyme non-covalently adsorbed to an insoluble particle;
(b) enzyme covalently attached to an insoluble particle;
(c) enzyme entrapped within an insoluble particle by a cross-linked polymer;
(d) enzyme confined within a semipermeable membrane



(a) enzyme non-covalently adsorbed to an insoluble particle;
(b) enzyme covalently attached to an insoluble particle;
(c) enzyme entrapped within an insoluble particle by a cross-linked polymer;
(d) enzyme confined within a semipermeable membrane

Fig 13.2 Immobilised enzyme systems

13.4 Utility of Enzyme Immobilization

The use of immobilized enzyme eliminate the enzyme separation step from the main process thus simplifying and increasing the overall process yield.

- Easy separation from reaction mixture, providing the ability to control reaction times and minimize the enzymes lost in the product.
- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.
- Ability of enzymes to replace multiple standard chemical steps and provide commercially pure products.

13.5 Properties of Immobilized Enzymes

It is important to understand the changes in physical and chemical properties which an enzyme would be expected to undergo upon insolubilization if the best use is to be made of the various insolubilization techniques available. Changes have been observed in the stability of enzymes and in their kinetic properties because of the microenvironment imposed upon them by the supporting matrix and by the products of their own action.

13.5.1 Stability

The stability of the enzymes might be expected to either increase or decrease on insolubilization, depending on whether the carrier provides a microenvironment capable of denaturing the enzymic protein or of

stabilizing it. Inactivation due to autodigestion of proteolytic enzymes should be reduced by isolating the enzyme molecules from mutual attack by immobilizing them on a matrix. It has been found that enzymes coupled to inorganic carriers were generally more stable than those attached to organic polymers when stored at 4 or 23 °C. Stability to denaturing agents may also be changed upon insolubilization.

13.5.2 Kinetic properties

Changes in activity of enzymes due to the actual process of insolubilization have not been studied in detail. There is usually a decrease in specific activity of an enzyme upon insolubilization, and this can be attributed to denaturation of the enzymic protein caused by the coupling process. Once an enzyme has been insolubilized, however, it finds itself in a microenvironment that may be drastically different from that existing in free solution. The new microenvironment may be a result of the physical and chemical character of the support matrix alone, or it may result from interactions of the matrix with substrates or products involved in the enzymatic reaction.

The diffusion of substrate from the bulk solution to the micro-environment of an immobilized enzyme can limit the rate of the enzyme reaction. The rate at which substrate passes over the insoluble particle affects the thickness of the diffusion film, which in turn determines the concentration of substrate in the vicinity of the enzyme and hence the rate of reaction.

The effect of the molecular weight of the substrate can also be large. Diffusion of large molecules will obviously be limited by steric interactions with the matrix, and this is reflected in the fact that the relative activity of bound enymes towards high molecular weight substrates has been generally found to be lower than towards low molecular weight substrates. This, however, may be an advantage in some cases, since the immobilized enzymes may be protected from attack by large inhibitor molecules.

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Lesson 14
ZYMOGENS AND RIBOZYMES

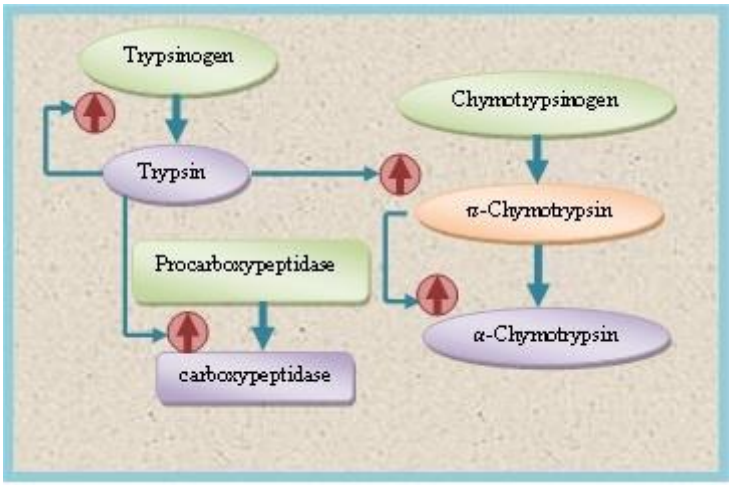
14.1 Introduction (Zymogens)

- Several enzymes are synthesised as larger inactive precursor forms called proenzymes or zymogens.
- Activation of zymogens involves irreversible hydrolysis of one or more peptide bonds.
- The biochemical change usually occurs in a lysosome where a specific part of the precursor enzyme is cleaved in order to activate it. The amino acid chain that is released upon activation is called the activation peptide.
- The pancreas secretes zymogens partly to prevent the enzymes from digesting proteins in the cells in which they are synthesised.
- Fungi also secrete digestive enzymes into the environment as zymogens. The external environment has a different pH than inside the fungal cell and this changes the zymogen's structure into an active enzyme.

14.2 Digestive Enzymes as Zymogens

The digestive enzymes trypsin, chymotrypsin and elastase produced as zymogens in the pancreas.

- They are transported to the small intestine as their zymogens forms and activated there by cleavage of specific peptide bonds.
- Trypsin is synthesized initially as zymogen trypsinogen. It is cleaved (and hence activated) in the intestine by the enzyme enteropeptidase which is only produced in the intestine. Once activated trypsin can cleave and activate further trysinogen molecules as well as other zymogens, such as chymotrysinogen and proelastases.
- The peptidase in the stomach is pepsin. Pepsin works optimally in the acidic environment of the stomach, being active at pH 2-3, but becoming inactivated when the pH is above 5. The chief cells at the base of the gastric glands secrete the zymogen, which is called pepsinogen. Pepsinogen is partially activated by hydrocholoric acid (HCl), which is secreted by the parietal cells. This partially active enzyme then cleaves the peptide from other pepsinogen molecules to form active pepsin.



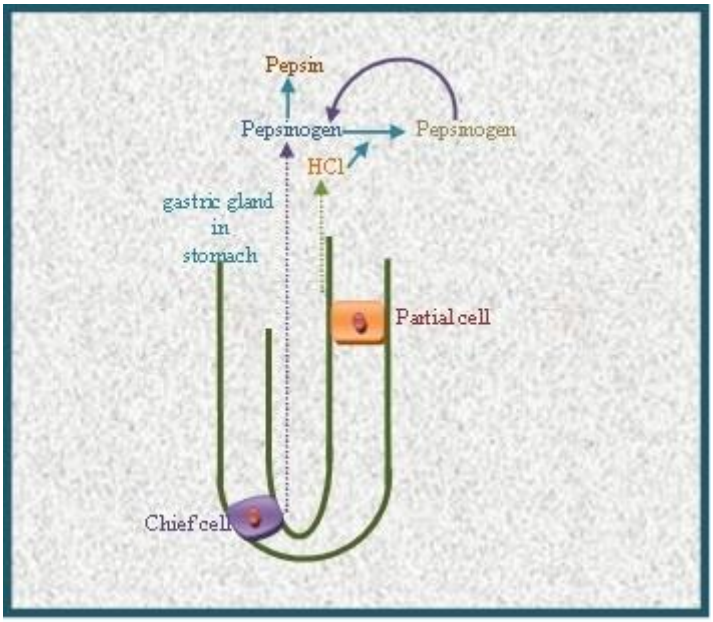


Fig 14.1 Digestive enzymes as zymogens

14.3 Biological Significance of Zymogens

- Zymogens: Inactive Precursor Proteins. A clinically important mechanism of controlling enzyme activity is the case of protease enzymes involved (predominantly) in food digestion and blood clotting.
- Activation of zymogens by proteolytic cleavage result in irreversible activation.
- Zymogen forms allow proteins to be transported or stored in inactive forms that can be readily converted to active forms in response to some type of cellular signal.
- Thus they represent a mechanism whereby the levels of an enzyme/protein can be rapidly increased (post-translationally). Other examples of zymogens include proinsulin, procollagen and many blood clotting enzymes

14.4 Ribozymes

- Before the discovery of ribozymes, enzymes, which are defined as catalytic proteins were the only known biological catalysts.
- The first ribozymes were discovered in the 1980s by Thomas R. Cech, who was studying RNA splicing in the ciliated protozoan Tetrahymena thermophila and Sidney Altman, who was working on the bacterial RNase P complex.
- These ribozymes were found in the intron of an RNA transcript, which removed itself from the transcript, as well as in the RNA component of the RNase P complex, which is involved in the maturation of pre-tRNAs. Ribozymes often have divalent metal ions such as Mg^{2+} as cofactors.
- A ribozyme (from ribonucleic acid enzyme, also called RNA enzyme or catalytic RNA) is an RNA molecule that catalyzes a chemical reaction. Many natural ribozymes catalyze either the hydrolysis of one of their own phosphodiester bonds, or the hydrolysis of bonds in other RNAs. They have also been found to catalyze the aminotransferase activity of the ribosome.
- Investigators studying the origin of life have produced ribozymes in the laboratory that are capable of catalyzing their own synthesis under very specific conditions, such as an RNA polymerase ribozyme.
- Some ribozymes may play an important role as therapeutic agents, as enzymes which tailor defined RNA sequences, as biosensors, and for applications in functional genomics and gene discovery.

14.5 Activity

- Although most ribozymes are quite rare in the cell, their roles are sometimes essential to life. For example, the functional part of the ribosome, the molecular machine that translates RNA into proteins, is fundamentally a ribozyme, composed of RNA tertiary structural motifs that are often coordinated to metal ions such as Mg^{2+} as cofactors. There is no requirement for divalent cations in a five-nucleotide RNA that can catalyze trans-phenylalanation of a four-nucleotide substrate which has three base complementary sequence with the catalyst

Lesson 15
DETERMINATION OF ENZYME ACTIVITY

15.1 Introduction

- Enzyme activity may be expressed as µmol of substrate transformed per minute (µmol min^{-1}).
- The standard unit of enzyme activity is enzyme unit and katal (kat).
- An enzyme unit is that amount of enzyme which catalyzes the transformation of 1 µmol of substrate per minute at 25^0C under optimal conditions for that enzyme.
- The Katal is the SI unit of enzyme activity and is defined as that catalytic activity which will raise the rate of reaction by one mole per second in a specified system. 1U=16.67 nanokatal.
- The term activity refers to total units of enzyme in the sample, whereas the specific activity is the number of enzyme units per milligram of protein (Units/mg).

15.2 Effect of Enzyme Concentration on Enzyme Activity

15.2.1 Alkaline phosphatase : E.C3.1.3.1 (Orthophosphoric-monoester phosphohydrolase)

15.2.1.1 Principle

Alkaline phosphates hydrolyzes p-nitrophenyl phosphate, which is colourless, to p-nitrophenol which is yellow in alkaline medium. Measurement of O.D, at 400-420 nm helps to determine the concentration of p-nitrophenol, which in turn is directly proportional to p-nitrophenyl phosphate. In alkaline solution, p-nitrophenol absorbs at 405 nm. The substrate p-nitrophenyl phosphate does not absorb at this wavelength, so that the progress of the enzyme catalyzed reaction can be readily followed by measuring the change in extinction at 405 nm. The optimum pHs for acid and alkaline phosphatases are 5.3 and 10.5 respectively.

15.2.1.2 Reagents

- 50 mM Carbonate-bicarbonate buffer (pH 10.5)
- Standard Solution: p-nitrophenol solution 0.5 mM, prepared in above buffer.
- Calculation: 1mM = 139 mg/l or 0.5mM = 69.5mg/l or 34.75 mg/500ml buffer.
- Substrate Solution : p-nitrophenyl phosphate (PNP)

10 mM, dissolved in above buffer (M.W. of PNP disodium salt = 371.16).

15.2.1.3 Calculation

1mM = 371.16 mg/l
10mM = 3711.6 mg/l or 371.16 mg/100 ml buffer

- 2% Na2CO3 solution – Dissolve 2g sodium carbonate in 100ml distilled water.
- Seminal Plasma: Buffalo or cattle semen is kept at room temperature for 10-15 minutes and then centrifuged at 1000 g* for 15 minutes, at room temperature. The sperms are pelleted at the bottom, while the clear supernatant obtained is the seminal plasma. This is diluted 1:30 with carbonate-bicarbonate buffer, 0.05 M, pH = 10.5.

• Method

- Preparation of Standard curve of p- nitrophenol

Concentration of Std.PNP = 0.5 mM
Add p- nitrophenol and distilled water to all the tubes as shown below.

Table 15.1 Preparation of Standard curve of p- nitrophenol

Test tube no.	B	1	2	3	4	5	6
PNP (ml)	-	0.1	0.2	0.3	0.4	0.5	0.6
PNP (µM)	-	0.05	0.1	0.15	0.20	0.25	0.3
D.W (ml)	2.0	1.9	1.8	1.7	1.6	1.5	1.4
Na_2CO_3	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Take reading at 405 nm							
OD							

- Plot Graph of absorption against PNP concentration

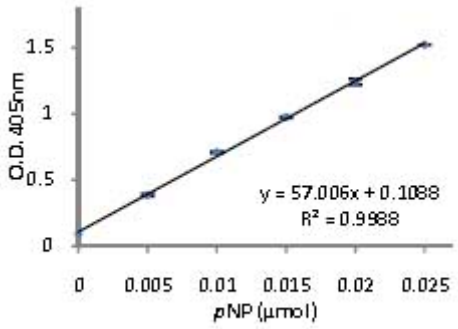


Fig 15.1 Plot Graph of absorption against PNP concentration

- Enzyme Assay

Table 15.2 Effect of enzyme concentration on enzyme activity

Test tube no	B	1	2	3	4	5
PNP Substrate (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Buffer (ml)	1.5	1.4	1.3	1.2	1.1	1.0
Enzyme (Seminal plasma)	-	0.1	0.2	0.3	0.4	0.5
Incubate at 37°C for 30 minutes						
Na_2CO_3	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**
Take reading at 405 nm						
OD						

- Absorbance (405 nm)

From the standard curve for p- nitrophenol, convert the absorbance readings into μ moles of substrate, converted by the enzyme and calculate the enzyme activity.

Plot a graph of absorbance against enzyme concentration

Enzyme activity may be calculated as “Number of micromoles of the substrate converted into product under defined conditions”.

Specific activity is defined as “micromoles of p-nitrophenol released per min per mg protein.”

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Module 3. Metabolism

Lesson 16
GLYCOLYSIS

16.1 Introduction

- Glycolysis is a set of reactions that take place in cytoplasm of prokaryotes and eukaryotes.
- Glycolysis is an almost universal central pathway of glucose catabolism .
- The major roles of glycolysis are to produce energy and to produce intermediates for biosynthetic pathways.

16.2 Glycolysis has two Phases

- Preparatory phase
- Pay Off Phase
    - In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates.
    - The payoff phase of glycolysis includes the energy-conserving phosphorylation steps in which some of the free energy of the glucose molecule is conserved in the form of ATP.
    - Remember that one molecule of glucose yields two molecules of glyceraldehyde 3-phosphate; both halves of the glucose molecule follow the same pathway in the second phase of glycolysis. The conversion of two molecules of glyceraldehyde 3-phosphate to two molecules of pyruvate is accompanied by the formation of four molecules of ATP from ADP.
    - However, the net yield of ATP per molecule of glucose degraded is only two, because two ATP were invested in the preparatory phase of glycolysis to phosphorylate the two ends of the hexose molecule.

- For each molecule of glucose degraded to pyruvate, two molecules of ATP are generated from ADP and Pi.

$$\text{Glucose} + 2\text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\text{NADH} + 2\text{H} \quad \Delta G1 = -146\ \text{kJ/mol}$$

and the formation of ATP from ADP and Pi, which is endergonic:

$$2\text{ADP} + 2\text{Pi} \longrightarrow 2\text{ATP} + 2\text{H}_2\text{O} \qquad \Delta G2 = +61.0\ \text{kJ/mol}$$

$$\Delta Gs = G1 + G2 \quad (-146\ \text{kJ/mol} + 61.0\ \text{kJ/mol} = -85\ \text{kJ/mol})$$

However, complete oxidation of glucose to carbon dioxide and water proceeds with a standard free-energy change of -2,840 kJ/mol.

16.3 Irreversible / Regulatory steps in glycolysis

16.3.1 Hexokinase

Hexokinase is present in all cells of all organisms. Hepatocytes also contain a form of hexokinase called hexokinase IV or glucokinase, which differs from other forms of hexokinase in kinetic and regulatory properties. Two enzymes that catalyze the same reaction but are encoded in different genes are called isozymes.

16.3.2 Phosphofructokinase

The PFK-1 reaction is essentially irreversible under cellular conditions, and it is the first "committed" step in the glycolytic pathway; glucose 6-phosphate and fructose 6-phosphate have other possible fates, but fructose 1,6-bisphosphate is targeted for glycolysis. Phosphofructokinase-1 is a regulatory enzyme. The activity of PFK-1 is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP (particularly the latter), are in excess. The enzyme is inhibited whenever the cell has ample ATP and is well supplied by other fuels such as fatty acids.

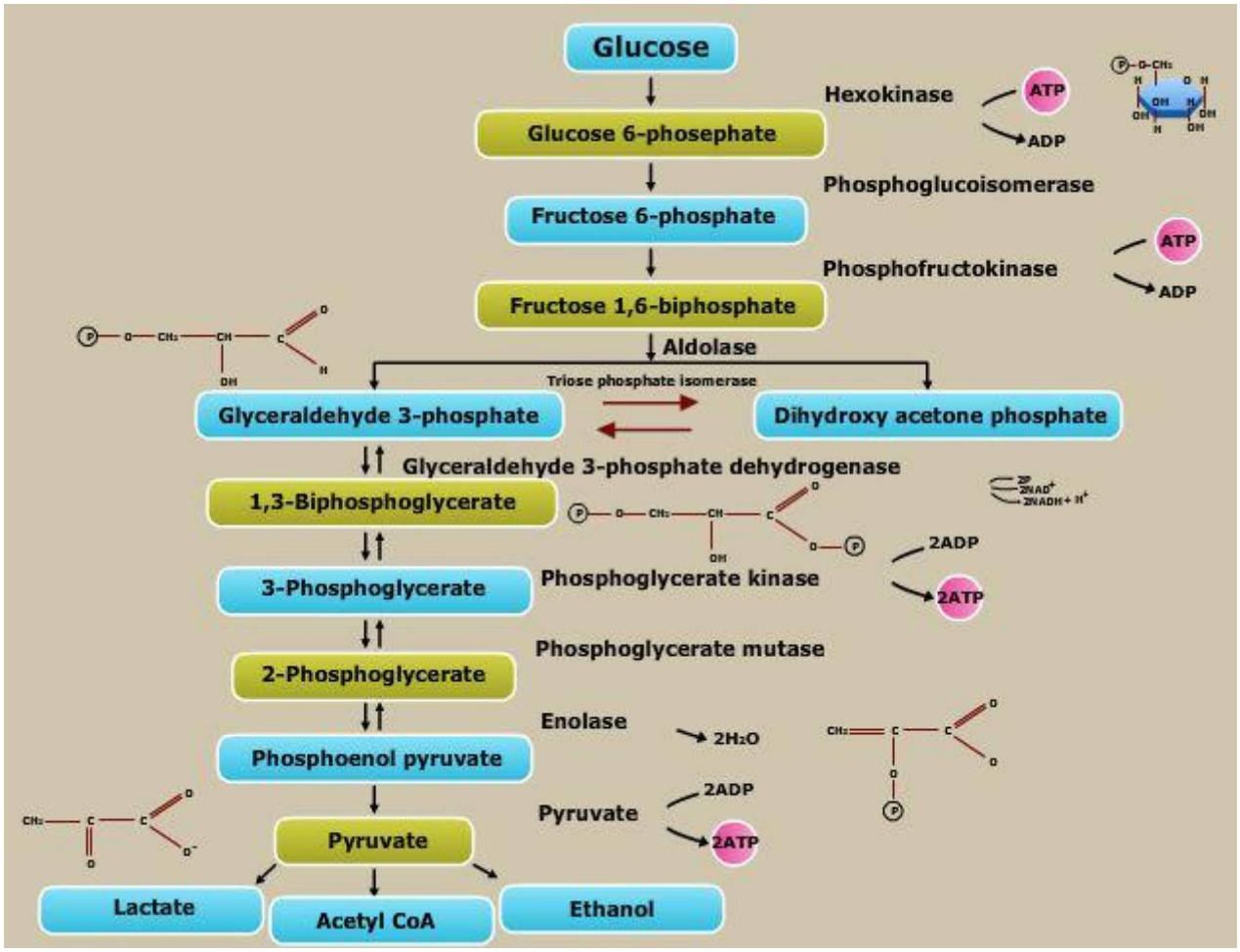

Fig 16.1 Glycolysis

16.3.3 Pyruvate kinase

The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by pyruvate kinase, which requires K+and either Mg^{2+} or Mn^{2+}

16.4 Pasteur Effect

Louis Pasteur discovered that both the rate and the total amount of glucose consumption were many times greater under anaerobic than aerobic conditions. The ATP yield from glycolysis under anaerobic conditions (2 ATP per molecule of glucose) is much smaller than that from the complete oxidation of glucose to CO_2 under aerobic conditions (30 or 32 ATP per glucose). About 15 times as much glucose must therefore be consumed anaerobically as aerobically to yield the same amount of ATP.

16.5 Substrate-level phosphorylation

The enzyme phosphoglycerate kinase transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and 3- phosphoglycerate. Thus by consuming the product of 1,3-bisphosphoglycerate of previous step, keeps [1,3-bisphosphoglycerate] relatively low in the steady state. The outcome of these coupled reactions, both reversible under cellular conditions, is that the energy released on oxidation of an aldehyde to a carboxylate group is conserved by the coupled formation of ATP from ADP and Pi. The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a substrate-level phosphorylation, to distinguish this mechanism from respiration-linked phosphorylation. Substrate-level phosphorylations involve soluble enzymes and chemical intermediates (1,3-bisphosphoglycerate in this case). Respiration-linked phosphorylations, on the other hand, involve membrane-bound enzymes and transmembrane gradients of protons

$$\text{Glucose} + 2ATP + 2NAD^{+} + 4ADP\ \_ + 2Pi \longrightarrow 2\ \text{pyruvate} + 2ADP + 2NADH + 2H + 4ATP + 2H2O$$

In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate (the pathway of carbon). Two molecules of ADP and two of Pi are converted to two molecules of ATP (the pathway of phosphoryl groups). Four electrons, as two hydride ions, are transferred from two molecules of glyceraldehydes 3-phosphate to two of NAD+ (the pathway of electrons).

16.6 Fate of Pyruvate

16.6.1 Entry into the citric acid cycle

Glycolysis releases relatively little of the energy present in a glucose molecule; much more is released by the subsequent operation of citric acid cycle and oxidative phosphorylation. Under aerobic conditions, pyruvate is converted to acetyl Co-A by the enzyme pyruvate dehydrogenase which enters the citric acid cycle.

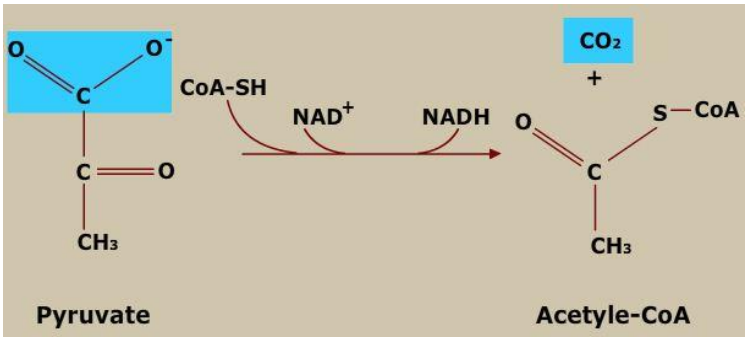

Fig 16.2 Entry of Pyruvate into the TCA cycle

16.6.2 Conversion to fatty acids or ketone bodies

When the cellular energy level is high (ATP in excess), the rate of citric acid cycle decreases and acetyl Co-A begins to accumulate and is used for fatty acid or ketone body synthesis

16.6.3 Conversion to lactate

The NAD+ used during glycolysis in the formation of 1,3 biphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase must be regenerated if glycolysis has to continue. Under aerobic conditions NAD+ is regenerated by reoxidation of NADH via electron transport chain. However, when oxygen is limiting as in muscle during exercise reoxidation of NADH to NAD+ by ETC is insufficient to maintain glycolysis . Hence NAD+ is regenerated by conversion of the puruvate to lactate by lactate dehydrogenase.

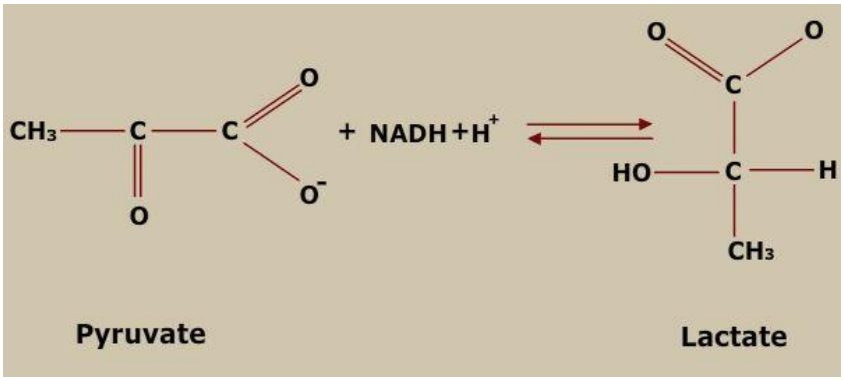

Fig. 16.3 Conversation of Pyruvate to lactate

16.6.4 Alcoholic fermentation

In microbes, NAD+ is required for continuation of glycolysis under anaerobic conditions. So, pyruvate is converted to acetaldehyde by pyruvate decarboxylase and then to ethanol by alcohol dehydrogenase. The last reaction simultaneously reoxidizes the NADH to NAD

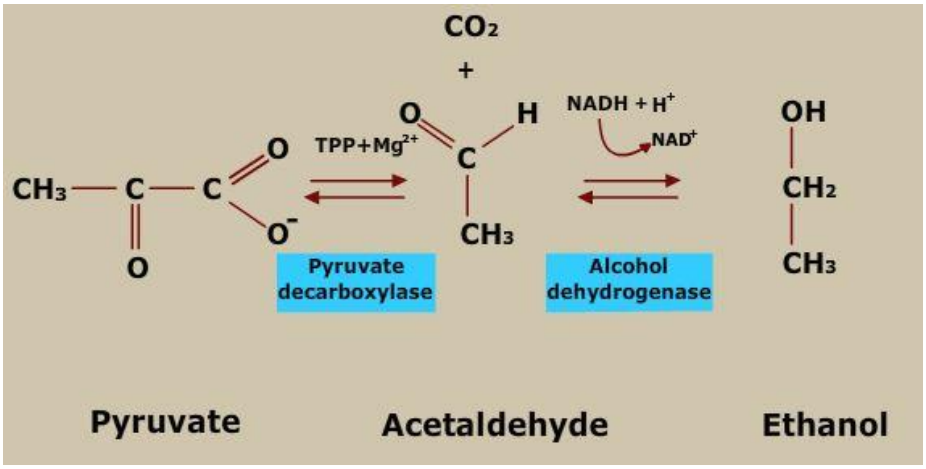

Fig 16.4 Involvement of Pyruvate into Alcoholic fermentation

16.7 Entry of other Carbohydrates in Glycolysis

Many carbohydrates besides glucose meet their catabolic fate in glycolysis, after being transformed into one of the glycolytic intermediates. The most significant are the storage polysaccharides glycogen and starch; the disaccharides maltose, lactose, trehalose, and sucrose; and the monosaccharides fructose, mannose, and galactose .

Glycogen in animal tissues can be mobilized for use within the same cell by a phosphorolytic reaction

catalyzed by glycogen phosphorylase. This enzyme catalyzes an attack by Pi on the (α1-4) glycosidic linkage that joins the last two glucose residues at a nonreducing end, generating glucose 1-phosphate and a polymer one glucose unit shorter. A debranching enzyme removes the branches α1-6 glucosidic linkage.

Glucose 1-phosphate ⇌ Glucose 6-phosphate

phosphoglucomuta

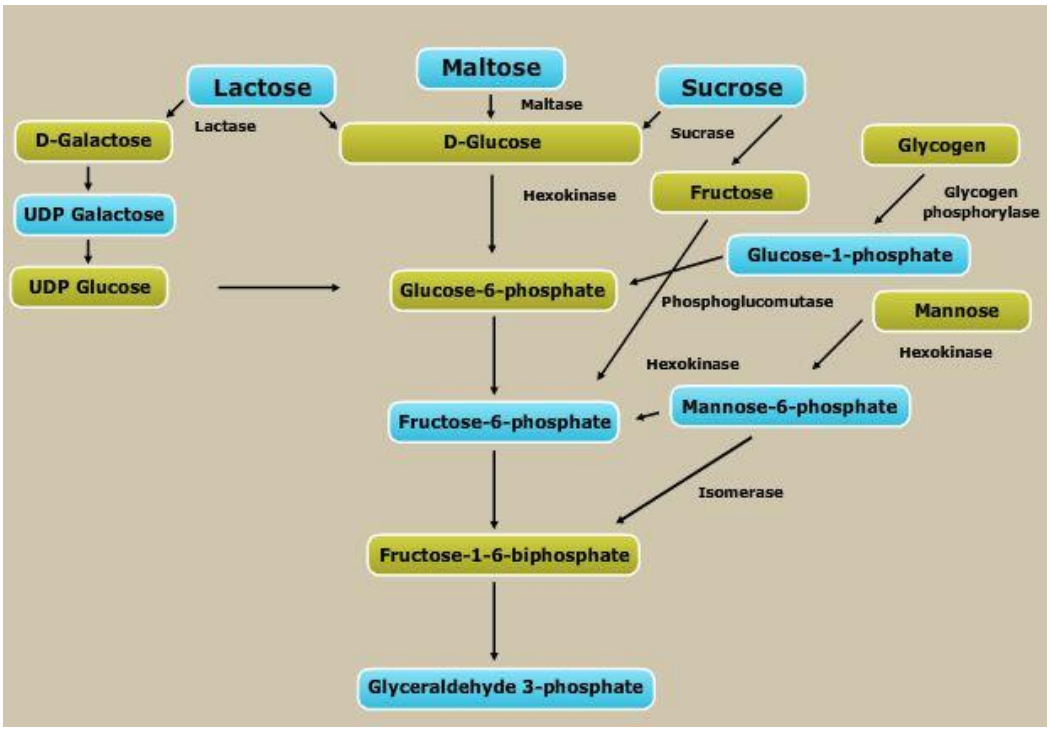

Fig 16.5 Entry of carbohydrates in glycolysis

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Lesson 17
GLUCONEOGENESIS

17.1 Introduction

- Gluconeogenesis is especially important in periods of starvation or vigorous exercise.
- During starvation, the formation of glucose via gluconeogenesis particularly uses amino acids from protein breakdown and glycerol from fat breakdown.
- During exercise, the blood glucose levels required for brain and skeletal muscle function are maintained by gluconeogenesis in the liver using lactate produced by the muscle.
- Gluconeogenesis synthesizes glucose from noncarbohydrate precursors, including lactate and pyruvate, citric acid cycle intermediates, the carbon skeletons of most amino acids and glycerol.
- This is extremely important since the brain and erythrocytes rely almost exclusively as their energy source under normal conditions. The store of liver glycogen is sufficient to supply the brain with glucose for only about half a day during fasting.
- The main site of gluconeogenesis is the liver, although it also occurs to a far lesser extent in the kidneys. Very little gluconeogenesis occurs in brain or muscle.

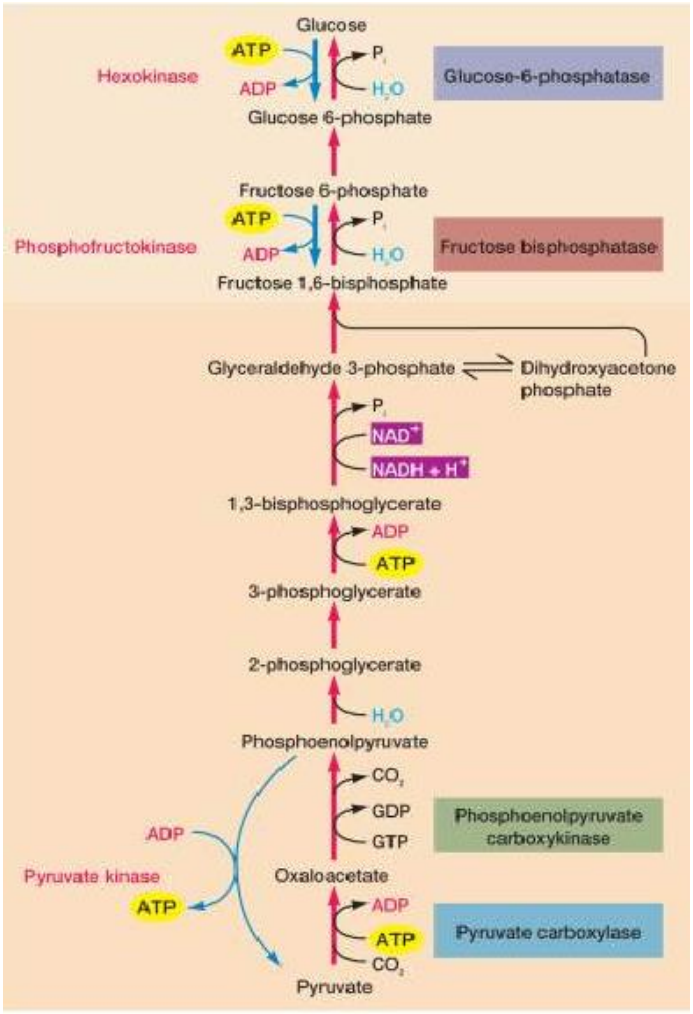

Fig 17.1 Gluconeogenesis

17.2 The Pathway

In principle, gluconeogenesis appears to be a reversal of glycolysis. Indeed, some of the reactions of glycolysis are reversible and so the two pathways have these steps in common.

- However, three steps in glycolysis are essentially irreversible; those catalyzed by the enzymes hexokinase, phosphofructokinase (PFK) and pyruvate kinase.
- Indeed it is the large negative free-energy change in these reactions that normally drives glycolysis forward towards pyruvate formation.
- Therefore, in gluconeogenesis, these three steps have to be reversed by using other reactions so gluconeogenesis is not a simple reversal of glycolysis.

17.2.1 Precursors for gluconeogenesis

- Glycerol can act as a substrate for glucose synthesis by conversion to dihydroxy-acetone phosphate, an intermediate in gluconeogenesis.
- Lactate, pyruvate, citric acid cycle intermediates and the carbon skeletons of most amino acids to act as precursors for gluconeogenesis, these compounds must first be converted to oxaloacetate. Some of the carbon skeletons of the amino acids give rise to oxaloacetate directly.
- Other precursors feed into the citric acid cycle as intermediates and the cycle then converts these molecules to oxaloacetate.
- Lactate is converted to pyruvate by the lactate dehy-drogenase reaction and some amino acids also give rise to pyruvate.
- Therefore, for these precursors, the first step in the gluconeogenic pathway is the conversion of pyruvate to oxaloacetate.

17.2.2 The steps in gluconeogenesis are as follows

- Pyruvate is converted to oxaloacetate by carboxylation using the enzyme pyruvate carboxylase that is located in the mitochondrial matrix. The other enzymes of the pathway are located in the cytosol.
    - This enzymes uses biotin as an activated carrier of CO_2, the reaction occurring in two stages:

$$E-biotin + ATP + HCO_3^- \rightarrow E-biotin-CO_2 + ADP + P_i$$

$$E-biotin-CO_2 + pyruvate \rightarrow E-biotin + oxaloacetate$$

    - The oxaloacetate is now acted on by phosphenolpyruvate carboxykinase which simultaneously decarboxylates and phosphorylates it to form phosphoenolpyruvate (PEP), releasing CO2 and using GTP in the process:

$$Oxaloacetate + GTP \rightleftharpoons PEP + CO_2 + GDP$$

    - In the conversion of PEP to pyruvate in glycolysis synthesizes ATP, it is not surprising that the overall reversal of this step needs the input of a substantial amount of energy, one ATP for the pyruvate carboxylase step and one GTP for the PEP carboxykinase step.

- PEP is converted to fructose 1,6-bisphosphate in a series of steps that are a direct reversal of those in glycolysis, using the enzymes enolase, phosphoglycerate mutase, phosphoglycerate kinase, glyceraldehydes 3-phosphate dehydrogenase, triose phosphate isomerase and aldolase. This sequence of reactions uses one ATP and one NADH for each PEP molecule metabolized.
- Fructose 1,6-bisphosphate is dephosphorylated to form fructose 6-phosphate by the enzyme fructose 1,6-bisphosphatase, in the reaction:

$$fructose\ 1,6-bisphosphate + H_2O \rightarrow fructose\ 6-phosphate + P_i$$

- Fructose 6-phosphate is converted to glucose 6-phosphate by the glycolytic enzyme phosphoglucoisomerase.
- Glucose 6-phosphate is converted to glucose by the enzyme glucose 6-phosphatase. This enzyme is bound to the smooth endoplasmic reticulum and catalyzes the reaction:

$$glucose\ 6-phosphate + H_2O \rightarrow glucose + P_i$$

17.3 Energy used

As would be expected, the synthesis of glucose by gluconeogenesis needs the input of energy. Two pyruvate molecules are required to synthesize one molecule of glucose. Energy is required at the following step

$$pyruvate\ carboxylase\ \ 1\ ATP\ (\times 2) = 2\ ATP$$
$$PEP\ carboxykinase\ \ \ 1\ GTP\ (\times 2) = 2\ ATP$$
$$phosphoglycerate\ kinase\ 1\ ATP\ (\times 2) = 2\ ATP$$
$$Total = 6\ ATP$$

This compares with only two ATPs as the net ATP yield from glycolysis. Thus an extra four ATPs per glucose are required to reverse glycolysis.

- In fact, the glyceraldehydes 3-phosphate dehydrogenase reaction also consumes NADH, equivalent to two molecules of NADH for each molecule of glucose synthesized.
- Since each cytosolic NADH would normally be used to generate approximately three ATP molecules via the glycerol 3-phosphate shuttle and oxidative phosphorylation, this is equivalent to the input of another six ATPs per glucose synthesized.

17.4 Transport of Oxaloacetate

- Pyruvate carboxylase is a mitochondrial matrix enzyme whereas the other enzymes of gluconeogenesis are located outside the mitochondrion.
- Thus oxaloacetate, produced by pyruvate, needs to exit the mitochondrion. However, the inner mitochondrial membrane is not permeable to this compound.
- Thus oxaloacetate is converted to malate inside the mitochondrion by mitochondrial malate dehydrogenase, the malate is transported through the mitochondrial membrane by a special transport

protein and then the malate is converted back to oxaloacetate in the cytoplasm by a cytoplasmic malate dehydrogenase.

17.5 Reciprocal Regulation of Glycolysis and Gluconeogenesis

- Glycolysis generates two ATPs net per glucose whereas gluconeogenesis uses four ATPs and two GTPs per glucose.
- Thus, if both glycolysis and gluconeogenesis were allowed to operate simultaneously, converting glucose to pyruvate and back again, the only net result would be the utilization of two ATPs and two GTPs, a so-called futile cycle.
- This is prevented by tight coordinate regulation of glycolysis and gluconeogenesis. Since many of the steps of the two pathways are common, the steps that are distinct in each pathway are the sites of this regulation, in particular the inter conversions between fructose 6-phosphate and fructose 1,6-bisphosphate and between PEP and pyruvate.

17.5.1 Regulation of PFK and fructose 1,6-bisphosphatase

- When the level of AMP is high, this indicates the need for more ATP synthesis. AMP stimulates PFK, increasing the rate of glycolysis, and inhibits fructose 1,6- bisphosphatase, turning off gluconeogenesis. Conversely, when ATP and citrate levels are high, this signals that no more ATP need be made. ATP and citrate inhibit PFK, decreasing the rate of glycolysis, and citrate stimulates fructose 1,6-bisphosphatase, increasing the rate of gluconeogenesis.
- Glycolysis and gluconeogenesis are made responsive to starvation by the level of the regulatory molecule fructose 2,6-bisphosphate (F-2,6-BP).
- F-2,6-BP is synthesized from fructose 6-phosphate and hydrolyzed back to fructose 6-phosphate by a single polypeptide with two enzymatic activities (PFK2 and FBPase2). Since F-2, 6-BP strongly stimulates PFK and inhibits fructose 1,6-bisphosphatase, glycolysis is stimulated and gluconeogenesis is inhibited in the fed animal. Conversely, during starvation, the low level of F-2,6-BP allows gluconeogenesis to predominate.

17.5.2 Regulation of pyruvate kinase, pyruvate carboxylase and PEP carboxykinase

- In liver, pyruvate kinase is inhibited by high levels of ATP and alanine so that glycolysis is inhibited when ATP and biosynthetic intermediates are already plentiful. Acetyl CoA is also abundant under these conditions and activates pyruvate carboxylase, favoring gluconeogenesis. Conversely, when the energy status of the cell is low, the ADP concentration is high and this inhibits both pyruvate carboxylase and PEP carboxykinase, switching off gluconeogenesis. At this time, the ATP level will be low so pyruvate kinase is not inhibited and glycolysis will operate.
- Pyruvate kinase is also stimulated by fructose 1,6-bisphosphate so that its activity rises when needed, as glycolysis speeds up. During starvation, the priority is to conserve blood glucose for the brain and muscle. Thus, under these conditions, pyruvate kinase in the liver is switched off. This occurs because the hormone glucagon is secreted into the bloodstream and activates a cAMP cascade that leads to the phosphorylation and inhibition of this enzyme.

17.6 The Cori Cycle

Under the limiting oxygen conditions experienced during vigorous exercise, the formation of NADH by glycolysis exceeds the ability of the respiratory chain to oxidize it back to NAD^+. The pyruvate produced by glycolysis in muscle is then converted to lactate by lactate dehydrogenase, a reaction that regenerates. NAD^+ and so allows glycolysis to continue to produce ATP. However, lactate is a metabolic dead-end in

that it cannot be metabolized further until it is converted back to pyruvate. Lactate diffuses out of the muscle and is carried in the bloodstream to the liver. Here it diffuses into liver cells and is converted back to pyruvate by lactate dehydrogenase. The pyruvate is then converted to glucose by gluconeogenesis and the glucose is released back into the bloodstream ready to be taken up by skeletal muscle (and brain). This cycle of reactions is called the Cori Cycle.

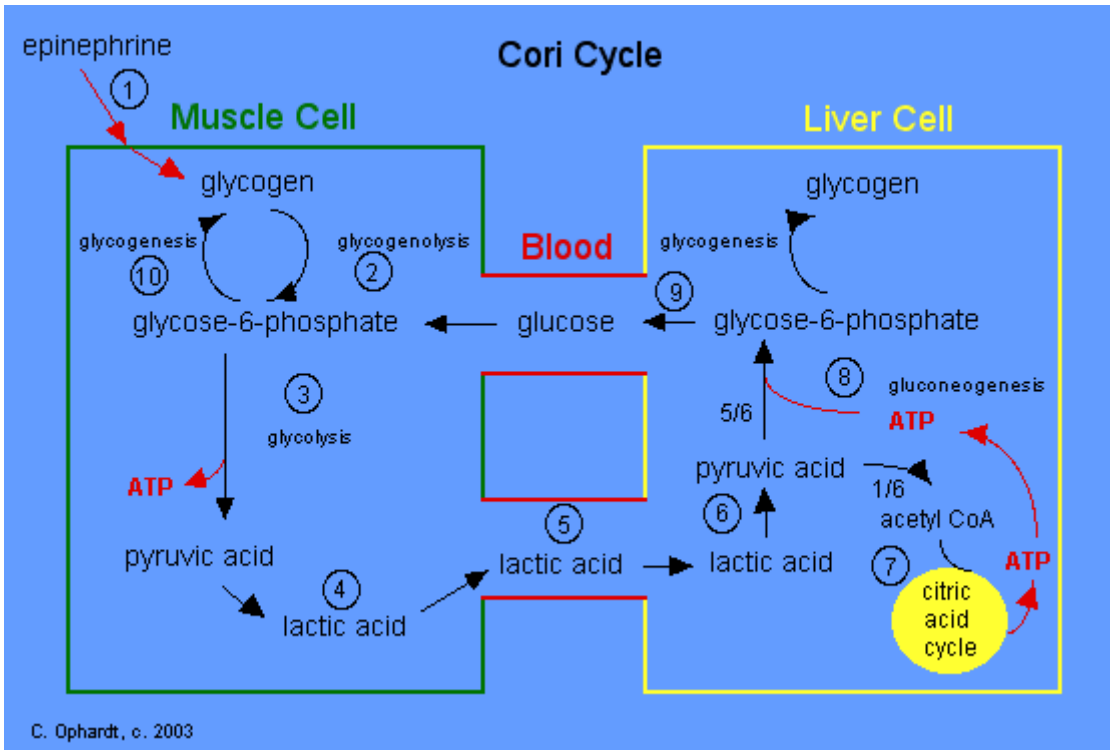

Fig 17.2 Cori Cycle

Lesson 18
TCA CYCLE

18.1 Introduction

- The citric acid cycle, also known as the TCA (tricarboxylic acid) cycle or Krebs cycle (after its discoverer in 1937), is used to oxidize the pyruvate formed during the glycolytic breakdown of glucose into CO_2 and H_2O. The cycle is a major energy source in the form of ATP and also produces precursors for many biosynthetic pathways.
- The citric acid cycle operates in the mitochondria of eukaryotes and in the cytosol of prokaryotes. Succinate dehydrogenase, the only membrane-bound enzyme in the citric acid cycle, is embedded in the inner mitochondrial membrane in eukaryotes and in the plasma membrane in prokaryotes.

18.2 The cycle

The cycle forms the central part of a three-step process which oxidizes organic fuel molecules into C_O2 with the concomitant production of ATP.

18.2.1 Step 1 – Oxidation of fuel molecules to acetyl CoA

A major source of energy is glucose which is converted by glycolysis into pyruvate. Pyruvate dehydrogenase (a complex of three enzymes and five coenzymes) then oxidizes the pyruvate (using NAD^+ which is reduced to NADH) to form acetyl CoA and CO_2. Since the reaction involves both an oxidation and a loss of CO_2, the process is called oxidative decarboxylation.

18.2.2 Step 2 – The citric acid cycle

The cycle carries out the oxidation of acetyl groups from acetyl CoA to C_O2 with the production of four pairs of electrons, stored initially in the reduced electron carriers NADH and $FADH_2$.

The cycle has eight stages:

1. Citrate (6C) is formed from the irreversible condensation of acetyl CoA (2C) and oxaloacetate (4C) – catalyzed by citrate synthase.
2. Citrate is converted to isocitrate (6C) by an isomerization catalyzed by aconitase. This is actually a two-step reaction during which cis-aconitate is formed as an intermediate. It is the cis-aconitate which gives the enzyme its name.
3. Isocitrate is oxidized to α-ketoglutarate (5C) and C_O2 by isocitrate dehydrognase. This mitrochondrial enzyme requires NAD^+, which is reduced to NADH.
4. A-Ketoglutarate is oxidized to succinyl CoA (4C) and C_O2 by the α-ketoglutarate dehydrogenase complex. Like pyruvate dehydrogenase, this is a complex of three enzymes and uses NAD^+ as a cofactor.
5. Succinyl CoA is converted to succinate (4C) by succinyl CoA synthetase. The reaction uses the energy released by cleavage of the succinyl-CoA bond to synthesize either GTP (mainly in animals) or ATP (exclusively in plants) from Pi and, respectively, GDP or ADP.
6. Succinate is oxidized to fumarate (4C) by succinate dehydrogenase. FAD is tightly bound to the enzyme and is reduced to produce FADH2.

7. Fumerate is converted to malate (4C) by fumarase; this is a hydration reaction requiring the addition of a water molecule.
8. Malate is oxidized to oxaloacetate (4C) by malate dehydrogenase. NAD^+ is again required by the enzyme as a cofactor to accept the free pair of electrons and produce NADH.

18.2.3 Step 3 – Oxidation of NADH and FADH2 produced by the citric acid cycle

The NADH and FADH2 produced by the citric acid cycle are reoxidized and the energy released is used to synthesize ATP by oxidative phosphorylation.

18.3 Energy Yield

- Each of the three NADH molecules produced per turn of the cycle yields three (2.5) ATPs and the single (1.5) $FADH_2$ yields two ATPs by oxidative phosphorylation.
- One GTP (or ATP) is synthesized directly during the conversion of succinyl CoA to succinate.
- Thus the oxidation of a single molecule of glucose via the citric acid cycle produces 12 (10) ATP molecules.

18.4 Regulation

- Regulation of the cycle is governed by substrate availability, inhibition by accumulating products, and allosteric feedback inhibition by subsequent intermediates in the cycle.
- Three enzymes in the cycle itself are regulated (citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) and so is the enzyme which converts pyruvate to acetyl CoA to enter the cycle, namely pyruvate dehydrogenase (fig. 2).
- Citrate synthase is inhibited by citrate and also by ATP (the Km for acetyl CoA is raised as the level of ATP rises);
- Isocitrate dehydrogenase is inhibited by NADH and ATP but activated by ADP;
- A-ketoglutarate dehydrogenase is inhibited by NADH and acetyl CoA (i.e. product inhibition). However, in eukaryotes the enzyme is also controlled by phosphorylation/ dephosphorylation via pyruvate dehydrogenase kinase and a phosphatase.
- Overall, the cycle speeds up when cellular energy levels are low (high ADP concentration, low ATP and NADH) and slows down as ATP (and then NADH2, succinyl CoA and citrate) accumulates.

18.5 Anaplerotic nature of TCA cycle

The intermediates in the cycle provide precursors for many biosynthetic pathways besides being involved in degradation of acetyl CoA.Anabolic reactions that initiate from TCA cycle are:

- Synthesis of fatty acids from citrate
- Amino acid synthesis following transamination of α-ketoglutarate
- Synthesis of purine and pyrimidine nucleotides from α-ketoglutarate and oxaloacetate
- Oxaloacetate can be converted to glucose by gluconeogenesis
- Succinyl CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups

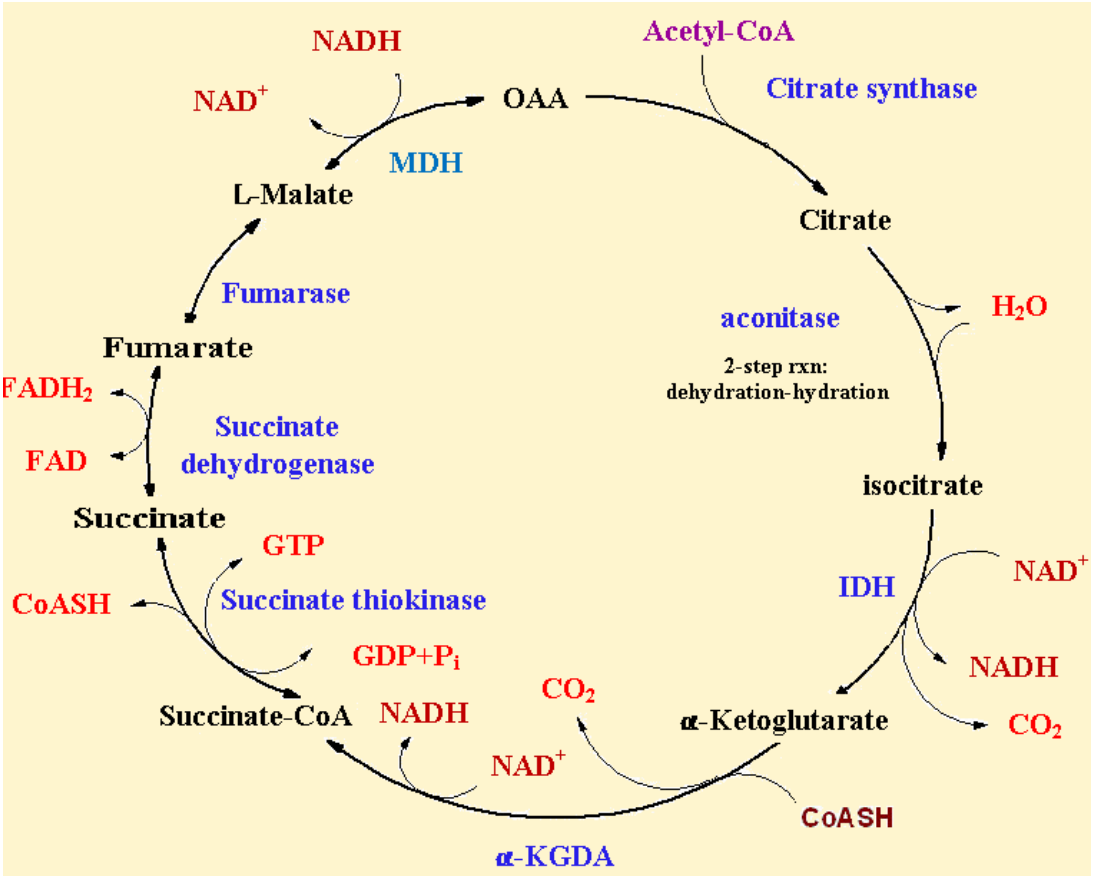

Fig. 18.1 Citric acid cycle

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Lesson 19
GLYCOGEN DEGRADATION AND SYNTHESIS

19.1 Introduction

- Glycogen is a large polymer of glucose residues linked by α 1-4 glucosidic bonds with branches every 10 residues or so via α 1-6 glucosidic bonds. Glycogen provides an important energy reserve for the body.
- The two main storage sites are the liver and skeletal muscle where the glycogen is stored as granules in the cytosol. The granules contain not only glycogen but also the enzymes and regulatory proteins that are required for glycogen degradation and synthesis.
- Glycogen metabolism is important because it enables the blood glucose level to be maintained between meals (via glycogen stores in the liver) and also provides an energy reserve for muscular activity. The maintenance of blood glucose is essential in order to supply tissues with an easily metabolizable energy source, particularly the brain which uses only glucose except after a long starvation period.

19.2 Glycogen Degradation

- Glycogen degradation requires two enzymes; glycogen phosphorylase and glycogen-debranching enzyme.
- Glycogen phosphorylase (often called simply phosphorylase) degrades glycogen by breaking α 1-4 glycosidic bonds to release glucose units one at a time from the nonreducing end of the glycogen molecule (the end with a free 4' OH group) as glucose 1-phosphate.
- The other substrate required is inorganic phosphate (Pi). The reaction is an example of phosphorolysis, that is breakage of a covalent bond by the addition of a phosphate group. The (reversible) reaction is as follows:

$$\underset{\text{(n residues)}}{glycogen} + P_i \rightleftharpoons \underset{\text{(n-1 residues)}}{glycogen} + glucose\ 1-phosphate$$

- Glycogen phosphorylase can remove only those glucose resides that are more than five residues from a branchpoint. Glycogen-debranching enzyme removes the α1-6 branches and so allows phosphorylase to continue degrading the glycogen molecule. The glucose 1-phosphate produced is converted to glucose 6-phosphate by the enzyme phosphoglucomutase:

$$glucose\ 1-phosphate \rightleftharpoons glucose\ 6-phosphate$$

- The fate of the glucose 6-phosphate depends on the tissue.
    - Liver contains the enzyme glucose 6-phosphatase which converts the glucose 6-phosphate to glucose, which then diffuses out into the bloodstream and so maintains the blood glucose concentration:

$$glucose\ 6-phosphate + H_2O \rightarrow glucose + P_i$$

- During glycogen degradation in muscle is required to produce energy quickly and so the glucose 6-phosphate is metabolized immediately via glycolysis. This tissue does not contain glucose 6-phosphatase.

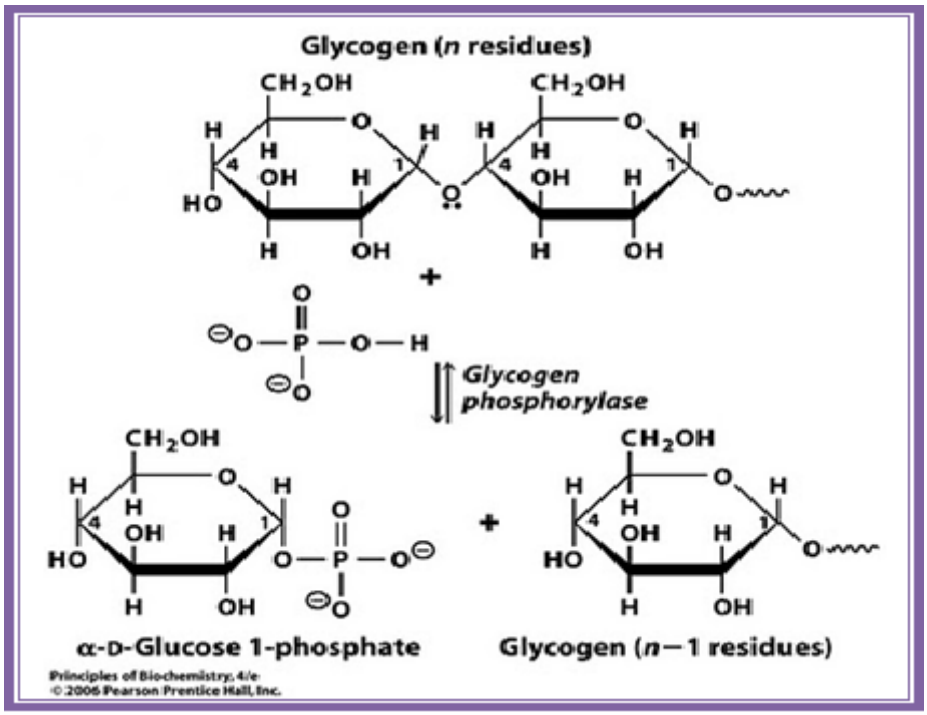

Fig. 19.1 Glycogen degradation

19.3 Glycogen Synthesis

- Three enzymes are needed to synthesize glycogen:
- UDP –glucose pyrophosphorylase catalyzes the synthesis of UDP-glucose from UTP and glucose 1-phosphate:

$$UTP + glucose\ 1-phosphate \rightarrow UDP-glucose + PP_i$$

The pyrophosphate (PPi) is immediately hydrolyzed by inorganic pyrophosphatase, releasing energy. Thus the overall reaction is highly exergonic and essentially irreversible.

- Glycogen synthase now transfers the glucosyl residue from UDP-glucose to the C4 OH group at the nonreducing end of a glycogen molecule, forming an α1-4 glycosidic bond.
- Glycogen synthase can only extend an existing chain. Thus it needs a primer; this is a protein called glycogenin. Glycogenin contains eight glucosyl units linked via α1-4 linkages, which are added to the protein by itself (i.e. autocatalysis). It is this molecule that glycogen synthase then extends. Each glycogen granule contains only a single glycogenin molecule at its core. The fact that glycogen synthase is fully active only when in contact with glycogenin .
- Branching enzyme [amylo-(1-4→1-6) transglycosylase is a different enzyme from glycogen-debranching enzyme. After a number of glucose units have been joined as a straight chain with α1-4 linkages, branching enzyme breaks one of the α1-4 bonds and transfers a block of residues (usually about seven) to a more interior site in the glycogen molecule, reattaching these by creating an α1-6 bond. The branches are important because the enzymes that degrade and synthesize glycogen (glycogen synthase and glycogen phosphorylase, respectively) work only at the ends of the glycogen molecule. Thus the existence of many termini allows a far more rapid rate of synthesis and degradation than would be possible with a nonbranched polymer.

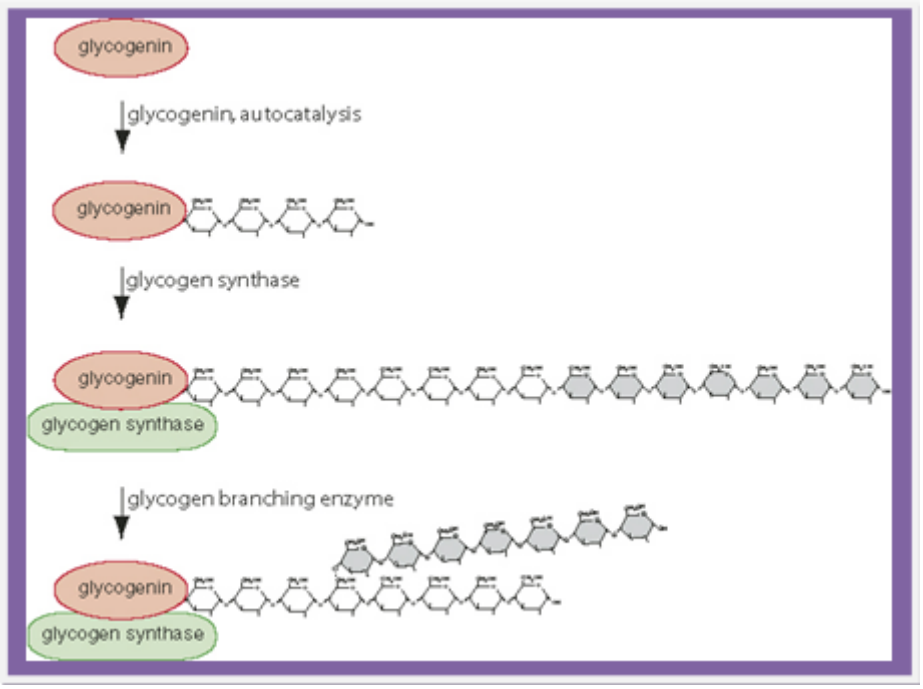

Fig. 19.2 Glycogen Synthesis

19.4 Control of Glycogen Metabolism

Glycogen degradation and glycogen synthesis are controlled both by allosteric regulation, covalent and by hormonal control.

19.4.1 Allosteric control and covalent modification

- Phosphorylase exists in a phosphorylated active a form and a dephosphorylated normally inactive b form. The two forms are interconverted by phosphorylase kinase and protein phosphatase I.
- In muscle, phosphorylase b is activated by the high concentrations of AMP generated by strenuous exercise and thus degrades glycogen, but the AMP stimulation is opposed by high concentrations of ATP and glucose 6-phosphate and so the enzyme is inactive in resting muscle.
- In liver, phosphorylase b is not responsive to AMP but phosphorylase a is deactivated by glucose so that glycogen degradation. Hence glucose production from glycogen occurs only when glucose levels are low.
- Conversely to phosphorylase glycogen synthase exists as a phosphorylated normally inactive b form and a dephosphorylated active a form.

19.4.2 Hormonal control by epinephrine and glucagon

- Epinephrine (adrenaline) stimulates glycogen degradation in skeletal muscle. Epinephrine and glucagon stimulate glycogen degradation in liver. The hormone binds to a plasma membrane receptor and activates adenylate cyclase via a G protein. Adenylate cyclase synthesizes cAMP from ATP which in turn activates protein kinase A. Protein kinase A phosphorylates phosphorylase kinase which activates it. The phosphorylase kinase then converts inactive phosphorylase b to active phosphorylase a by phosphorylation. The same active protein kinase A inactivates glycogen synthase by phosphorylation, converting active glycogen synthase a to glycogen synthase b. When hormone levels fall, stimulation of glycogen degradation is turned off by degradation of cAMP to 5'AMP by phosphodiesterase and by dephosphorylation of the phosphorylated forms of phosphorylase and synthase by protein phosphatase I.
- Insulin is released into the bloodstream when the blood glucose concentration is high and it stimulates glycogen synthesis. It binds to and activates a receptor protein kinase in the plasma membrane of target cells. This leads to activation of an insulin-responsive protein kinase then

activates protein phosphatase I by phosphorylation. Activated protein phosphatase I ensures that phosphorylase and glycogen synthase are dephosphorylated, thus inhibiting glycogen degradation and activating glycogen synthesis.

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Lesson 20
FATTY ACID OXIDATION

20.1 Introduction

- The yield of completely burning fatty acids is approximately 9000 calories per gram. The yield of burning carbohydrates and proteins is approximately 4000 calories per gram only.
- This is the result of the fact that fatty acids are more reduced than carbohydrates and proteins.
- Fatty acids are because of their non-polar character (not soluble in water) stored in a water free form. Carbohydrates and proteins in contrast, do bind water when stored. Because of this 1 gram of fat contains six times more energy than 1 gram glycogen in which water is bound.
- Most fatty acids are degraded by the sequential removal of two-carbon fragments from the carboxyl end of fatty acids. During this process, referred to as β-oxidation, acetyl-CoA is formed as the bond between the α- and β-carbon atoms is broken. β-Oxidation is so named because the β-carbon of fatty acids is oxidized. β-oxidation occurs primarily within mitochondria.

20.2 Hydrolysis of Triglycerides

- The first event in the use of fat as energy source is the hydrolysis (i.e break down by water) of triglycerides by the enzymes that are called lipases. This process is also called lipolysis. Lipases convert triglycerides into glycerol and fatty acids as below.

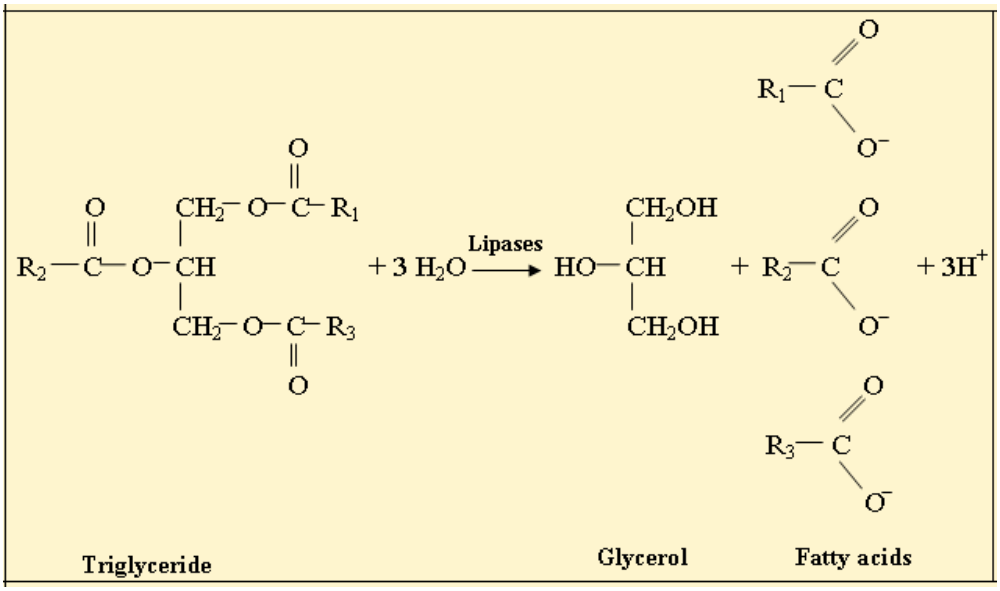


Fig 20.1 Hydrolysis by lipases of triglycerol in glycerol and fatty acids.

- The activity of lipase in fat cells is regulated by hormones like epinephrine and glucagon. These hormones activate the enzyme adenylate cyclase which produces cAMP from ATP. This cAMP activates the enzyme protein kinase A (PKA). The enzyme PKA phosphorylates the lipase enzyme and gets activated because of this phosphorylation.
- The hormone insulin inhibits the hydrolysis of triglycerids. Glycerol, that by the breakdown of triglyceride arise, is phosphorylated by glycerolkinase and is then oxidised by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate. This is an intermediary of the glycolysis and will be broken down further in the glycolysis.

20.3 Fatty Acid Activation and Transport Across the Mitochondrial Membrane

Before β-Oxidation begins, each fatty acid is activated in a reaction with ATP and CoA. Fatty acids undergo for ATP dependent acylation reaction to form fatty acyl-CoA. This activation process is catalyzed by acyl-CoA synthetase in the cytosol.

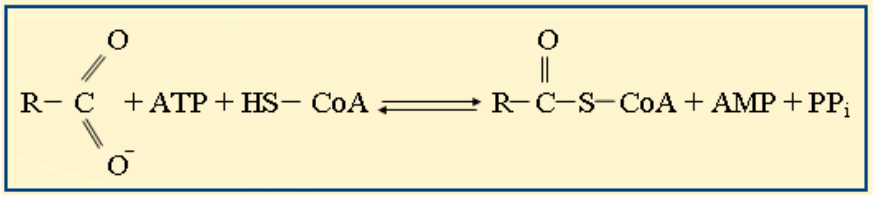
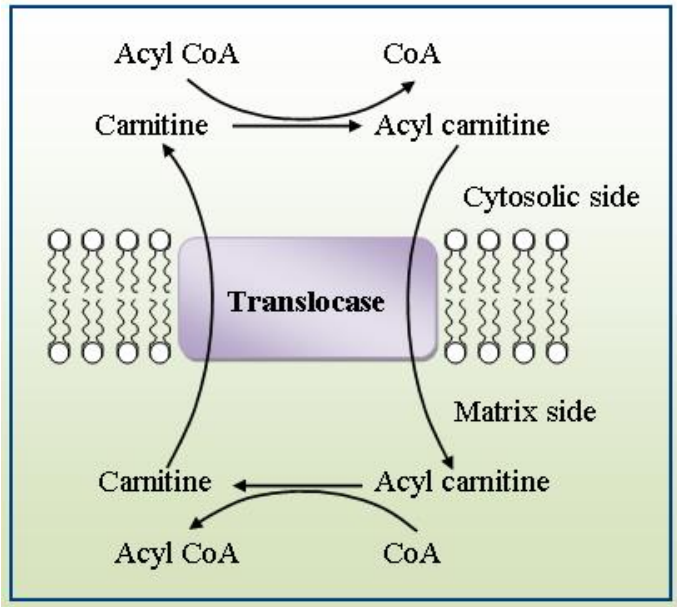


Fig. 20.2 Activation of Fatty acid

The enzyme acyl CoA synthetase has been bound at the outer membrane of the mitochondria. Hydrolysis of pyrophosphate moves the reaction in forward direction.

20.3.1 Translocation of long-chain activated fatty acids into the mitochondrial matrix

Transport of activated long chain fatty acid into the mitochondria for oxidation is brought out by carnitine mediated specialized mechanism. The acyl group is transferred by the sulphur atom of coenzyme A on the hydroxyl group of carnitine under formation of acylcarnitine. This reaction is catalysed by carnitine acyltransferase I that is bound at the outer face of the inner membrane of the mitochondria.



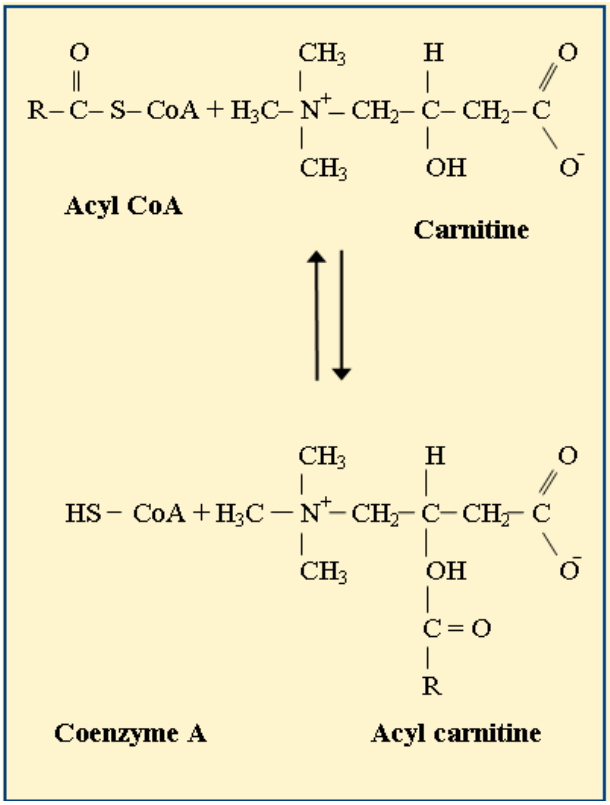


Fig 20.3 Activated long-chain fatty acids are combined with carnitine.

Acylcarnitine is then moved through the inner membrane by a translocase enzyme (membrane protein). The acyl group is transferred back to coenzyme A at the matrix side (in the mitochondria) by the membrane. This reaction is catalysed by carnitine acyltransferase II. Ultimately carnitine is transported back into the inter membrane space by the enzyme translocase in exchange for a coming in of acylcarnitine.

20.4 Fatty Acid Oxidation

20.4.1 Fatty Acids are Broken by Splitting Off Always Two Carbon Atoms.

Fatty acids are broken down by repetitions of separations of parts of two carbon atoms. The reactions that repeat are oxidation, hydration, oxidation (dehydrogenation) and thiolyse. See the figure below.

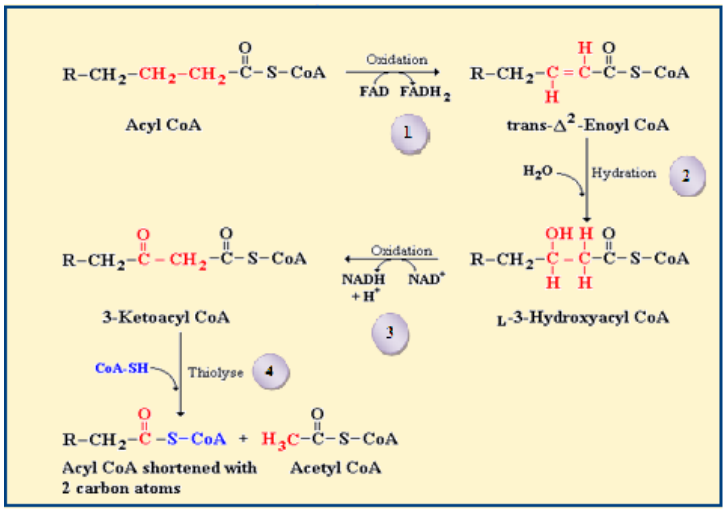


Fig 20.4 Reaction order for the breakdown of fatty acids: Oxidation, hydration, oxidation and thiolyse.

The three reactions from acyl CoA to 3-ketoacyl CoA are comparable to the reactions of succinate to oxalacetate in the citric acid cycle.

20.4 Excess Acetyl CoA are Converted into Ketone Bodies

All by the fatty acid break down formed active acetyl CoA can only be sufficient fast broken down in the citric acid cycle when sufficient oxalacetate is present. By fasting or by diabetes oxalacetate is used for the gluconeogenesis. Then there is insufficient oxalacetate available to react with acetyl CoA. Under these circumstances, from two molecules of acetyl CoA one molecule of acetoacetyl CoA is formed and from that the ketone bodies are formed: acetolacetate, D-3-hydroxybutyrate and acetone.

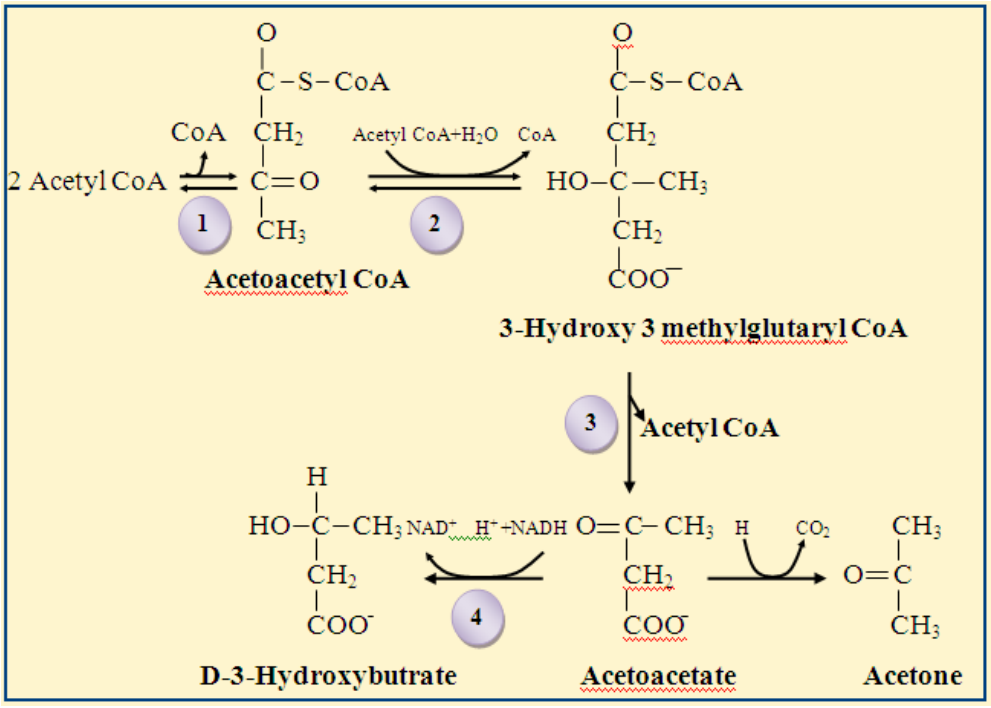


Fig. 20.5 Two molecules acetyl CoA form one acetoacetyl CoA and from this the ketone bodies are formed

The enzymes that accelerate these reactions in the liver are (1) 3- ketothiolase, (2) hydroxymethylglutaryl CoA synthetase, (3) hydroxymethylglutaryl CoA lyase and (4) the mitochondrial enzyme D-3-hydroxybutyratedehydrogenase. Acetoacetate decarboxylates spontaneously to acetone. Acetone is a volatile compound and its smell is observed in the breath of men with diabetes or with people that fast.

The ketone bodies appear to be important energy sources, It is the primary fuels for the heart muscle and kidney. By fasting or diabetes the brains change from the use of glucose to the use of acetoacetate as fuel. Acetoacetate is activated by the transfer of the CoA of succinyl CoA to acetoacetate. Acetoacetyl CoA is then thiolysed to two molecules of acetyl CoA that go into the citric acid cycle.

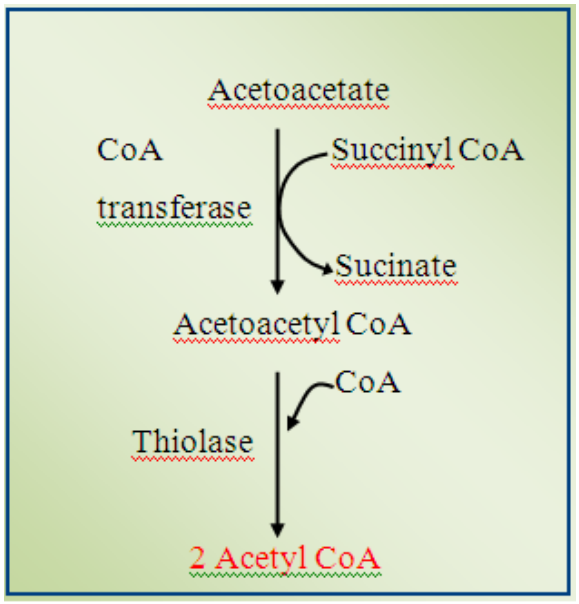


Fig. 20.6 The use of acetoacetate as a fuel by TCA cycle after conversion in acetyl CoA

Humans and animals can not convert fatty acids into glucose because they cannot use the acetyl CoA to make pyruvate or oxalacetate. The both carbon atoms are taken up in the citric acid cycle, and are converted to CO_2 or acetyl CoA is converted to ketone bodies, so acetyl CoA does not provide oxaloacetate to be needed for gluconeogenesis.

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Lesson 21
BIOSYNTHESIS OF FATTY ACIDS

21.1 Introduction

- Fatty acid synthesis in animal/yeast cells occurs in cytosol and in case of plant cells in chloroplast stroma.
- Biosynthesis of fatty acids involves stepwise addition of two carbon units. The two carbon units are supplied by acetyl-CoA, which in turn is derived from the oxidation of glucose.
- Acetyl-CoA formed in the matrix of mitochondria cannot cross the inner mitochondrial membrane, and an acetyl CoA shuttle system is required to transport the two-carbon units out of the mitochondria and into the cytosol.

21.2 Fatty acid Synthesis

21.2.1 Carboxylation of acetyl CoA

- The formation of malonyl coenzyme A is the speed determining step in the fatty acid synthesis.

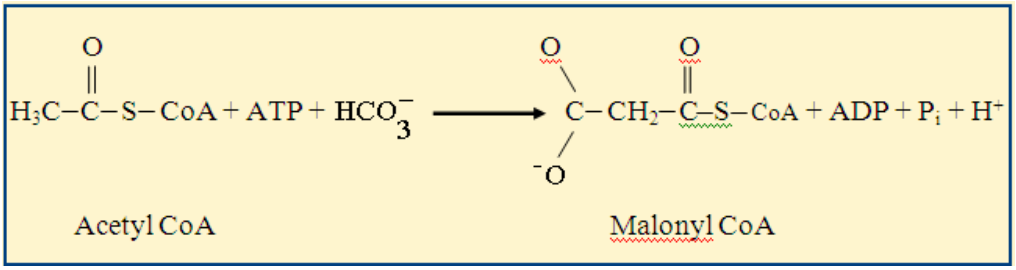

Fig. 21.1 Carboxylation of acetyl CoA

- The fatty acid synthesis begins with the carboxylation of acetyl CoA to malonyl CoA by the enzyme acetyl CoA carboxylase with biotin as help group. The carboxyl group of the formed malonyl CoA originates from a bicarbonate ion.

21.2.2 The cycle of the chain extension in the fatty acid synthesis.

- The enzyme system that catalyses the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA and NADPH are called the fatty acid synthase.
- Fatty acid synthase (FAS) carries out the chain elongation steps of fatty acid biosynthesis. FAS is a large multienzyme complex. In mammals, FAS contains two subunits, each containing multiple enzyme activities. In bacteria and plants, individual proteins, which associate into a large complex, catalyze the individual steps of the synthesis scheme.
- The intermediate products in the fatty acid synthesis are bound to an acyl transport protein (ACP = acyl carrier protein). The extensions phase of the fatty acid synthesis begins with the formation of acetyl-ACP and malonyl-ACP by respectively acetyl transacylase and malonyl transacylase:

Acetyl CoA + ACP ⟶ Acetyl-ACP + CoA
Malonyl CoA + ACP ⟶ Malonyl-ACP + CoA

The four reactions of the chain extension in the fatty acid synthesis are a condensation, reduction, dehydration and a reduction.

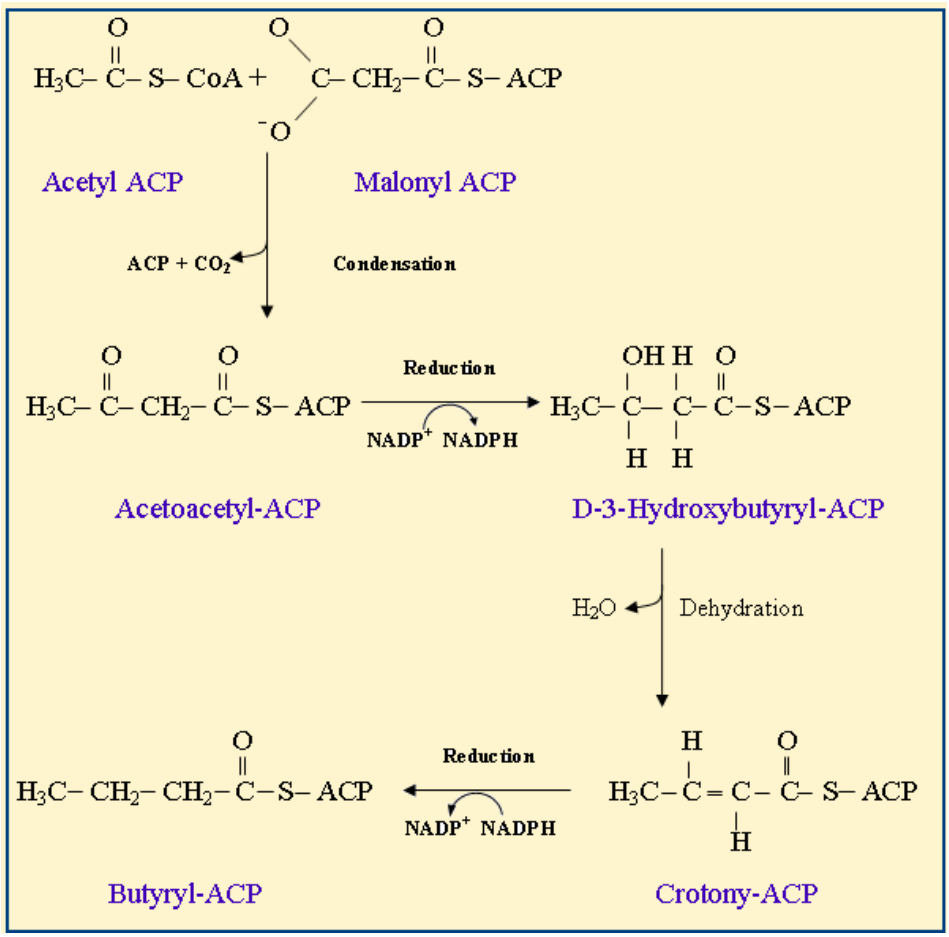

Fig. 21.2 Fatty acid synthesis.

- Acetyl-ACP and malonyl-ACP condense to acetoacetyl-ACP under influence of the enzyme acyl-malonyl-ACP condensing enzyme. In this condensation reaction, a C4-unit is formed from a C2- and a C3-unit while a CO_2-group is split off.
- In the three next reactions of the fatty acid synthesis, the keto-group (C with double tied oxygen) at the third carbon atom is reduced to a methylene group (-CH2-). In the first reaction, acetoacetyl-ACP is reduced to D -3-hydroxybutyryl-ACP.
- Here the reaction differs in two respects from the similar reaction in the fatty acid break down:
    - (1) instead of the L-epimer the D-epimer is formed.
    - (2) NADPH is the reducing compound in synthesis, while NAD+ is the oxidising compound in the Beta-oxidation. This difference is an example of the general principle that NADPH is used for synthetic (build up) reactions and NADH is made in energy supplying reactions.

- Then D-3-hydroxybutyryl-ACP is dehydrated to crotonyl-ACP. In the last step, crotonyl-ACP is reduced to butyryl-ACP, with which the first extension cycle is completed.
- After the first round, butyryl-ACP is formed. In the second round, butyryl-ACP condenses with malonyl-ACP. Below go the reactions as in the first round. As to it are added each round 2 carbon atoms. This goes on until palmitate (C16) has been formed.

21.3 Citrate Transports Acetyl Groups from the Mitochondria to the Cytoplasm

- Fatty acids are formed from acetyl CoA in the cytoplasm, while acetyl CoA from pyruvate is anabolised in the mitochondria. Acetyl CoA is transported from the mitochondria to the cytoplasm in the form of citrate and involves an additional enzyme, citrate ATP-lyase.

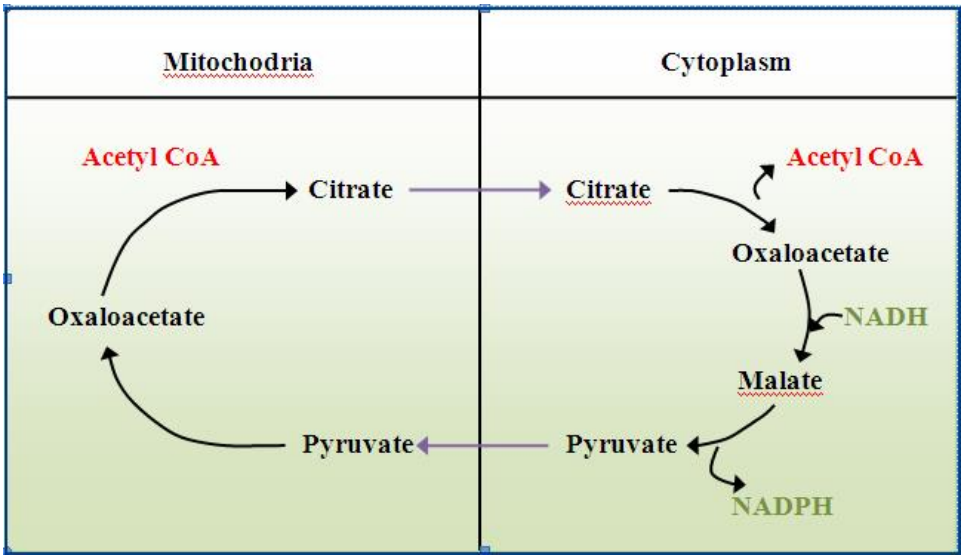

Fig. 21.3 Acetyl CoA Shuttle system

21.4 Stoichiometry of the Synthesis of Palmitate

7 Acetyl CoA + 7 CO_2 + 7 ATP + 7 H_2O → 7 Malonyl CoA + 7 ADP
Acetyl CoA + 7 malonyl CoA + 14 NADPH → Palmitate + 7 CO_2 + 14 $NADP^+$ + 6 H_2O
Sum: 8 Acetyl CoA + 7 ATP + 14 NADPH + H_2O → palmitate + 7 ADP + 14 $NADP^+$

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Lesson 22
ELECTRON TRANSPORT CHAIN AND ATP SYNTHESIS

22.1 Introduction

- In eukaryotes, electron transport and oxidative phosphorylation occur in the inner membrane of mitochondria.
- These processes re-oxidize the NADH and $FADH_2$ that arise from the citric acid cycle (located in the mitochondrial matrix), glycolysis (located in the cytoplasm) and fatty acid oxidation (located in the mitochondrial matrix) and trap the energy released as ATP.
- Oxidative phosphorylation is by far the major source of ATP in the cell. In prokaryotes, the components of electron transport and oxidative phosphorylation are located in the plasma membrane.

22.2 Redox Potential

- The oxidation of a molecule involves the loss of electrons. The reduction of a molecule involves the gain of electrons. Since in a chemical reaction, if one molecule is oxidized, another must be reduced (i.e. it is an oxidation-reduction reaction). Thus, by definition, oxidation-reduction reactions involve the transfer of electrons.
- In the oxidation-reduction reaction when the NADH is oxidized to NAD^+, it loses electrons. When the molecular oxygen is reduced to water, it gains electrons.

$$NADH + H^+ + \tfrac{1}{2}\, O_2 \rightleftharpoons NAD^+ + H_2O$$

- The oxidation-reduction potential, E, (or redox potential) is a measure of the affinity of a substance for electrons and is measured relative to hydrogen. A positive redox potential means that the substance has a higher affinity for electrons than does hydrogen and so would accept electrons from hydrogen. A substance with a negative redox potential has a lower affinity for electrons than does hydrogen and would donate electrons to H+, forming hydrogen. In the example above, NADH is a strong reducing agent with a negative redox potential and has a tendency to donate electrons. Oxygen is a strong oxidizing agent with a positive redox potential and has a tendency to accept electrons.
- For biological systems, the standard redox potential for a substance (E0') is measured under standard conditions, at pH 7, and is expressed in volts. In an oxidation-reduction reaction, where electron transfer is occurring, the total voltage change of the reaction (change in electric potential, ΔE) is the sum of the voltage changes of the individual oxidation-reduction steps. The standard free energy change of a reaction at pH 7, $\Delta G0'$, can be readily calculated from the change in redox potential $\Delta E0'$ of the substrates and products:

$$\Delta G^{0'} = -nF\ \Delta E_0'$$

Where n is the number of electrons transferred, $\Delta E_0'$ is in volts (V), $\Delta G^{0'}$ is in kilocalories per mole (kcal mol^{-1}) and F is a constant called the Faraday (23.06 kcal V^{-1} mol^{-1}). Note that a reaction with a positive $\Delta E0'$ has a negative $\Delta G^{0'}$ (i.e., is exergonic).
Thus for the reaction:

$$NADH + H^+ + \tfrac{1}{2}\, O_2 \rightleftharpoons NAD^+ + H_2O$$

$$\Delta E_0' = +\ 1.14\ V$$

$$\Delta G^{0'} = -\ 52.6\ kcal\ mol^{-1}.$$

22.3 Electron Transport from NADH

Comparing the energetic of the oxidation of NADH:

$$NADH + H^+ + \tfrac{1}{2} O_2 \rightleftharpoons NAD^+ + H_2O \qquad \Delta G^{0'} = -52.6 \text{ kcal mol}^{-1}$$

and the synthesis of ATP:

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O \qquad \Delta G^{0'} = +7.3 \text{ kcal mol}^{-1}$$

Thus, the oxidation of NADH releases sufficient energy to drive the synthesis of several molecules of ATP.

- NADH oxidation and ATP synthesis do not occur in a single step. Electrons are not transferred from NADH to oxygen directly. Rather the electrons are transferred from NADH to oxygen along a chain of electron carriers collectively called the electron transport chain (also called the respiratory chain).

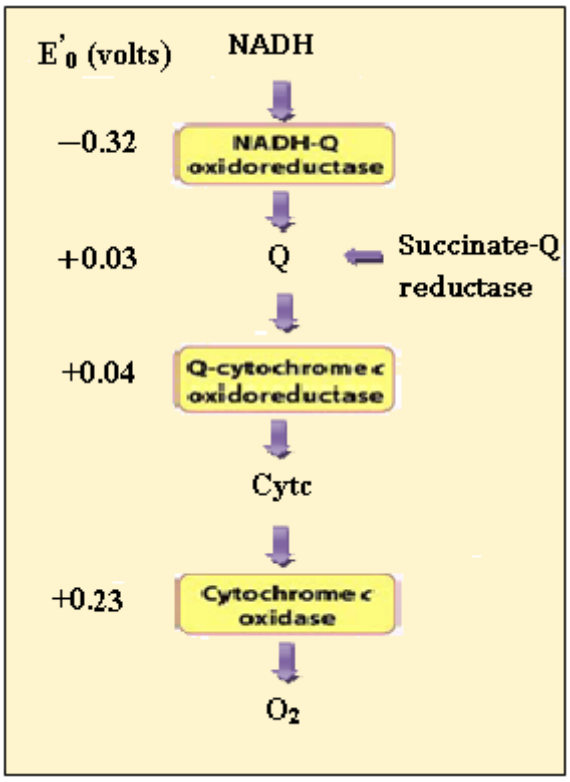


Fig 22.1 Organization of ETC Complexes

22.4 Electron Transport Chain

Electron transport chain consists of three large protein complexes embedded in the inner mitochondrial membrane,

- NADH dehydrogenase complex (Complex I)
- Succinate Q reductase (Complex II)
- The cytochrome bc1 complex (Complex III)
- cytochrome oxidase (Complex IV)

Electrons flow from NADH to oxygen through these three complexes as shown in Fig 22.1

Each complex contains several electron carriers that work sequentially to carry electrons down the chain. Two free electron carriers are also needed to link these large complexes;

- Ubiquinone also called as coenzyme Q (CoQ)
- cytochrome c

22.5 ATP Synthesis (Oxidative Phosphorylation)

- Oxidative phosphorylation is also the name given to the synthesis of ATP (phosphorylation) that occurs when NADH and FADH2 are oxidized (hence oxidative) by electron transport through the respiratory chain. Unlike substrate level phosphorylation, it does not involve phosphorylated chemical intermediates.
- This proposes that energy liberated by electron transport is used to create a proton gradient across the mitochondrial inner membrane and that is used to drive ATP synthesis (chemiosmotic hypothesis). Thus the proton gradient couples electron transport and ATP synthesis, not a chemical intermediate as in substrate level phosphorylation.
- The actual synthesis of ATP is carried out by an enzyme called ATP synthase located in the inner mitochondrial membrane. (Note that the enzyme was originally called an ATPase because, without the input of energy from electron transport, the reaction can reverse and actually hydrolyzes ATP.)

22.6 Summary

- In brief, Electron transport down the respiratory chain from NADH oxidation causes H^+ ions to be pumped out of the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space by the three H^+ pumps; NADH dehydrogenase, the cytochrome bc1 complex and cytochrome oxidase.
- The free energy change in transporting an electrically charged ion across a membrane leads to the formation of electrochemical proton gradient. The pumping out of the H^+ ions generates a higher concentration of H+ ions in the intermembrane space and an electrical potential. Thus, the side of the inner mitochondrial membrane facing the intermembrane space being positive.
- The protons flow back into the mitochondrial matrix according to electrochemical gradient through the ATP synthase and this drives ATP synthesis. The ATP synthase is driven by proton-motive force, which is the sum of the pH gradient (i.e. the chemical gradient of H^+ ions) and the membrane potential (i.e. the electrical charge potential across the inner mitochondrial membrane).
- FADH2 is reoxidized via ubiquinone , its oxidation causes H^+ ions to be pumped out only by the cytochrome bc1 complex and cytochrome oxidase and so the amount of ATP made from $FADH_2$ is less than from NADH. Measurements made have shown that 2.5 ATP molecules are synthesized per NADH oxidized whereas 1.5 ATPs are synthesized per $FADH_2$ oxidized.

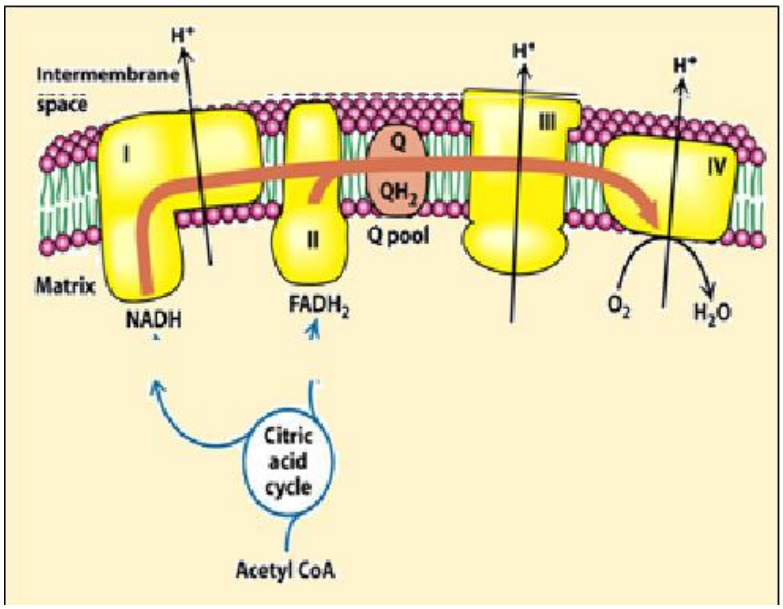


Fig. 22.2 Summary of electron flow

Lesson 23
AMINO ACID CATABOLISM

23.1 Introduction

- The catabolism of the amino acids usually begins by removing the amino group.
- Amino groups can then be disposed of in urea synthesis.
- The carbon skeletons produced from the standard amino acids are then degraded to TCA intermediates or their precursors so that they can be metabolized to CO_2 and H_2O or used in gluconeogenesis.

23.2 Catabolic Pathway of Amino Acids Involves three Common Stages

- Removal of alpha-amino group from amino acids (amino acid deamination) and conversion of amino group to ammonia.
- Incorporation of ammonia into urea.
- Conversion of amino acid's carbon skeletons to common metabolic intermediate.

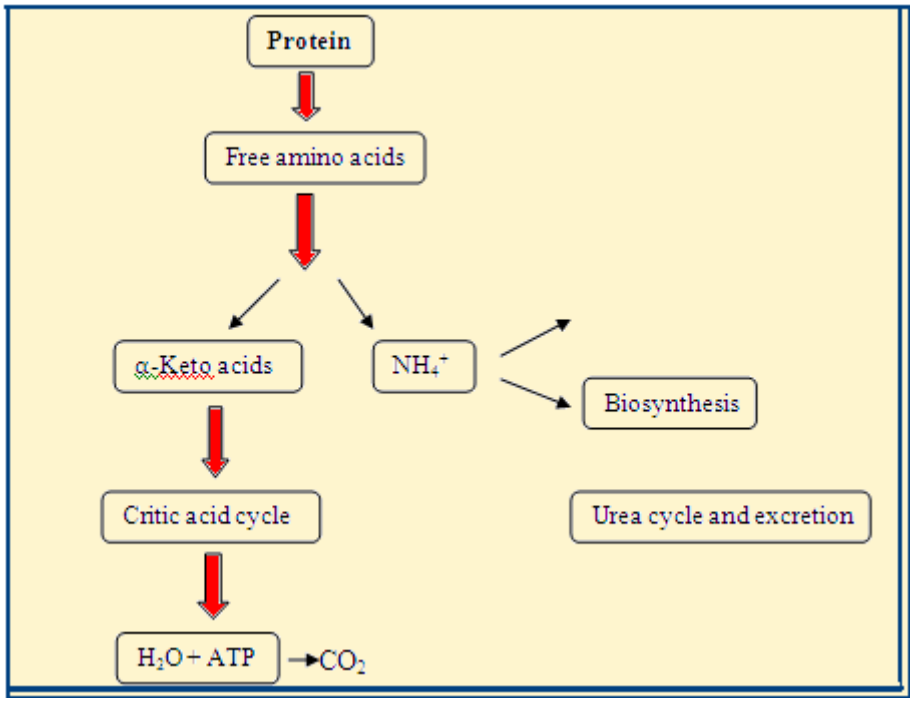


Fig. 23.1 General pathway showing the stages of amino acid catabolism

23.3 Amino Acid Deamination

The removal of the α-amino group from amino acids involve two types of biochemical reactions: transamination and oxidative deamination.

23.3.1 Transamination

- The dominant reactions involved in removing amino acid nitrogen are known as transaminations.
- This class of reactions funnel nitrogen from all free amono acids into a small number of compounds; then, either they are oxidatively deaminated, producing ammonia, or their amino groups are converted to urea by the urea cycle.

- Transaminations involve moving an α-amino group from a donor α-amino acid to the keto carbon of an acceptor α-keto acid. As a result of transfer, α-keto derivatives of amino acid and corresponding amino acid forms.
- All amino acids except lysine, threonine and proline participate in transamination during catabolism.
- Transamination is readily reversible. This reaction is catalyzed by enzyme called aminotransferase (also called transaminase). Each aminotransferase is specific for one or at most a few amino group donors. Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate, that get aminated to glutamate

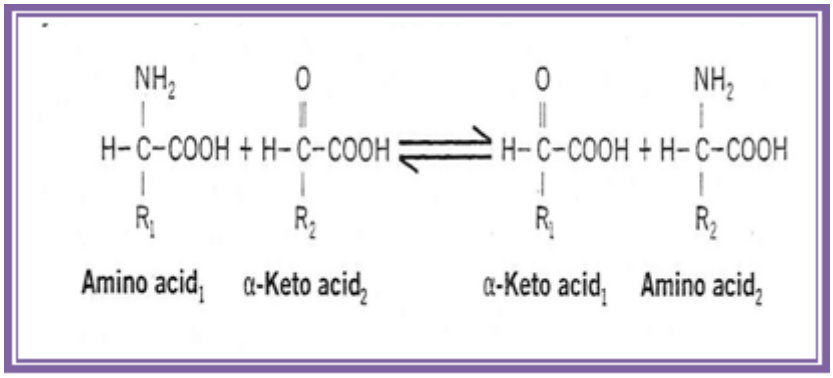
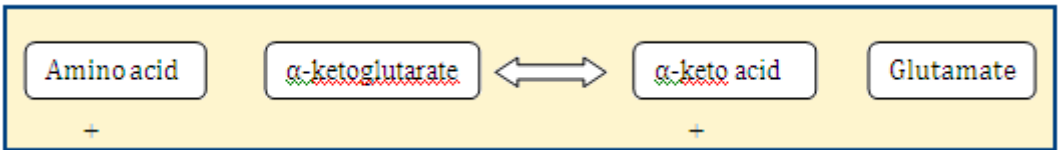


Fig 23.2 Transamination

- Aminotransferases require participation of an aldehyde-containing coenzyme, pyridoxal-5-phosphate, a derivative of pyridoxine (vitamin B6¬).
- Pyridoxal -5-phosphate is covalently attached to the enzyme via a schiff base linkage formed by the condensation of its aldehyde group with the α-amino group of lysine residue.
- Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pryidoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid and regenerates the original aldehyde form of the coenzyme.
- The most common compounds involved as a donor/acceptor pair in transamination reactions are glutamate and α-ketoglutarate, which participate in reactions with many different aminotransferases.



- All the amino nitrogen from amino acid that undergo transamination can be concentrated in glutamate. This is important because L-glutamate is the only amino acid that undergoes oxidative deamination at an appreciable rate.

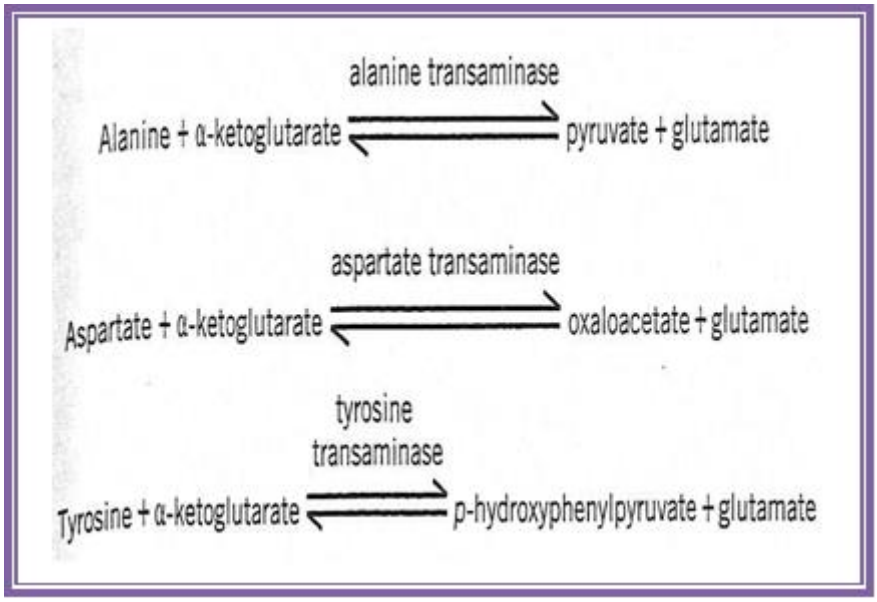


Fig. 23.3 Example of Transamination

23.3.2 Glucose-alanine cycle

In skeletal muscle, excess amino groups are generally transferred to pyruvate to form alanine, which finally enters into the liver where it undergoes transamination to yield pyruvate for use in gluconeogenesis. The resulting glucose is returned to the muscles, where it is glycolytically degraded to pyruvate.

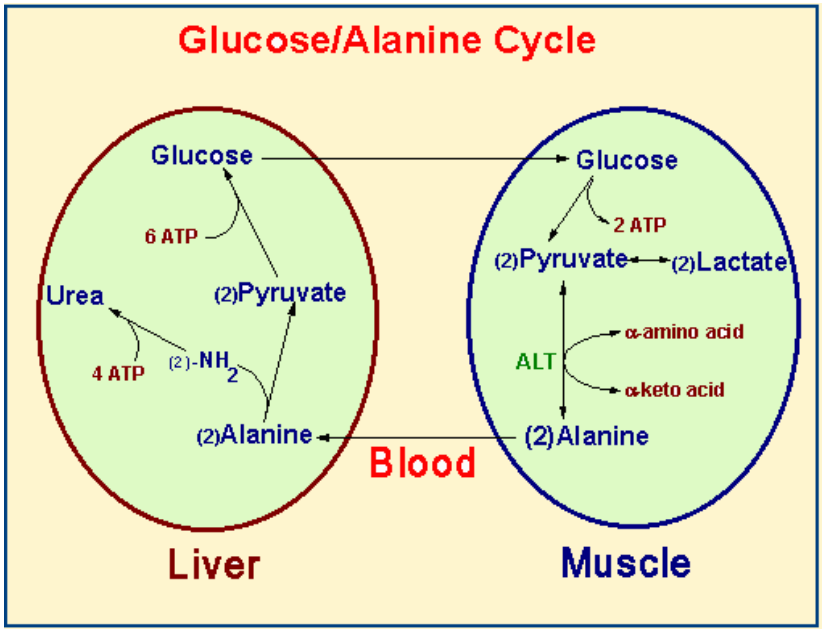


Fig. 23.4 Glucose Alanine Cycle

23.3.3 Oxidative deamination

- Transamination does not result in any net deamination. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia and the amine functional group is replaced by the ketone group. the ammonia eventually goes into the urea cycle.
- As the recipient of amino groups from many sources, glutamate now sheds it as ammonia for excretion, and the product a-ketoglutarate can recycle as a nitrogen acceptor, enter the TCA cycle, or serve as a precursor in gluconeogenesis

- Deamination occurs mainly through the oxidative deamination of glutamate by glutamate dehydrogenase. The reaction requires an oxidizing agent NAD^+ or $NADP^+$. Glutamate dehydrogenase is allosterically inhibited by GTP and NADH and activated by ADP and NAD^+.

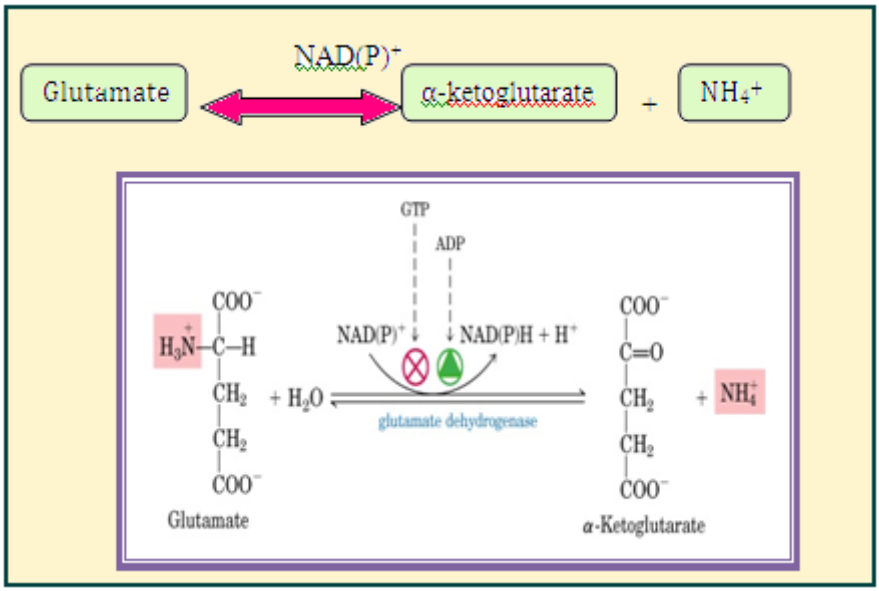


Fig 23.5 Oxidative deamination of Glutamate

23.4 Urea Cycle

- Living organisms excrete the excess nitrogen resulting from the metabolic breakdown of amino acids in one of three ways.
- Many aquatic animals simply excrete ammonia. Where water is less plentiful, however, processes have evolved that convert ammonia to less toxic waste products that therefore require less water for excretion. One such product is urea and other is uric acid.
- Accordingly, living organisms are classified as being either ammonotelic (ammonia excreting), ureotelic (urea excreting) or uricotelic (uric acid excreting).
- Urea is formed from ammonia, CO_2 and aspartate in a cyclic pathway referred to as the urea cycle. Because the urea cycle was discovered by Krebs and Henseleit, it is often referred to as Krebs-Henseleit cycle.
- Urea synthesis, which occurs in the hepatocytes (liver cells), consists of five sequential enzymatic reactions. The first two reactions occur in the mitochondria and the remaining three reactions take place in the cytosol.
- Urea cycle begins with the formation of carbamoyl phosphate in the mitochondria. The substrates for this reaction, catalyzed by carbamoyl phosphate synthetase I (CPSI), are NH_4^+ and HCO_3^-. Because two molecules of ATP are required in carbamoyl phosphate synthesis, this reaction is essentially irreversible (one is used to avtivate HCO_3^- and the second molecule is used to phosphorylate carbamate).
- Carbamoyl phosphate subsequently reacts with ornithine to form citrulline. Citrulline passes into the cytosol.

- Next three steps that occur in cytosol involves formation of argininosuccinate by ATP dependent reaction of citrulline with aspartate (aspartate provides second nitrogen that is ultimately incorporated into urea).
- Formation of arginine from argininosuccinate. This reaction releases fumarate, which enters the critic acid cycle. Formation of urea and regeneration of ornithine.

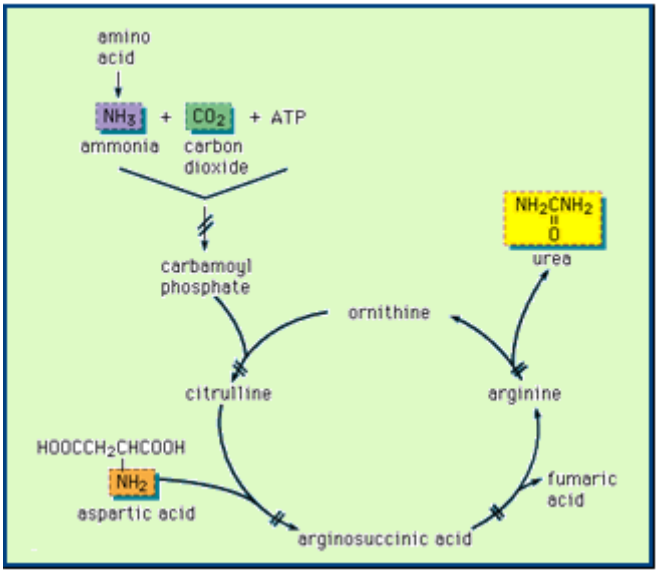


Fig. 23.6 Urea Cycle

Net reaction of urea cycle;

$CO2 + NH4^{+} + Aspartate + 3ATP + 2H_2O \Rightarrow Urea + Fumarate + 2ADP + AMP$

i.e., four high energy phosphates are consumed in the synthesis of one molecule of urea.

Module 4. Human nutrition

Lesson 24
HUMAN NUTRITION

24.1 Introduction

- Most foods contain a mix of some or all of the nutrient classes, together with other substances, such as toxins of various sorts. Some nutrients can be stored internally while others are required more or less continuously. Poor health can be caused by a lack of required nutrients or, in extreme cases, too much of a required nutrient. For example, both salt and water (both absolutely required) will cause illness or even death in excessive amounts.
- There are five basic components in a adequate diet that can be grouped into two category

1. Macronutrient (needed in relatively large amounts)

    - Carbohydrates
    - Fats
    - Proteins

- The macronutrients (excluding water) provide structural material (amino acids from which proteins are built, and lipids from which cell membranes and some signaling molecules are built).
- Some of the structural material can be used to generate energy internally, and in either case it is measured in Joules or kilocalories (often called "Calories" and written with a capital C to distinguish them from little 'c' calories). Carbohydrates and proteins provide 17 kJ approximately (4 kcal) of energy per gram, while fats provide 37 kJ (9 kcal) per gram. Though the net energy from either depends on such factors as absorption and digestive effort.

2. Micronutrient (needed in smaller quantities)

    - Vitamins
    - Minerals and trace elements

- Micronutrients also include antioxidants and phytochemicals, which are said to influence (or protect) some body systems Their necessity is not as well established as in the case of, for instance, vitamins.

24.2 Carbohydrates

- Carbohydrates include sugars, starches and fiber. They constitute a large part of foods such as rice, noodles, bread, and other grain-based products. They are the major source of biological energy through their oxidation. They furnish base material for the biosynthesis of many cell components.
- Carbohydrates rich foods are abundant & cheap. They form a major part of the diet of the world. 4/5 world's population rely on plant food. Carbohydrates provide 70-90% of calorie intake. But in affluent countries, where meat & dairy products are consumed it contribute 45% of total daily caloric intake. 40 % of carbohydrate is furnished by sucrose to the refined sugar.
- Dietary fiber is a carbohydrate (or a polysaccharide) that is incompletely absorbed in humans and in some animals. Like all carbohydrates, when it is metabolized it can produce four Calories (kilocalories) of energy per gram. However, in most circumstances it accounts for less than that because of its limited absorption and digestibility. Dietary fiber consists mainly of cellulose, a large carbohydrate polymer that is indigestible because humans do not have the required enzymes to disassemble it.

24.3 Fats

- A molecule of dietary fat typically consists of several fatty acids (containing long chains of carbon and hydrogen atoms), bonded to a glycerol. They are typically found as triglycerides (three fatty acids attached to one glycerol backbone).Triacylglycerols from animal and plant sources rank close behind carbohydrates as major sources of energy.
- Saturated fats (typically from animal sources) have been a staple in many world cultures for millennia. Unsaturated fats (e. g., vegetable oil) are considered healthier, while trans fats are to be avoided. Saturated and some trans fats are typically solid at room temperature (such as butter or lard), while unsaturated fats are typically liquids (such as olive oil or flaxseed oil). Trans fats are very rare in nature, and have been shown to be highly detrimental to human health, but have properties useful in the food processing industry, such as rancidity resistance.
- Most fatty acids are non-essential, meaning the body can produce them as needed, generally from other fatty acids and always by expending energy to do so. However, in humans, at least two fatty acids are essential and must be included in the diet An appropriate balance of essential fatty acids—omega-3 and omega-6 fatty acids—seems also important for health. Both of these "omega" long-chain polyunsaturated fatty acids are substrates for a class of eicosanoids known as prostaglandins, which have roles throughout the human body. They are hormones, in some respects.
- The omega-3 eicosapentaenoic acid (EPA), which can be made in the human body from the omega-3 essential fatty acid alpha-linolenic acid (LNA), or taken in through marine food sources, serves as a building block for series 3 prostaglandins (e.g. weakly inflammatory PGE3). The omega-6 dihomo-gamma-linolenic acid (DGLA) serves as a building block for series 1 prostaglandins (e.g. anti-inflammatory PGE1), whereas arachidonic acid (AA) serves as a building block for series 2 prostaglandins (e.g. pro-inflammatory PGE 2). Both DGLA and AA can be made from the omega-6 linoleic acid (LA) in the human body, or can be taken in directly through food. An appropriately balanced intake of omega-3 and omega-6 partly determines the relative production of different prostaglandins, which is one reason why a balance between omega-3 and omega-6 is believed important for cardiovascular health. In industrialized societies, people typically consume large amounts of processed vegetable oils, which have reduced amounts of the essential fatty acids along with too much of omega-6 fatty acids relative to omega-3 fatty acids.

24.4 Protein

- Most meats such as chicken contain all the essential amino acids needed for humans. Proteins are the basis of many animal body structures (e.g. muscles, skin, and hair). They also form the enzymes that control chemical reactions throughout the body.
- Each molecule is composed of amino acids, which are characterized by inclusion of nitrogen and sometimes sulphur (these components are responsible for the distinctive smell of burning protein, such as the keratin in hair). The body requires amino acids to produce new proteins (protein retention) and to replace damaged proteins (maintenance). As there is no protein or amino acid storage provision, amino acids must be present in the diet. Excess amino acids are discarded, typically in the urine.
- For all animals, some amino acids are essential (an animal cannot produce them internally) and some are non-essential (the animal can produce them from other nitrogen-containing compounds). About twenty amino acids are found in the human body, and about eight of these are essential and, therefore, must be included in the diet (HITPMTLLV). A diet that contains adequate amounts of amino acids (especially those that are essential) is particularly important in some situations: during early development and maturation, pregnancy, lactation, or injury (a burn, for instance). A complete protein source contains all the essential amino acids; an incomplete protein source lacks one or more of the essential amino acids.
- It is possible to combine two incomplete protein sources (e.g. rice and beans) to make a complete protein source, and characteristic combinations are the basis of distinct cultural cooking traditions. Sources of dietary protein include meats, tofu and other soy-products, eggs, legumes, and dairy

products such as milk and cheese. Excess amino acids from protein can be converted into glucose and used for fuel through a process called gluconeogenesis.

24.5 Minerals

Dietary minerals are the chemical elements required by living organisms, other than the four elements carbon, hydrogen, nitrogen, and oxygen that are present in nearly all organic molecules. Some dietitians recommend that these be supplied from foods in which they occur naturally, or at least as complex compounds, or sometimes even from natural inorganic sources (such as calcium carbonate from ground oyster shells). Some minerals are absorbed much more readily in the ionic forms found in such sources. On the other hand, minerals are often artificially added to the diet as supplements; the most famous is likely iodine in iodized salt which prevents goiter.

24.6 Macro minerals

Many elements are essential in relative quantity; they are usually called "bulk minerals". Some are structural, but many play a role as electrolytes. Following elements with recommended dietary allowance (RDA) greater than 200 mg/day are,

- Calcium, a common electrolyte, but also needed structurally (for muscle and digestive system health, bone strength, some forms neutralize acidity, may help clear toxins, provides signaling ions for nerve and membrane functions)
- Chlorine as chloride ions; very common electrolyte; see sodium, below
- Magnesium, required for processing ATP and related reactions (builds bone, causes strong peristalsis, increases flexibility, increases alkalinity)
- Phosphorus, required component of bones; essential for energy processing
- Potassium, a very common electrolyte (heart and nerve health)
- Sodium (also see salt), a very common electrolyte; not generally found in dietary supplements, despite being needed in large quantities, because the ion is very common in food: typically as sodium chloride, or common salt. Excessive sodium consumption can deplete calcium and magnesium, leading to high blood pressure and osteoporosis
- Sulfur, for three essential amino acids and therefore many proteins (skin, hair, nails, liver, and pancreas). Sulfur is not consumed alone, but in the form of sulfur-containing amino acids

24.7 Trace Minerals

Many elements are required in trace amounts, usually because they play a catalytic role in enzymes. Some trace mineral elements (RDA < 200 mg/day) are, in alphabetical order:

- Cobalt required for biosynthesis of vitamin B12 family of coenzymes. Animals cannot biosynthesize B12, and must obtain this cobalt-containing vitamin in the diet
- Copper required component of many redox enzymes, including cytochrome c oxidase and blood protein ceruloplasmin. Copper toxicity causes Wilson’s disease.
- Chromium required for sugar metabolism
- Iodine required not only for the biosynthesis of thyroxine, but probably, for other important organs as breast, stomach, salivary glands, thymus etc. (see Extrathyroidal iodine); for this reason iodine is needed in larger quantities than others in this list, and sometimes classified with the macrominerals
- Iron required for many enzymes, and for hemoglobin and some other proteins
- Manganese (processing of oxygen)
- Molybdenum required for xanthine oxidase and related oxidases
- Nickel present in urease
- Selenium required for peroxidase (antioxidant proteins)
- Vanadium (Speculative: there is no established RDA for vanadium. No specific biochemical function has been identified for it in humans, although vanadium is required for some lower organisms.

- Zinc required for several enzymes such as carboxypeptidase, liver alcohol dehydrogenase, glutamate dehydrogenase and carbonic anhydrase

24.8 Vitamins

As with the minerals discussed above, some vitamins are recognized as essential nutrients, necessary in the diet for good health. (Vitamin D is the exception: it can be synthesized in the skin, in the presence of UVB radiation.)
Water Soluble – B1, B2, B6, B12
Fat Soluble – A, D, E K

- Serve as essential components of specific coenzymes and enzymes participating in metabolism and other specialized activities like NADPH, FAD.
- Vitamin deficiencies may result in disease conditions, including goitre, scurvy, osteoporosis, impaired immune system, disorders of cell metabolism, certain forms of cancer, symptoms of premature aging, and poor psychological health (including eating disorders), among many others Excess levels of some vitamins are also dangerous to health (notably vitamin A), and for at least one vitamin, B6, toxicity begins at levels not far above the required amount.

24.9 Water
It is not fully clear how much water intake is needed by healthy people, although some assert that 6–8 glasses of water daily is the minimum to maintain proper hydration.The original water intake recommendation in 1945 by the Food and Nutrition Board of the National Research Council read: "An ordinary standard for diverse persons is 1 milliliter for each calorie of food. Most of this quantity is contained in prepared foods." The latest dietary reference intake report by the United States National Research Council recommended, generally, (including food sources): 2.7 liters of water total for women and 3.7 liters for men. Specifically, pregnant and breastfeeding women need additional fluids to stay hydrated.

Water is sometimes called the “silent nutrient” and is taken for granted in nutritional consideration. A deficient intake, however, can produce death faster than that of any other nutrient. Total body water in humans varies from 55% to 65% of body weight depending on body composition. Lean body tissues contain approximately 75% of water, but adipose tissue has very little water. Therefore, the percentage of water is greater in lean than in obese individuals.

Most of the body water is found within three major body compartments: intracellular fluid (within the cells) has about 70%, interstitial fluid (e.g., lymph) has about 20%, and blood plasma has about 7%. The latter two compartments together come under the extracellular fluid category. The remaining 3% of body water is in the intestinal lumen, cerebrospinal fluid and other body compartments. The body controls the amount of water in each compartment mainly by controlling the ion concentrations in each compartment. Intracellular water volume depends primarily on intracellular potassium and phosphate concentration. Extracellular water volume depends primarily on extracellular sodium and chloride concentration.

The body has three sources of water: ingested water and beverages, the water content of solid foods, and metabolic water which is derived from the oxidation of carbohydrate, fat, and protein. The latter amounts to some 300-350 g per day in an average adult male. According to composite estimates, 100 g of starch yields 55 g of water, 100 g of fat yields 107 g of water, and 100 g of protein gives 41 g of water. Water is absorbed in the upper small intestine and is distributed by way of the lymph and blood into and from the various tissues and cells of the body. Eventually water is excreted via the kidneys, sweat, expired air, feces, and so on.

Under ordinary conditions, the water balance between the cells and the fluids of the body is maintained at a constant level. The loss of water equals the intake and endogenous formation, and is in the range of 2-4 L. Water intake is regulated mainly by “thirst” and the output is controlled by antidiuretic hormone and the kidneys. If excessive amounts of water are ingested, the kidneys excrete the excess. On the other hand, if the

fluid intake is low, the kidneys excrete a more concentrated urine so that less water is lost from the body. Starvation or a carbohydrate-restricted regimen is associated with an acute loss of body water (e.g., 1-1.5L) which represents the water normally held by glycogen storage in the tissues.

Table 24.1 List of forty different substances which are essential for human

Energy sources	
Carbohydrates	Pantothenic acid
Fats	Folic acid
Proteins	Biotin
Essential amino acids	Vitamin B_{12}
Argenine Arg R	Ascorbic acid (Vit C)
Histidene His H	Vit A D E & K
Isoleucine ILe I	
Leucine Leu L	Essential fatty acid
Lysine Lys K	Linoteic acid
Methionine Net M	Lenotenic acid
Phenylalamine Phe F	Mineral elements
Threonine Thr T	Arsenic Magnesium
Tryptophan Trp W	Calcium Manganese
Valine Val V	Chlorine Sodium
(Vitamins)	Nickel Molybdenum
Theamine (B_1)	Copper Phosphorus
Resoflanin (B_2)	Fluorine Potassium
Micotenanide	Iodine Selenium Zinc
Pyridoxine (B_c)	Iron Silicon, Sulphur

24.10 Energy provided by oxidation of bulk organic nutrient

The first requisite of an adequate diet is a source of energy- provided by oxidation of the three bulk nutrients

- Carbohydrates
- Fats
- Proteins

Unit—Kilocalorie (kcal)
The amount of energy which in the form of heat is required to raise the temperature of 1.0Kg of water by 1.0 for 15 to 16^{o}C.
Recommended daily energy allowances by Food & Nutrition Board.

College-age male require—2900 kcal/d
Female – 2100 kcal/d

Compare these values with basal caloric requirement i.e. the amount of energy needed by the body at complete rest 12h after a meal.

For college age males Basal Requirement-1800 kcal/d

Females-1300kcal/d

The excess energy-for physical work.

Amount of energy released by the oxidation of carbohydrates, fat, proteins has been determined by burning weighed samples in an atmosphere of oxygen in a Bomb calorimeter and total amount of heat produced is measured.

Caloric equivalent of Major Nutrient
Energy equivalent kcal/g
Carbohydrates 4.2
Fat 9.5
Protein 4.3

These foods when oxidized in body i.e. they are completely digested and absorbed yield an amount of heat equal to the heat released when they are oxidized in a calorimeter.

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Lesson 25
NUTRIENT REQUIREMENTS OF DIFFERENT AGE GROUPS

25.1 Fundamentals of Nutrition

The science of nutrition deals with the processes by which components of food are made available to an organism for meeting energy requirements, for building and maintaining tissues, and, in a more general sense, for maintaining the organism in optimal functional health. Thus nutrition is concerned with many issues traditionally considered to be digestion, absorption, transport, metabolism, and biochemical functions performed by individual chemical substances.

- Nutrients are those chemical substances needed for growth and maintenance of normal cells, both in animals and plants. Clinical nutrition is a medical specialty dealing with the relationship between disease and nutrition. Acute and chronic illnesses are caused by deficiencies of dietary components and others by their excesses.
- Malnutrition is a condition characterized by inappropriate quality, quantity, digestion, absorption or utilization of ingested nutrients. It includes: undernutrition – low food intake (calorie deficiency) leading to growth suppression or other deficiency signs, and overnutrition to consume too much food and/or single nutrients leading to specific toxicities.
- Some 45-50 chemical entities are now known to be required by humans, either preformed in food or added as an appropriate chemical substitute. These can be divided into six main categories: carbohydrates, fats, proteins, vitamins, inorganic elements, and are important in maintaining good health. The term essential or dietary essential means that we must obtain the nutrient from our diet either because we lack the biochemical machinery to manufacture it or we cannot make enough of it.

25.2 Recommended Dietary Allowances (RDA)

- RDA are developed by the Food and Nutrition Board of the National Academy of Sciences.
- RDAs are defined as the “levels of intake of essential nutrients considered, in the judgment of Committee of Dietary Allowances of the Food and Nutrition Board, on the basis of available scientific knowledge to be adequate to meet the known nutritional needs of practically all healthy persons.”
- The RDAs are meant to apply only to a healthy population and should be met from the consumption of a wide variety of readily available foods.
- RDAs should not be confused with nutrient requirements of individuals because these are too variable. Rather, an RDA represents an average level of daily intake of a nutrient which over time approximates the RDA, and thus the nutritional inadequacy will be rare in that population.
- RDAs do not provide the needs that have been altered as a result of disease states, chronic usage of certain drugs, or other factors that require specific individual attention.

Table 25.1 Summary of RDA for Indians 2010

Group	Particulars	Body Wt. (kg)	Net energy (kcal/d)	Protein (g/d)	Visible Fat (g/d)
Man	Sedentary work	60	2320	60.0	25
	Moderate work		2730		30
	Heavy work		3490		40
Woman	Sedentary work	55	1900	55.0	20
	Moderate work		2230		25
	Heavy work		2850		30
Children	1-3 years	12.9	1060	16.7	27
	4-6 years	18.0	1350	20.1	25
	7-9 years	25.1	1690	29.5	30
Boys	10-12 years	34.3	2190	39.9	35
Girls	10-12 years	35.0	2010	40.4	35

- Energy RDA Each individuals food energy intake must equal the energy expended, in order for the person to maintain their body weight.
- Protein RDA Protein recommendations are mainly based on the individuals body weight. The protein RDA is high, to cover most person's needs. The average requirement for protein is 0.6 grams per kilogram of body weight; the RDA is 0.8 grams this is said to meet 97.5% of the population's needs.
- No RDA for Carbohydrate and Fat The amount of protein recommended represents a small percentage of a person's energy allowance; with the remainder acquired from carbohydrates and fats. The general guideline for carbohydrate and fat is that more than half of daily energy should come from carbohydrates, with no more than one-third from fat.

25.3 Minimal Daily Requirement (MDR)

- (MDR) is the minimum amount of a nutrient from exogenous sources required to sustain normality .
- Individuals consume food more for satiation of energy needs than for individual nutrients.
- To express the quality of any food in relation to its content of specific nutrient, the term nutrient density is used. It is defined as the concentration of a nutrient per unit of energy (e.g., 1,000 calories) in a specific food.
- For any nutrient the higher the nutrient density the better the food source; for example, one whole green pepper contains 20 mg of vitamin C and provides 4 calories, while one medium sweet potato also contains 20 mg of vitamin C but provides 100 calories. Therefore, green pepper is a much better source of vitamin C than sweet potato.

25.4 Metabolism

All cells have in common two major general functions: energy generation and energy utilization for growth and/ or maintenance. These may be termed metabolic reactions or simply metabolism.

- Anabolism broadly refers to processes in which relatively large molecules such as proteins are biosynthesized from small nutrient materials such as amino acids. These reactions require energy which is available in cells in the form of stored chemical energy in high energy phosphate compounds.
- Catabolism is the degradation of relatively large molecules to smaller ones. Catabolic reactions serve to capture chemical energy (in the form of adenosine triphosphate, ATP) from the degradation of energy-rich molecules. Catabolism also allows nutrients (in the diet or stored in cells) to be converted into the building blocks needed for the synthesis of complex molecules.

- Intermediary metabolism refers to all changes that occur in a food substance beginning with absorption and ending with excretion.
- In the adult there is a delicate regulated balance between anabolic (synthetic) and catabolic (degradative) processes. In the growing child, input of nutrients and anabolism exceed catabolism so that the growth of tissues may occur. In the aging process or in wasting diseases, the catabolic processes exceed anabolic ones.
- Bio-availability: Bio-availability of a given nutrient from a diet, that is, the release of the nutrient from the food, its absorption in the intestine and bioresponse have to be taken into account. It is the level of the nutrient that should be present in the diet to meet the requirement. This bio-availability factor is quite important in case of calcium and protein and trace elements like iron and zinc. In case of iron, the amount to be present in the diet is 20-30 times higher than the actual iron requirement to account for the low bio-availability of iron from a given diet, particularly a cereal-based diet.

Table 25.2 Basic four food groups

Group	Food	Major nutrients
Milk	Milk and other dairy products	Calcium, protein riboflavin
Meat	Meat, poultry, fish, eggs Beans, peas, nuts, seeds (meat substitutes)	Protein, fat, iron, other minerals
Fruits and vegetable	All varieties of fruits and vegetables, green yellow vegetables	Vitamin C, vitamin A precursors
Bread and cereal	Bread, cooked cereal, dry cereal, rice, pasta	B vitamins, iron, carbohydrate

25.5 The Need for Energy

- The human body needs a continuous regulated supply of nutrients. Energy is required for all body processes, growth, and physical activity. Even at rest the body requires energy for muscle contraction, active transport of molecules and ions, and synthesis of macromolecules and other biomolecules from simple precursors.
- For example, the heart pumps approximately 8,000 L/day of blood in about 80,000 pulsations. The daily energy required for this heart function alone is estimated to be equivalent to lifting a weight of 1,000 kg to a height of 10 meters.
- In most processes the energy is supplied by adenosine triphosphate (ATP). Energy is liberated when ATP is hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate. A resting human consumes about 40 kg of ATP in 24 hours. The amount of ATP in the body tissues is limited but is generated continuously from the fuel stores to supply the required energy. These fuel stores must be replenished via food intake.

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Lesson 26
EVALUATION OF NUTRITIVE VALUE OF FOOD

26.1 Nutritive value

It is the indication of the contribution of a food to the nutrient content of the diet. This value depends on the quantity of a food which is digested and absorbed and the amounts of the essential nutrients (protein, fat, carbohydrate, minerals, vitamins) which it contains. This value can be affected by soil and growing conditions, handling and storage, and processing.

26.2 Calorie Content of Food (Bomb Calorimeter)

A bomb calorimeter is a type of constant-volume calorimeter used in measuring the heat of combustion of a particular reaction. Bomb calorimeters have to withstand the large pressure within the calorimeter as the reaction is being measured. Electrical energy is used to ignite the fuel; as the fuel is burning, it will heat up the surrounding air, which expands and escapes through a tube that leads the air out of the calorimeter. When the air is escaping through the copper tube it will also heat up the water outside the tube. The temperature of the water allows for calculating calorie content of the fuel.

Basically, a bomb calorimeter consists of a small cup to contain the sample, oxygen, a stainless steel bomb, water, a stirrer, a thermometer, the dewar (to prevent heat flow from the calorimeter to the surroundings) and ignition circuit connected to the bomb (Figure 26.1)

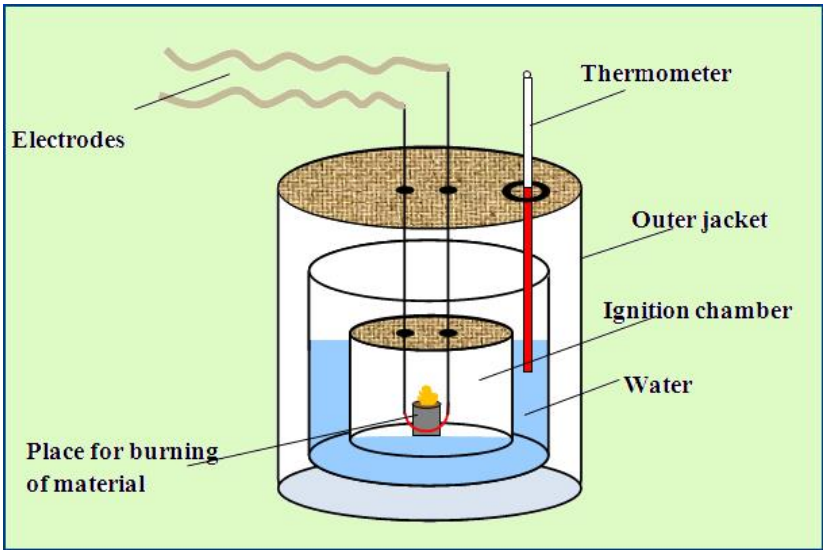

Fig. 26.1 Bomb calorimeter

Unit—Kilocalorie (kcal)
The amount of energy which in the form of heat is required to raise the temperature of 1.0 Kg of water by 1.0 from 15 to 16^{o}C.

26.3 Crude Protein

The classic methods for protein concentration in food are the Kjeldahl method and the Dumas method. These tests determine the total nitrogen in a sample. The only major component of most food which contains nitrogen is protein (fat, carbohydrate and dietary fibre do not contain nitrogen). If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected in the food, the total protein can be determined. This value is known as the "crude protein" content. On food labels the protein is given by the

nitrogen multiplied by 6.25, because the average nitrogen content of proteins is about 16%. The Kjeldahl test is typically used because it is the method the AOAC International has adopted and is therefore used by many food standards agencies around the world.

- Kjeldahl method

The method consists of digestion of the substance with sulfuric acid in presence of potassium sulphate that increases the boiling point of the medium (from 337°F to 373°F / 169°C to 189°C). Digestion decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. Chemical decomposition of the sample is complete when the medium has become clear and colorless (initially very dark).

The solution is then distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia. The amount of ammonia present (hence the amount of nitrogen present in the sample) is determined by back titration. The end of the condenser is dipped into a solution of standard boric acid solution. The ammonia reacts with the acid and the remainder of the acid is then titrated with a standard sodium carbonate solution with a methyl orange pH indicator.

Digestion: Protein + $H_2SO_4 \rightarrow (NH_4)_2SO_4(aq) + CO_2(g) + SO_2(g) + H_2O(g)$

(a) Liberation of ammonia: $(NH_4)_2SO_4(aq) + 2NaOH \rightarrow Na_2SO_4(aq) + 2H_2O(l) + 2NH_3(g)$

(b) Capture of ammonia: $B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^- + B(OH)_4^-$

Back-titration: $B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3(aq) + NaB(OH)_4(aq) + CO_2(g) + H_2O$

Nowadays, the Kjeldahl method is largely automated and makes use of specific catalysts (mercury oxide or copper sulfate) to speed up the decomposition.

26.4 Carbohydrate

The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured: % carbohydrates = 100 – (% moisture + %protein + %lipid + % mineral). Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to directly measure the carbohydrate content for accurate measurements.

The amount of preparation needed to prepare a sample for carbohydrate analysis depends on the nature of the food being analyzed. Aqueous solutions, such as fruit juices, syrups and honey, usually require very little preparation prior to analysis. On the other hand, many foods contain carbohydrates that are physically associated or chemically bound to other components, e.g.,seeds, nuts, cereals, fruit, woody material breads and vegetables. In these it is usually necessary to isolate the carbohydrate from the rest of the food before it can be analyzed. The precise method of carbohydrate isolation depends on the carbohydrate type, the food matrix type and the purpose of analysis, however, there are some procedures that are common to many isolation techniques. For example, foods are usually dried under vacuum (to prevent thermal degradation), ground to a fine powder (to enhance solvent extraction) and then defatted by solvent extraction.

One of the most commonly used methods of extracting low molecular weight carbohydrates is to boil a defatted sample with an 80% alcohol solution. Monosaccharides and oligosaccharides are soluble in alcoholic solutions, whereas proteins, polysaccharides and dietary fiber are insoluble. The soluble components can be separated from the insoluble components by filtering the boiled solution and collecting the filtrate (the part which passes through the filter) and the retentate (the part retained by the filter). These two fractions can then be dried and weighed to determine their concentrations. In addition, to monosaccharides and oligosaccharides various other small molecules may also be present in the alcoholic extract that could interfere with the subsequent analysis e.g., amino acids, organic acids, pigments, vitamins,

minerals etc. It is usually necessary to remove these components prior to carrying out a carbohydrate analysis. This is commonly achieved by treating the solution with clarifying agents or by passing it through one or more ion-exchange resins.

A number of chemical methods used to determine monosaccharides and oligosaccharides are based on the fact that many of these substances are reducing agents that can react with other components to yield precipitates or colored complexes which can be quantified. The concentration of carbohydrate can be determined gravimetrically, spectrophotometrically or by titration. Non-reducing carbohydrates can be determined using the same methods if they are first hydrolyzed to make them reducing. It is possible to determine the concentration of both non-reducing and reducing sugars by carrying out an analysis for reducing sugars before and after hydrolysis. Many different chemical methods are available for quantifying carbohydrates. Most of these can be divided into three catagories: titration, gravimetric and colorimetric

- Colorimetric Methods

The Anthrone method is an example of a colorimetric method of determining the concentration of the total sugars in a sample. Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. The sample is mixed with sulfuric acid and the anthrone reagent and then boiled until the reaction is completed. The solution is then allowed to cool and its absorbance is measured at 620 nm. There is a linear relationship between the absorbance and the amount of sugar that was present in the original sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid. Like the other methods it is non-stoichemetric and therefore it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.

The Phenol - Sulfuric Acid method is an example of a colorimetric method that is widely used to determine the total concentration of carbohydrates present in foods. A clear aqueous solution of the carbohydrates to be analyzed is placed in a test-tube, then phenol and sulfuric acid are added. The solution turns into yellow-orange color as a result of the interaction between the carbohydrates and the phenol. The absorbance at 420 nm is proportional to the carbohydrate concentration initially in the sample. The sulfuric acid causes all non-reducing sugars to be converted to reducing sugars, so that this method determines the total sugars present. This method is non-stoichemetric and so it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration.

- Gravimetric Method
- Crude Fiber Method

The crude fiber method gives an estimate of indigestible fiber in foods. It is determined by sequential extraction of a defatted sample with 1.25% H2SO4 and 1.25% NaOH. The insoluble residue is collected by filtration, dried, weighed and ashed to correct for mineral contamination of the fiber residue. Crude fiber measures cellulose and lignin in the sample, but does not determine hemicelluloses, pectins and hydrocolloids, because they are digested by the alkali and acid and are therefore not collected. For this reason many food scientists believe that its use should be discontinued. Nevertheless, it is a fairly simple method to carry out and is the official AOAC method for a number of different foodstuffs.

26.5 Ether Extraction for Lipid Estimation

The method described by Soxhlet in 1879 is the most commonly used example of a semi-continuous method applied to extraction of lipids from foods. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware as shown in figure.

In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber (2), which is suspended above a flask containing the solvent (1) and below a condenser (4). The flask is heated and the solvent evaporates and moves up into the condenser

where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and lipid is removed. In some device a funnel (3) allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask (1) is then evaporated and the mass of the remaining lipid is measured. The percentage of lipid in the initial sample can then be calculated.

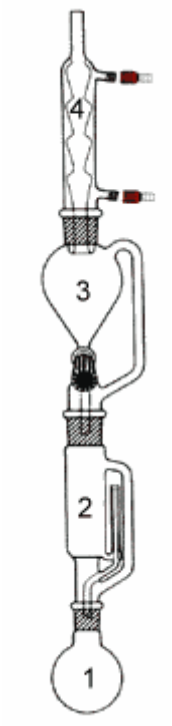

Fig. 26.2 Soxhlet appratus

Despite disadvantages of this procedure (poor extraction of polar lipids, long time involved, large volumes of solvents, hazards of boiling solvents, several methods involving automatic solvent extraction were described. Different automated or semi-automated extraction instruments may be found on the market.

26.6 Nutrition Value of Milk

Milk is a prefect food as it provides vital nutrients like proteins, EFA,vitamins, minerals and lactose in balanced proportions. It complements and supplements nutrients available from grains, legumes, vegetables, fruits, meat, seafoods and poultary. Milk has high nutrient density. High concentration of major nutrients at relatively low caloric value. Concentrations of nutrients and energy in milk are given in table 26.1

Table 26.1 Nutrition value of Cow, buffalo and goat milk per 100g

	Cow	Buffalo	Human
Water	87.99	83.39	87.50
Food energy (Kcal)	61.44	96.62	69.56
Protein (g)	3.29	3.75	1.03
Fat (g)	3.34	6.89	4.38
Carbohydrate (g)	4.66	5.18	6.89
Ash (g)	0.72	0.79	0.20
Ca^{2+}	119.4	169.0	32.2

26.7 Milk Fat

- The Good and the Bad
    - Despite the fact that the cholesterol level in milk is low, milk fat is considered hypercholesterolemic. This is mainly because of its high-saturated fatty acid content (60 to 65%). Palmitic (C16:0) and C14:0 acids have been shown to be hypercholesterolemic while shorter fatty acids (C4-C10) are neutral. Stearic, C18:1 and C18:2 acids are hypocholesterolemic.
    - Bovine milk fat contains significant amounts of short chain fatty acids and relatively lower concentrations of C18 fatty acids than are found in other sources of animal fat such as beef or pork. Bovine milk is also a poor source of polyunsaturated fatty acids. Milk fatty acids are derived in part from dietary long chain fatty acids, microbial synthesis of fatty acids and body stores of fat with the remainder coming from de novo synthesis in the mammary glands. Manipulating the diet of the dairy cow can substantially alters the balance between mammary de novo synthesis of short and medium chain fatty acids, and dietary long chain fatty acids presented to the mammary gland.

26.8 Biological Importance of Milk Proteins

- Milk proteins are the main source of amino acids for the newborn. Casein micelles also provide Ca and P for skeletal development. Casein micelles are highly digestible by the proteolytic enzymes of the newborn.
- -lactalbumin forms a part of the enzyme lactose snynthase.αSome milk proteins have intracellular functions. For instance,
- Milk contains proteins such as lactoferrin and lysozyme. The antibacterial properties of these materials, lysozyme digesting bacterial polysaccharides and lactoferrin sequestering iron required by bacteria emphasize their importance in reducing mastitis infections. Lactoferrin concentration is high in the dry bovine mammary gland.
- In many mammalian species including bovine colostrum is a vital way of transferring passive immunity from the mother to the newborn.

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Lesson 27
VITAMINS

Sources of Vitamins

27.1 Introduction

- A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. In other words, an organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and on the particular organism.
- The term vitamin was derived from "vitamine," a combination word made up by Polish scientist Casimir Funk from vital and amine, meaning amine of life, because it was suggested in 1912 that the organic micronutrient food factors that prevent beriberi and perhaps other similar dietary-deficiency diseases might be chemical amines. This proved incorrect for the micronutrient class, and the word was shortened to vitamin.
- By convention, the term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids (which are needed in larger amounts than vitamins), nor does it encompass the large number of other nutrients that promote health but are otherwise required less often. Thirteen vitamins are presently universally recognized. (Table 24.1)
- Vitamins have diverse biochemical functions. Some have hormone-like functions as regulators of mineral metabolism (e.g., vitamin D), or regulators of cell and tissue growth and differentiation (e.g., some forms of vitamin A). Others function as antioxidants (e.g., vitamin E and sometimes vitamin C).
- The largest number of vitamins (e.g., B complex vitamins) function as coenzymes, that help enzymes in metabolism. In this role, vitamins may be tightly bound to enzymes as part of prosthetic groups: For example, biotin is part of enzymes involved in making fatty acids. Vitamins may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules.

27.2 Classification of Vitamins

- Classically, vitamins have been divided into two groups based on their solubilities in fat solvents or in water.
- Fat-soluble vitamins include A, D, E and K, while vitamin of the B-complex and C are classified as water soluble.
- Fat soluble vitamins are found in foodstuffs in association with lipids. The fat soluble vitamins are absorbed along with dietary fats, apparently by mechanisms similar to those involve in fat absorption. Conditions favorable to fat absorption, such as adequate bile flow and good micelle formation, also favor absorption of the fat-soluble vitamins.
- Water soluble vitamins are not associated with fats, and alterations in fat absorption do not affect their absorption.
- Three of the four fat-soluble vitamins (vitamins A, D and E) are well stored in appreciable amounts in the animal body. Except for vitamin B12, water soluble vitamins are not well stored, and excesses are rapidly excreted.
- A continual dietary supply of the water soluble vitamins and vitamin K is needed to avoid deficiencies.
- Fat-soluble vitamins are excreted primarily in the feces via the bile, whereas water soluble vitamins are excreted mainly in the urine.

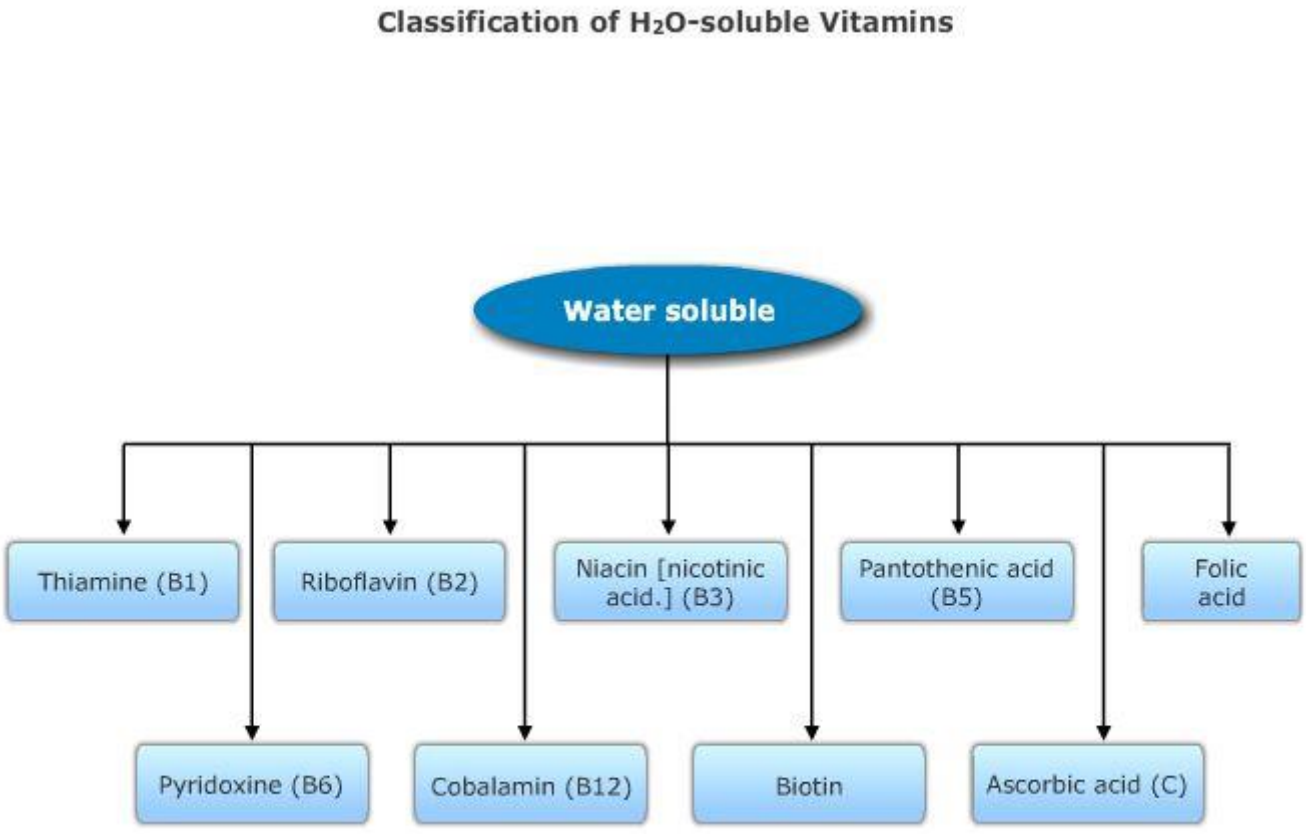


Fig. 27.2 Classification of Water Soluble Vitamins

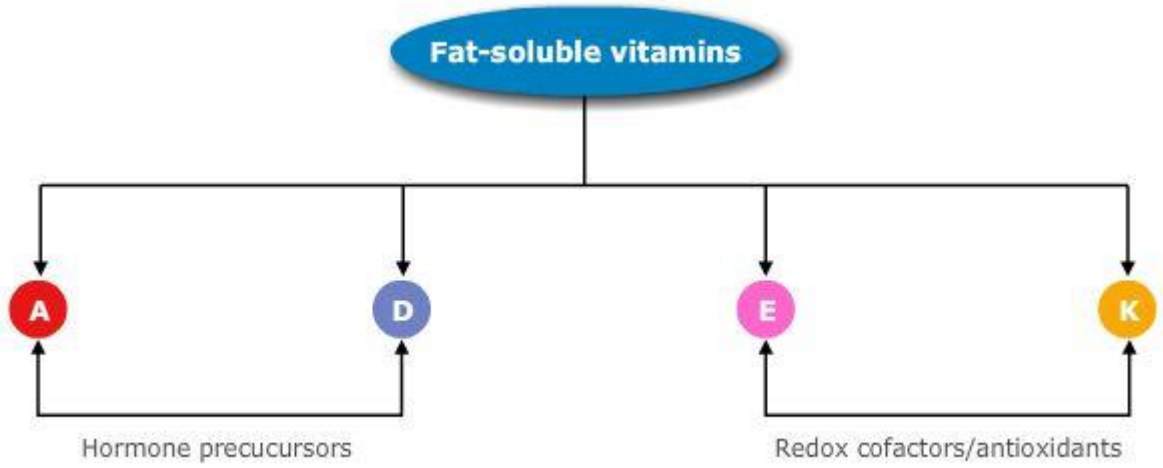
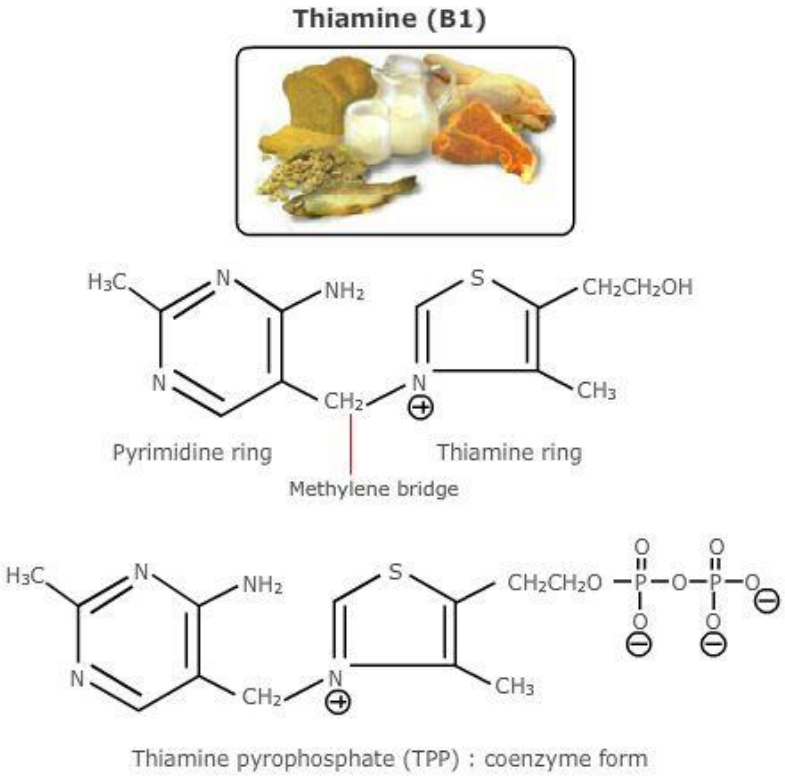


Fig. 27.3 Classification of Fat Soluble Vitamins

27.3 Water Soluble Vitamins

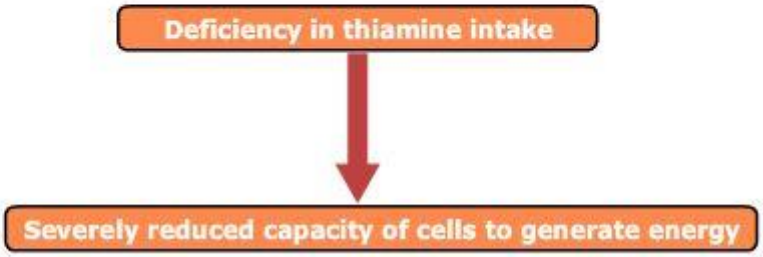
- B-complex vitamins and vitamin C are water-soluble vitamins that are not stored in the body and must be replaced each day.
- These vitamins are easily destroyed or washed out during food storage and preparation.
- The B-complex group is found in a variety of foods: cereal grains, meat, poultry, eggs, fish, milk, legumes and fresh vegetables.
- Citrus fruits are good sources of vitamin C.
- Use of megadoses of vitamins is not recommended.



Thiamine (B1)

Coenzyme for decarboxylation :

- **pyruvate dehydrogenase catalyzed** reactions
- **α-ketoglutarate dehydrogenase catalyzed** reactions
- **transketolase catalyzed reactions of the** pentose phosphate pathway



Thiamine (B1)

Deficiency of thiamine

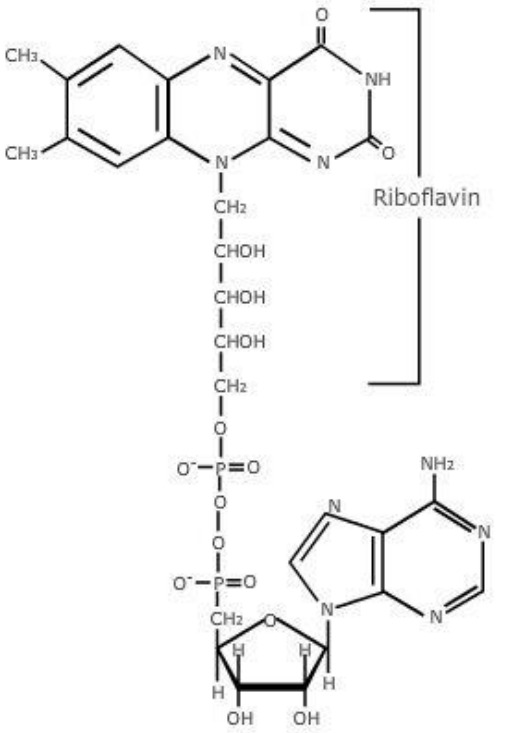
- dry beriberi (neuritic beriberi)
- wet beriberi (cardiac beriberi)
- infantile beriberi (acute beriberi)
- cerebral beriberi ; Wernicke's encephalopathy Korsakoff's psychosis

Fig. 27.4 Thiamine B1

Riboflavin (B2)

Precursor for the coenzymes

- flavin mononucleotide (FMN)
- flavin adenine dinucleotide (FAD)

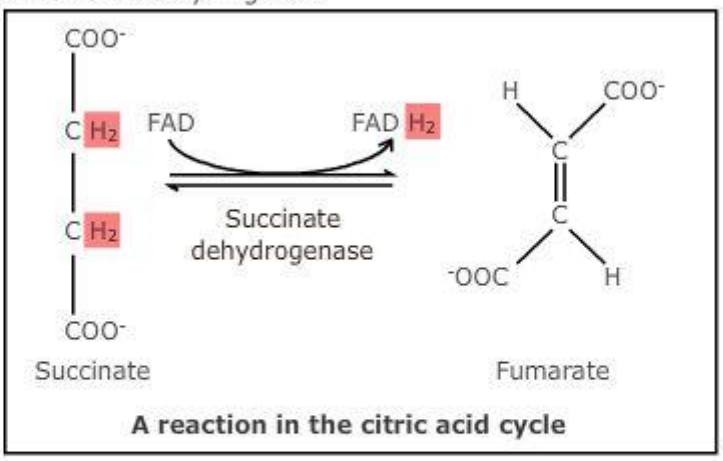


Flavin adenine dinucleotide (FAD)

Riboflavin (B2)

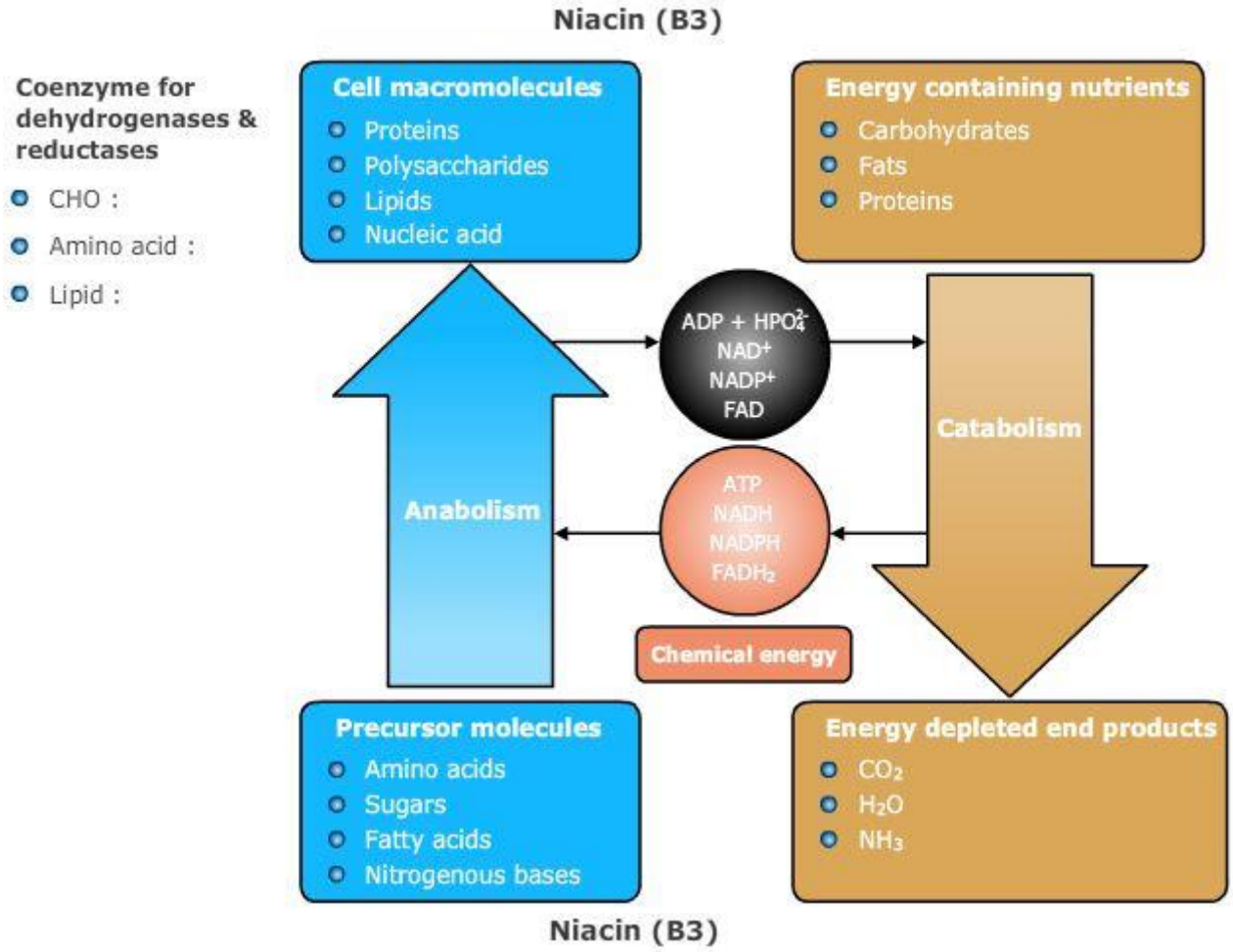
Hydrogen transfer : dehydrogenase, oxidase

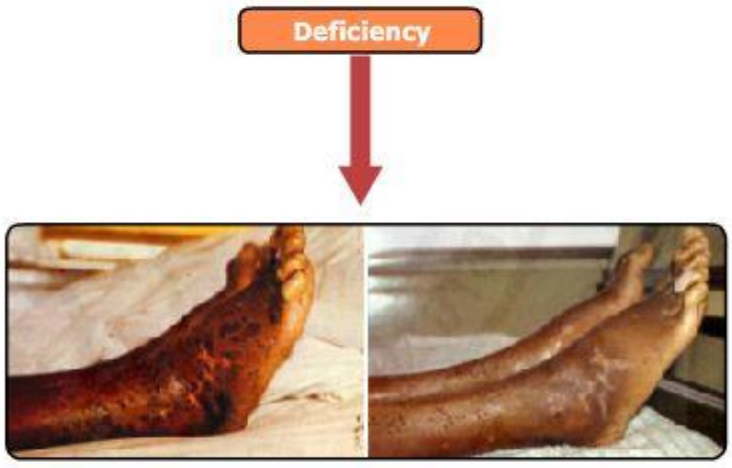
FAD is a coenzyme for succinate dehydrogenase



A reaction in the citric acid cycle

Fig. 27.5 Riboflavin B2





Pellagra

Symptomps :
- Diarrhoea
- Dementia
- Dermatitis

if not treated then death.

Fig. 27.6 Niacin [Nicotinic acid] B3

Pantothenic acid (B5)

Required for synthesis of CoA
A component of the acyl carrier protein (ACP) of fatty acid synthase

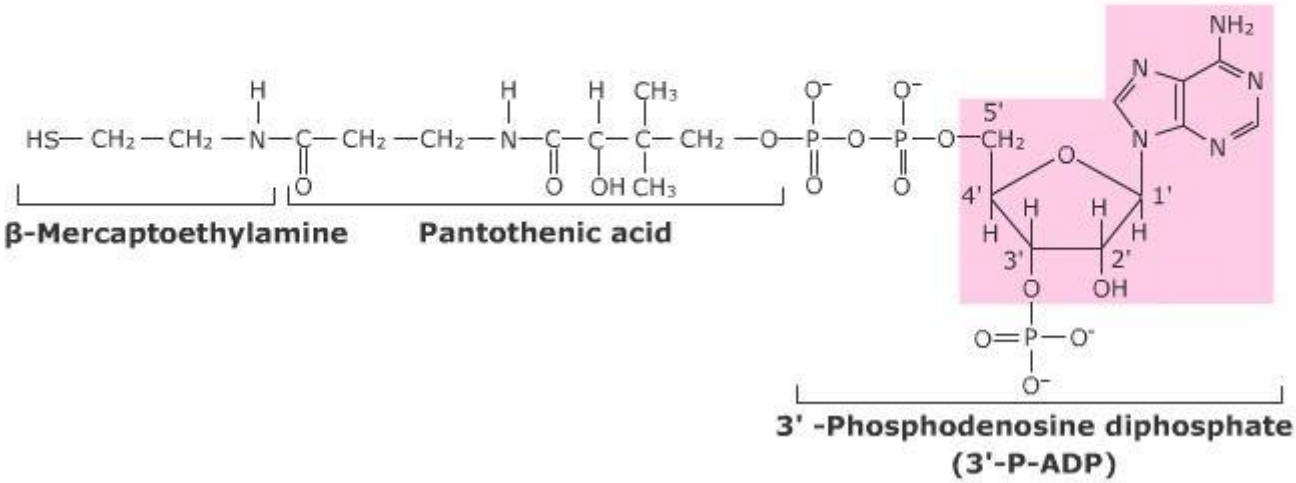
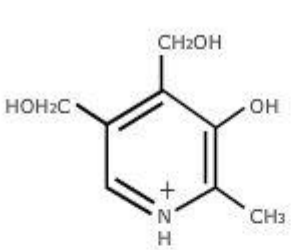
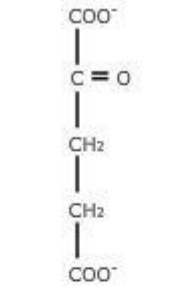


Fig. 27.7 Pantothenic acid B5

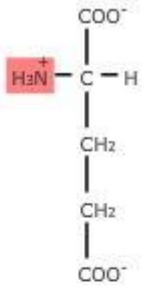
Pyridoxine (B6)



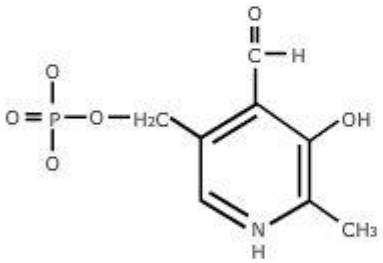
Vitamin B6 (Pyridoxine)



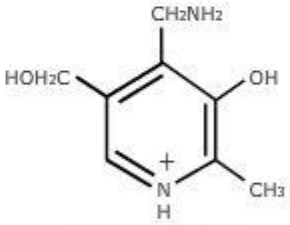
α-Ketoglutarate



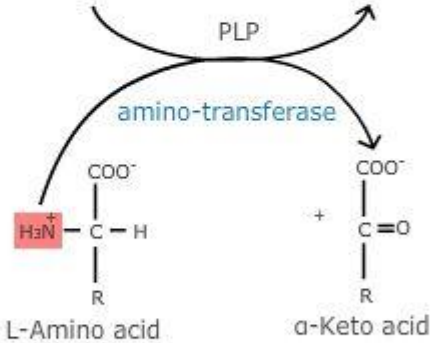
L-Glutamate



Pyridoxal phosphate (PLP)



Pyridoxamine



L-Amino acid

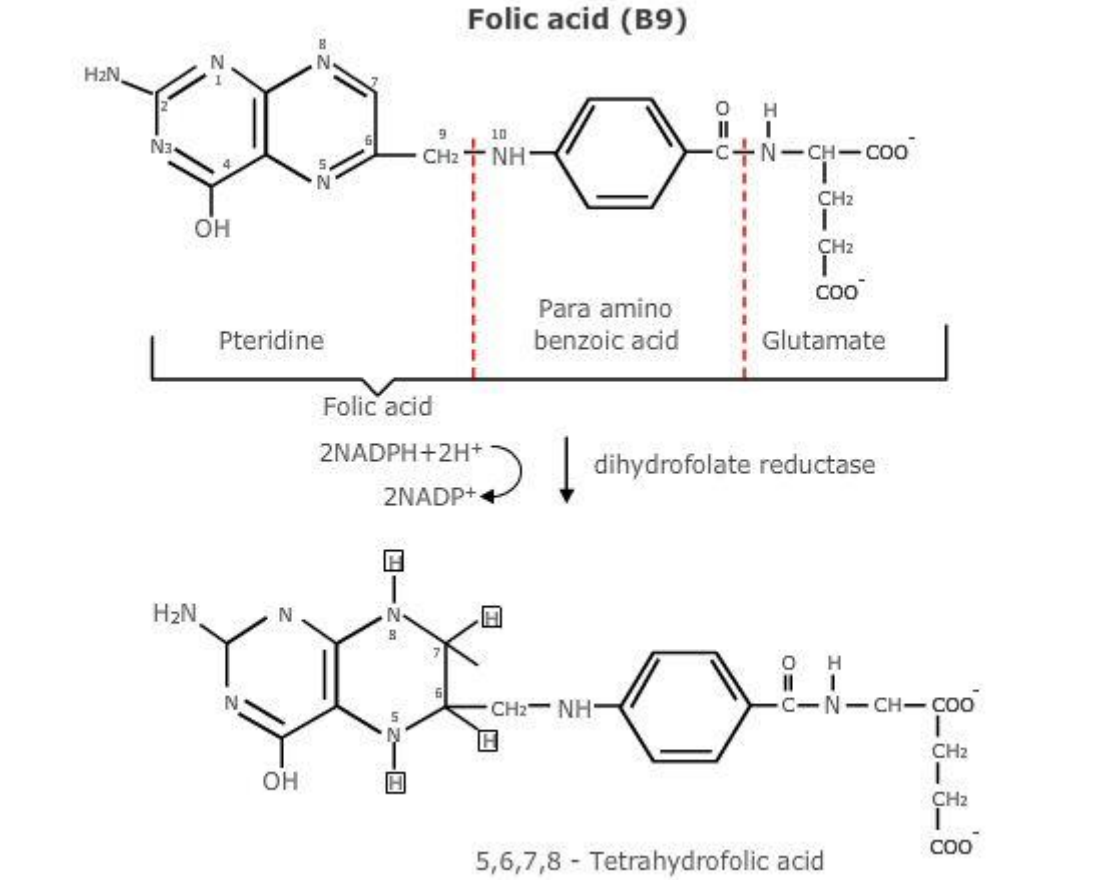
α-Keto acid

Coenzyme in enzymes involved in transamination reactions
(synthesis and catabolism of amino acids)

Deficiency

- Microcytic hypochromic anemia
- Peripheral neuropathy

Fig. 27.8 Pyridoxine B6



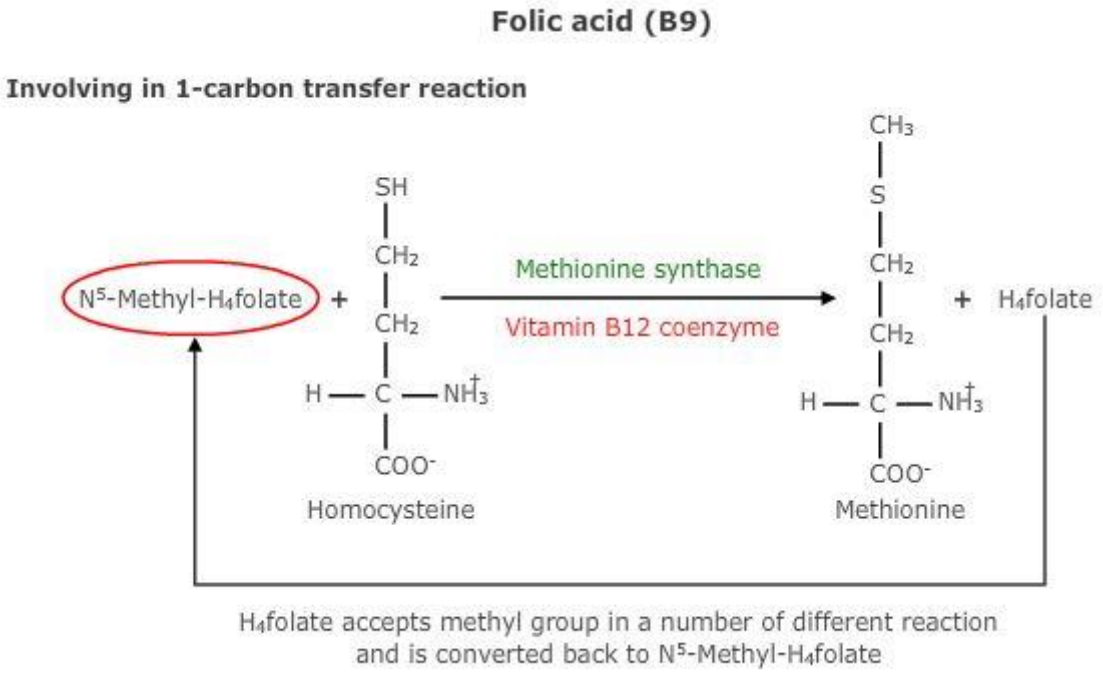
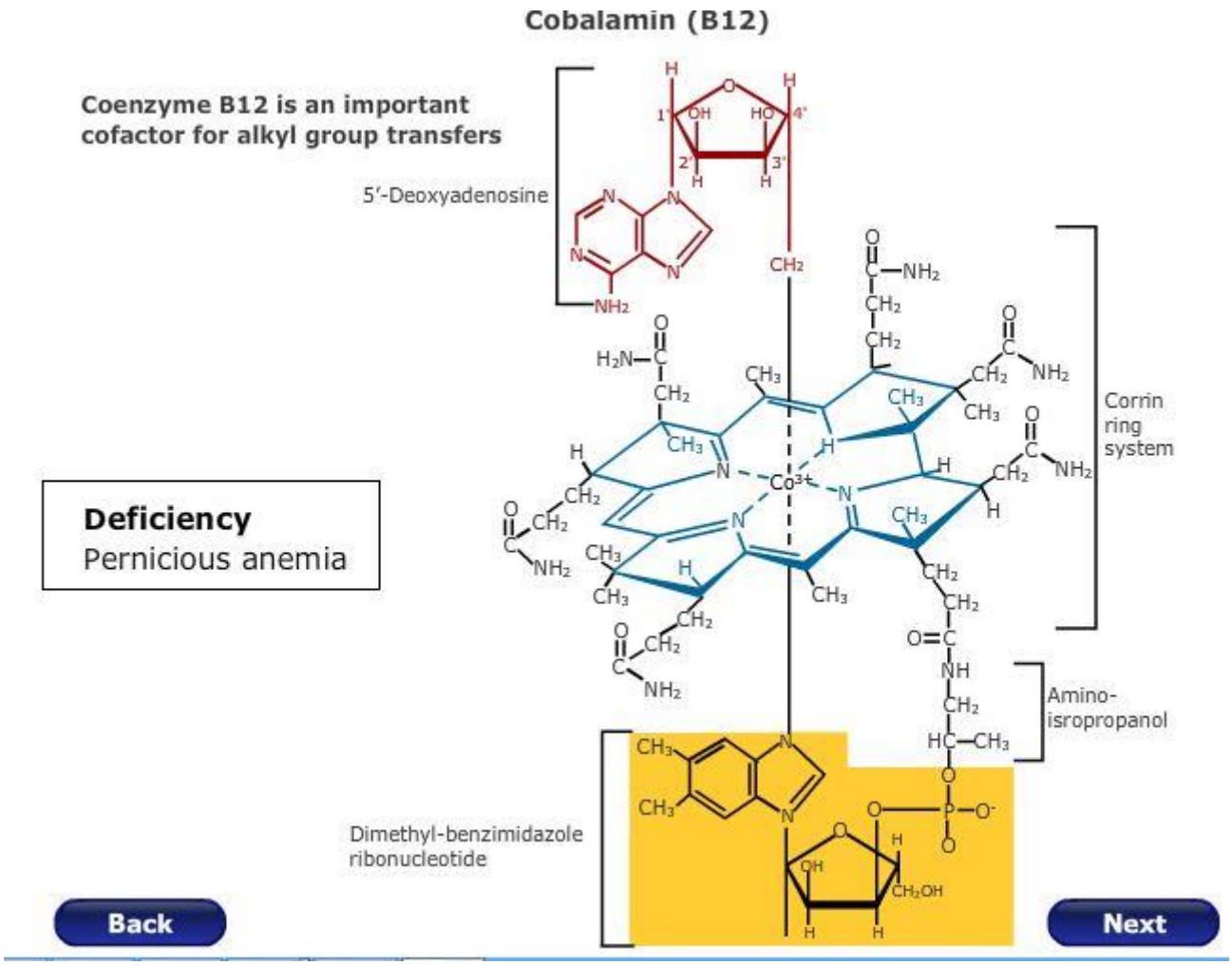


Fig. 27.9 Folic acid B9



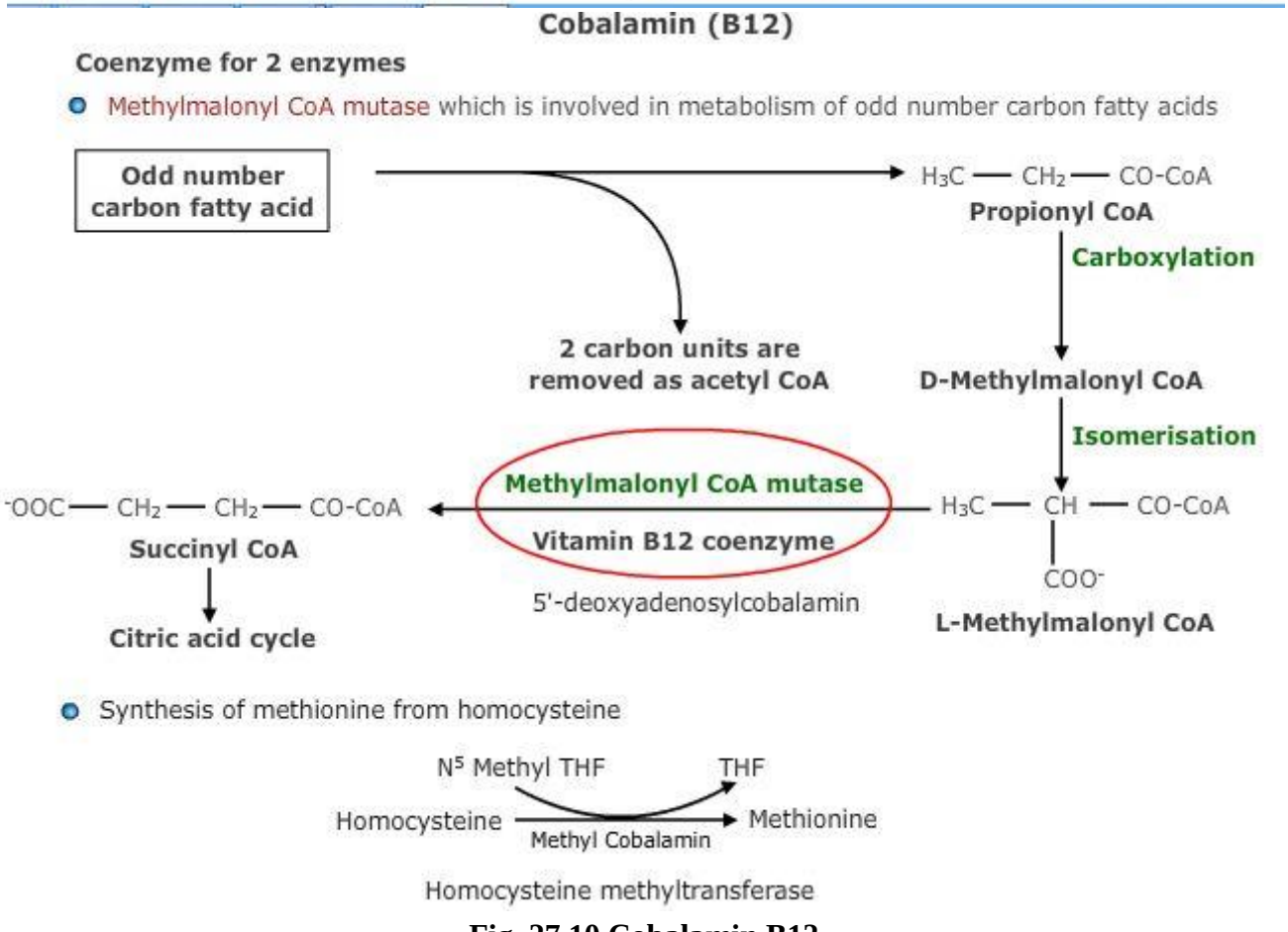
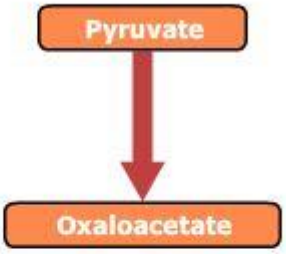


Fig. 27.10 Cobalamin B12

Biotin (B7)

carrier of activated CO2 (carboxyl transfer)

1. Pyruvate carboxylase
2. Acetyl COA carboxylase
3. Propionyl COA carboxylase
4. β-methylcrotonyl COA carboxylase



Gluconeogenesis in fatty acid synthesis

Biotin deficiency is rare

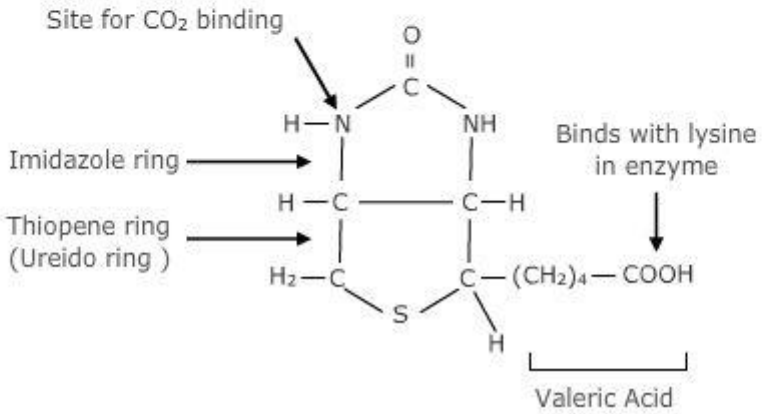
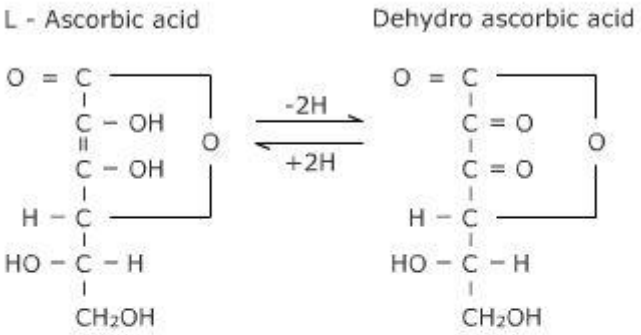


Fig. 27.11 Biotin B7

Ascorbic acid



A cofactor for hydroxylases
e.g prolyl 4-hydroxylase

↓

Collagen synthesis

- Collagen synthesis
- Bone formation
- Antioxidant

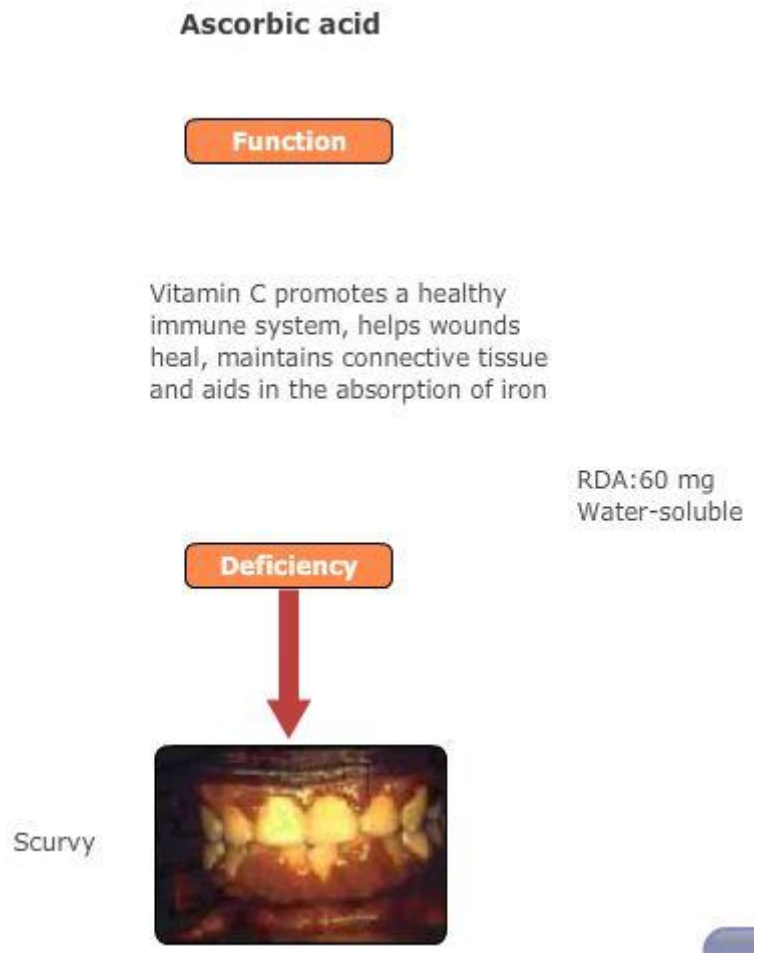


Fig. 27.12 Ascorbic acid (C)

27.4 Fat Soluble Vitamins

- Small amounts of vitamins A, D, E and K are needed to maintain good health.
- Foods that contain these vitamins will not lose them when cooked.
- The body does not need these every day and stores them in the liver when not used.
- Most people do not need vitamin supplements.
- Megadoses of vitamins A, D, E or K can be toxic and lead to health problems

*****☺*****

Lesson 28
HORMONES

28.1 Introduction

- The living body possesses a remarkable communication system to coordinate its biological functions. This is achieved by two distinctly organized functional systems.
    - The nervous system coordinates the body functions through the transmission of electrochemical impulses.
    - The endocrine system acts through a wide range of chemical messengers known as hormones.

- Hormones are the chemical messengers of the body having diverse structures and functions. They act either directly or through messengers to coordinate and perform biological functions such as growth, reproduction and digestion etc.
- Hormones are defined as organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells.
- Hormones may be regarded as the chemical messengers involved in the transmission of information from one tissue to another and from cell to cell. The major endocrine organs in human body are depicted in fig 28.1.

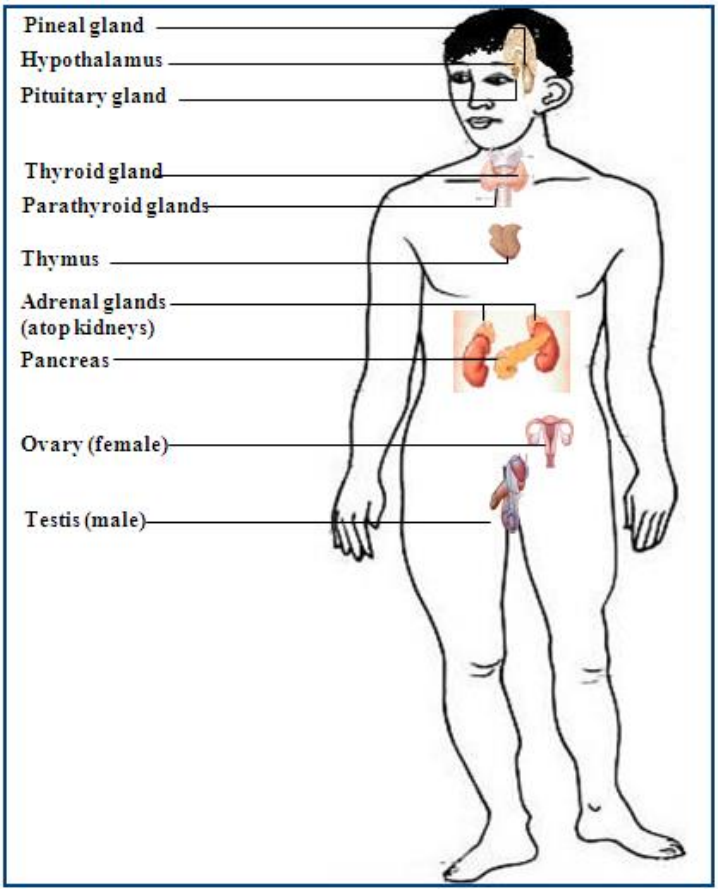


Fig. 28.1 Location of endocrine glands

28.2 Classification of Hormones

- Based on the chemical nature the hormones can be categorized into three groups

1. Protein or peptide hormones e.g. insulin, glucagon, antidiuretic hormone, oxytocin.
2. Steroid hormones e.g. glucocorticoids, mineralocorticoids, sex hormones.
3. Amino acid derivatives e.g. epinephrine, norepinephrine, thyroxine (T4), triiodothyronine (T3).

- Based on the mechanism of action

Hormones are classified into two broad groups (I and II) based on the location of the receptors to which they bind and the signals used to mediate their action.

- Group I hormones: These hormones bind to intracellular receptors to form receptor-hormone complexes (the intracellular messengers) through which their biochemical functions are mediated. Group I hormones are lipophilic in nature and are mostly derivatives of cholesterol (exception – T3 and T4). e.g. estrogens, androgens, glucocorticoids, calcitriol.
- Group II hormones: These hormones bind to cell surface (plasma membrane) receptors and stimulate the release of certain molecules, namely the second messengers which, in turn, perform the biochemical functions. Thus, hormones themselves are the first messengers.
- Group II hormones are subdivided into three categories based on the chemical nature of the second messengers.
    - The second messenger is cAMP e.g. ACTH, FSH, LH, PTH, glucagon, calcitonin.
    - The second messenger is phosphatidylinositol/calcium e.g. TRH, GnRH, gastrin, CCK.
    - The second messenger is unknown e.g. growth hormone, insulin, oxytocin, prolactin.

The principal human hormones, their classification based on the mechanism of action, and major functions are given in Table- 28.1

28.3 Mechanism of action

- Group I hormones

These hormones are lipophilic in nature and can easily pass across the plasma membrane. They act through the intracellular receptors located either in the cytosol or the nucleus. The hormone-receptor complex binds to specific regions on the DNA called hormone responsive element (HRE) and causes increased expression of specific genes (Fig-28.2). It is believed that the interaction of hormone receptor complex with HRE promotes initiation and, to a lesser extent, elongation and termination of RNA synthesis (transcription). The ultimate outcome is the production of specific proteins (translation) in response to hormonal action.

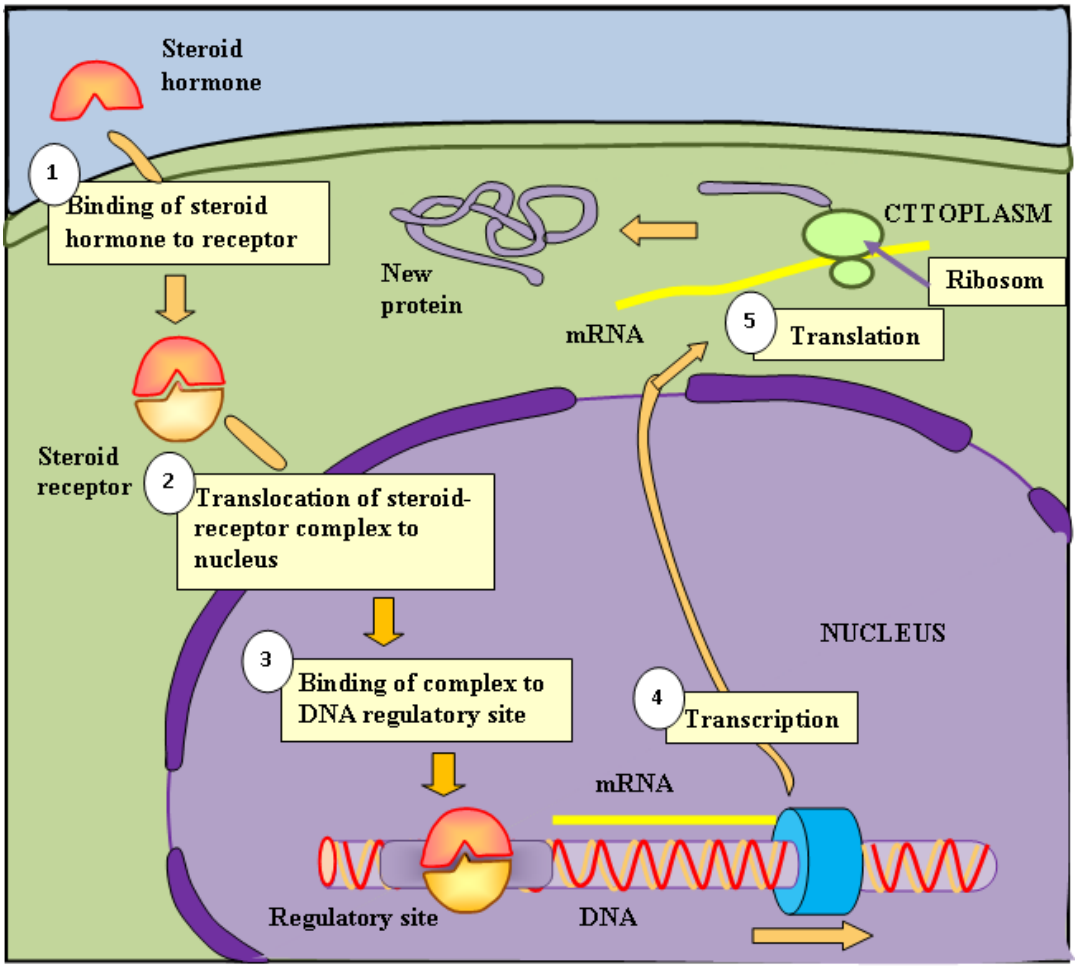


Fig. 28.2 Mechanism of action of intracellular receptor hormones

- **Group II hormones**

These hormones are considered as the first messengers. They exert their action through mediatory molecules, collectively called second messengers. Cyclic AMP (cAMP) is a ubiquitous nucleotide. It consists of adenine, ribose and a phosphate. cAMP acts as a second messenger for a majority of polypeptide hormones. The membrane-bound enzyme adenylate cyclase converts ATP to cyclic AMP. cAMP is hydrolysed by phosphodiesterase Fig-28.3 and 28.4.

- Adenylate cyclase system

A series of events occur at the membrane level that influence the activity of adenylate cyclase leading to the synthesis of cAMP. This process is mediated by G-proteins, so designed due to their ability to bind to guanine nucleotides.

- Action of cAMP – a general view

Once produced, cAMP performs its role as a second messenger in eliciting biochemical responses. cAMP activates protein kinase A (PKA). This enzyme is a hetrotetramer consisting of 2 regulatory subunits (R) and 2 catalytic subunits (C). cAMP binds to inactive protein kinase and causes the dissociation of R and C subunits. (Fig-28.4)

$4cAMP + R_2C_2 \rightarrow R_2(4cAMP) + 2C$
(interactive) (interactive) (active)

The active submit (C) catalyses phosphorylation of proteins (transfer of phosphate group to serine and

threonine residues). It is the phosphoprotein that ultimately causes the biochemical response.

It should, however, be remembered that cAMP does not act on all protein kinases. For instance, on protein kinase C (the second messenger is diacylglycerol).

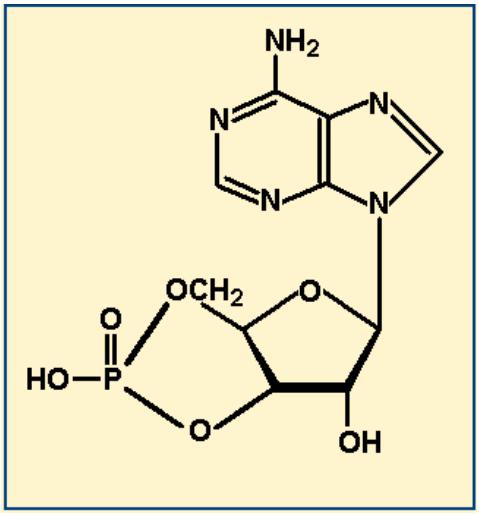


Fig. 28.3 cAMP – The second messenger

- Dephosphorylation of proteins: A group of enzymes called protein phosphatases hydrolyse and remove the phosphate group added to proteins.

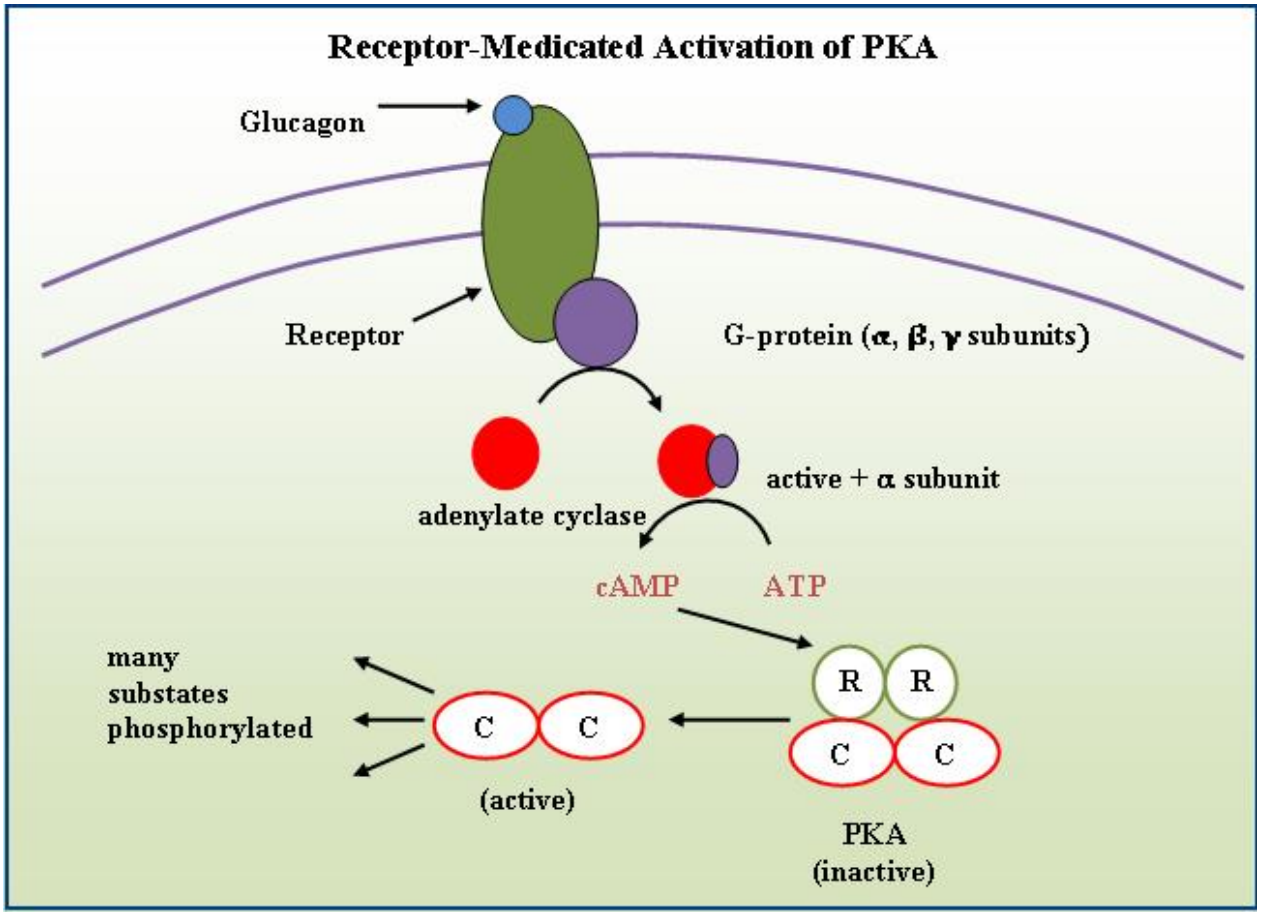


Fig. 28.4 Mechanism of action of cell surface receptor hormones

Table-28.1 Principal human hormones – classification (by mechanism of action), origin and major functions

Hormone (s)	*Origin*	*Major Function (s)*
Group I. HORMONES THAT BIND TO INTRACELLULAR RECEPTORS		
Estrogens	Ovaries and adrenal cortex	Female sexual characteristics, menstrual cycle
Progestins	Ovaries and placenta	Involved in menstrual cycle and maintenance of pregnancy.
Androgens	Testes and adrenal cortex	Male sexual characteristics, spermatogenesis.
Glucocorticoids	Adrenal cortex	Affect metabolisms, suppress immune system.
Mineralocorticoids	Adrenal cortex	Maintenance salt or water balance.
Calcitriol (1,25-DHCC)	Kidney (final form)	Promotes absorption of Ca^{2+} from intestine, kidney and bone.
Thyroid hormones (T_3, T_4)	Thyroid	Promote general metabolic rate.
Group II. HORMONES THAT BIND TO CELL SURFACE RECEPTORS		
A. The second messenger is cAMP		
Adrenocorticotropic hormone (ACTH)	Anterior pituitary	Stimulates the release of adrenocorticosteroids.
Follicle stimulating hormone (FSH)	Anterior pituitary	In females, stimulates ovulation and estrogen synthesis. In males, promotes spermatogenesis
Luteinizing hormone (LH)	Anterior pituitary	Stimulates synthesis of estrogens and progesterone and causes - ovulation. Promotes androgen synthesis by testes.
Chorionic gonadotropin (hCG)	Anterior pituitary	Stimulates progesterone release from placenta.
Thyroid stimulating hormone (TSH)	Anterior pituitary	Promotes the release of thyroid hormones (T_3, T_4).
β-Endorphins and enkephalins	Anterior pituitary	Natural endogenous analgesics (pain relievers).
Antidiuretic homone (ADH)	Posterior pituitary (stored)	Promotes water reabsorption by kidneys.
Glucagon	Pancreas	Increases blood glucose level, stimulates glycogenolysis & lipolysis
Parathyroid hormone (PTH)	Parathyroid	Increases serum calcium, promotes Ca^{2+} release from bone.
Calcitonin	Thyroid	Lowers serum calcium. Decreases Ca^{2+} uptake by bone and kidney.
Epinephrine	Adrenal medulla	Increases heart rate and blood pressure. Promotes glycogenolysis in liver and muscle and lipolysis in adipose tissue.
Norepinephrine	Adrenal medulla	Stimulates lipolysis in adipose tissue.
B. The second messenger is phosphatidlyinositol/calcium		
Thyrotropin-releasing hormone (TRH)	Hypothalamus	Promotes TSH release.
Gonadotropin-releasing hormone (TRH)	Hypothalamus	Stimulates release of FSH and LH.
Gastrin	Stomach	Stimulates gastric HCl and pepsinogen secretion.
Cholecystokinin (CCK)	Intestine	Stimulates gall bladder and secretion of pancreatic enzymes.
C. The second messenger is unknown/unsettled		
Growth hormone (GH)	Anterior pituitary	Promotes growth of the body (bones and organs).
Prolactin (PRL)	Anterior pituitary	Growth of mammary glands and lactation.
Oxytocin	Posterior pituitary (stored)	Stimulates uterine contractioin and milk ejection.
Insulin	Pancreas	Hypoglycemic effect, promotes protein synthesis and lipogenesis.
Somatomedins (insulin-like growth factors, IGF-I, IGF-II)	Liver	Stimulates growth of cartilage.

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Lesson 29
DIGESTION AND ABSORPTION OF CARBOHYDRATES, LIPIDS AND PROTEINS

29.1 Introduction

- Our normal food is a mixture of complex plant and animal materials that is composed largely of carbohydrates, fat, protein, vitamins, and minerals.
- The bulk of these ingested nutrients consist of large polymers that must be reduced to simpler components before they can be absorbed and thus made available to all the cells in the body.
- The disintegration of naturally occurring food stuffs into assimilable forms in the gastrointestinal tract constitutes the process of digestion and involves enzymes.
- The gastrointestinal tract constitutes the process of digestion and involves enzymes. The majority of the enzymes involved in the digestive process are hydrolases (i.e., they split bonds of esters, glycosides, or peptides by the addition of water).
- The powerful hydrolytic enzymes of the digestive tract catalyze the degradation of large molecules present in food (e.g., starch or protein) into small molecules that can be readily absorbed such as glucose or amino acids.

29.2 Digestion

- Food is taken through mouth where it is homogenized, mixed, and lubricated by saliva secreted by salivary glands. One constituent of human saliva is amylase which catalyses the hydrolysis of starch. Approximately 1.5 L of saliva is secreted daily.
- From the mouth the food contents pass via the esophagus to the stomach where they come in contact with gastric juice with a pH $\leq$ 2. Gastric juice contains hydrochloric acid, mucins, and the enzymes pepsin and lipase. Hydrochloric acid is secreted by parietal cells and pepsin by the chief cells. Chyme, the acidic food content in the stomach, is intermittantly introduced into the small intestine.
- The alkaline content of pancreatic (about 1.5 L/day) and biliary secretions (0.5 L/day) neutralize the acid of the chyme and change the pH to the alkaline side necessary for the optimum activity of pancreatic and intestinal enzymes.
- Most of the breakdown of food is catalyzed by the soluble enzymes and occurs within the lumen of the small intestine; however, the pancreas is the major organ that synthesizes and secretes the large amounts of enzymes needed to digest the food. Secreted enzymes amount to about 30 g/day of protein in a healthy adult. The pancreatic duct joins with the common bile duct to form the ampulla of Vater; thus, pancreatic juice and bile empty into the duodenum at the same point.

Table 29.1 Gastrointestinal Hormones

Hormone	Site of secretion	Biological action
Gastrin (34 amino acids)	Gastric antrum; stimulated by the presence of food in the stomach	Increased secretion of hydrochloric acid, pepsin
Secretin (27 amino acids)	Duodenum, jejunum; stimulated by the presence of acid in duodenum	Increased secretion of pancreatic juice rich in bicarbonate
Cholecystokinin (33 amino acids)	Duodenum, jejunum; stimulated by the presence of digestion products of fat and protein in the duodenum	Increased secretion of pancreatic juice rich in enzymes; increased contraction of gallbladder

- When fat and digestion products of protein reach the small intestine, the duodenal and jejunal mucosa release cholecystokinin, a peptide hormone. It stimulates the secretion of pancreatic juice rich in enzymes and also stimulates the contraction of the gall bladder and secretion of bile.
- The presence of acidic food in the small intestine causes the release of another peptide hormone, secretin, by the duodenal and jejuna mucosa; this stimulates secretion of pancreatic juice rich in bicarbonate and potentiates the action of cholecystokinin on the pancreas.
- The secretion of gastric juice is under the control of the hormone gastrin, a heptadecapeptide and its release is stimulated by the presence of food in the stomach. Gastrin is secreted by the antral region of the gastric mucosa and by the duodenal mucosa. The main function of gastrin is to stimulate the secretion of hydrochloric acid into the stomach, but it also stimulates pepsin secretion and increases the motility of the gastric antrum. Site of secretion and biological activity these hormones are summarized in Table 29.1.
- At the low pH of the gastric juice, proteins are denatured and this allows the polypeptide chains to unfold and makes them more accessible to the action of proteolytic enzymes. Some digestion of protein occurs within the lumen of the stomach and the acid environment also destroys most of the microorganisms swallowed or ingested with food. An average of 2-2.5 L/day of gastric juice is secreted, but the volume is reduced in atrophy of the gastric glands.
- The bulk of digestion occurs distal to the second (descending) part of the duodenum. The final result of the action of digestive enzymes is to reduce the nutrients to form that can be absorbed and assimilated. There is little absorption of nutrients from the stomach although alcohol can be absorbed to a significant extent by this organ. Even water passes through the stomach to be absorbed subsequently in the intestine. The main organ for the absorption of nutrients is the small intestine which has sites for the absorption of specific nutrients.

29.3 Carbohydrates

- The digestion of starch begins in the mouth when the food is mixed with salivary α-amylase but the hydrolysis stops in the stomach because of the change in pH and resumes in the duodenum where pancreatic α-amylase is secreted. Both salivary and pancreatic amylase are α-1, 4 glucosidases and serve to hydrolyze only the internal 1, 4 glucosidic bonds found in starch and glycogen. There is little activity at the 1, 4- linkages adjacent to the branching points, and the α-1, 6 bonds (or branch points) are not attached by amylase.
- Consequently, the products of digestion by α-amylase on starch or glycogen are maltose, isomaltose, maltotriose (a trisaccharide), and α-limit dextrins (containing on the average 8 glucose units with one or more α-1, 6 bonds). The final digestive process occurs at the mucosal lining and involves the action of α-dextrinase (isomaltase) which hydrolyzes the 1, 6 glucosidic bonds from limit dextrins and isomaltose.
- Maltase, another brush-border enzyme, breaks down maltose and maltotriose to glucose which is the end product of starch and glycogen digestion.
- Sucrose and lactose are similarly hydrolyzed by sucrase and lactase that are located on the brush border to their corresponding monosaccharides glucose and fructose, and glucose and galactose, respectively.
- Monosaccharides are absorbed from the intestinal lumen by passage through the mucosal epithelial cells into the blood stream.
- The transport of glucose and galactose across the brush border membrane of the mucosal cell occurs by an active, energy-requiring process that involves a specific transport protein and the presence of sodium ions.
- Fructose is absorbed by a facilitated diffusion process. Other sugars (e.g., pentoses) are absorbed by simple diffusion through the lipid bilayer of the membrane. In the normal individual, the digestion and absorption of usable carbohydrates are 95% or more complete.

29.4 Lipids

- Lipids include a wide variety of chemical substances such as neutral fat (e.g., triglycerides), fatty acids and their derivatives, phospholipids, glycolipids, sterols, carotenes, and fat-soluble vitamins. Fat constitutes about 90% of dietary lipids and provides energy in a highly concentrated form. It accounts for 40-45% of the total daily energy intake (100 g/day in the average western diet).
- The digestion of fat and other lipids poses a special problem because they are insoluble in water while the lipolytic enzymes, like other enzymes, are soluble in aqueous medium. The problem is solved by emulsification which is the intimate admixture of two phases, one dispersed in the other as fine droplets or micelles. In this context the two phases are water and fat, the later making up the micelles.
- Micelles tend to aggregate if they are not stabilized in some way; in the duodenum this role is performed by the bile salts. A bile salt molecule has two sides, one is hydrophobic and the other hydrophilic. So one side tends to be associated with aqueous phase and the other with lipid phase. Such molecules are said to be amphipathic and are powerful emulsifying agents.
- Little or no lipid digestion occurs in the mouth. There is some lipase in the stomach but the acidic environment and the absence of bile salts prevents any significant digestion of fat in this organ.
- The forceful contraction of the stomach (antrum) breaks up lipids into fine droplets and in the duodenum these droplets are exposed to the solubilizing effects of bile salts. A fat globule which has an average diameter of about 100 is reduced several fold after emulsification and the surface area is amplified about 10,000 times.
- Lipolytic enzymes cannot penetrate the lipid droplets, but function at the lipid-water interface. Emulsified triglycerides are readily attacked by lipase secreted in pancreatic juice. The bile salts and phospholipids present in bile normally adhere to the surface of triglyceride droplets, thereby displacing lipase from its substrate.
- Colipase (a small protein with a molecular weight of 10,000) which binds to both the water-lipid interface and to lipase, thereby anchoring and activating the enzyme. Colipase is secreted by the pancreas as procolipase (inactive) simultaneously with lipase in a 1:1 ratio and is activated by trypsin hydrolysis of an arginyl-glycyl bond in N-terminal region and removal of a small group (<12) of amino acids.
- Pancreatic lipase attacks the ester linkages at the 1- and 3-carbons of the triglyceride, leaving a monoglyceride with the fatty acid esterified at the 2-carbon position of glycerol. This linkage can be cleaved by an esterase to release the third fatty acid molecule and glycerol, but is not a necessary step for absorption.
- Monoglycerides, along with bile salts, play an important role in stabilizing and further increasing the emulsification of lipid in the small intestine. The emulsified lipid droplets (micelles) are further reduced in size which enhances the digestion of fats and other lipids solubilized in the micellar particles.
- Several other enzymes secreted in the pancreatic juice are involved in the digestion of certain lipids. For example, cholesterol esterase hydrolyzes cholesterol esters to cholesterol and fatty acids. Another less specific lipid esterase acts on short-chain triglycerides, monoglycerides or other lipid esters (e.g., esters of vitamin A) with fatty acids.
- Phospholipids are hydrolyzed by phospholipase A2 which is secreted as proenzyme (inactive) and is activated by trypsin. Phospholipase A2 releases the fatty acid at 2-carbon of the phospholipid leaving a lysophospholipid.
- In normal individuals lipid absorption occurs in the upper part of the small intestine. Monoacylglycerol, fatty acids, and cholesterol leave the micelles at the brush border of the epithelial cells of the intestinal mucosa and pass through the cell membrane by passive diffusion.
- The fate of the absorbed fatty acids depends on their size. Those with less than 10-12 carbon atoms pass directly from the mucosal cells into portal blood and are bound to albumin for transport as unesterified (free) fatty acids.
- The large fatty acids are reesterified with monoacylglycerol to the triglyceride level in the smooth endoplasmic reticulum. Some of the cholesterol that enters the mucosal cells from the micelles is also esterified. The newly synthesized triglycerides and cholesterol esters are complexed with a

specific protein, cholesterol, and phospholipids to produce particles called chylomicrons which are released from the mucosal cells by exocytosis and enter the lymph.

- The bile salts do not cross the mucosal barriers into the lymphatic system. Instead, they are reabsorbed in micellar form in the lower segment of the small intestine and are returned to the liver by the portal vein. This route is part of enterohepatic circulation and permits the bile salts to be salvaged for resecretion into the bile.

29.5 Proteins

- The total daily protein load to be digested includes about 70-100 g of dietary protein and 35-200 g of endogenous protein from digestive enzymes and sloughed cells.
- The overall process of proteolysis must occur without the body's own protein being digested. A protected compartment for the hydrolytic process is provided by the lumen of the gastrointestinal tract.
- In addition, the secretory cells that synthesize proteases (except dipeptidases and aminopeptidases) are protected because these enzymes are formed and sequestered in storage granules in inactive forms, the zymogens, until needed.
- The subsequent transformation of the zymogens to the active enzymes occurs largely in the lumen of the gastrointestinal tract and involves, in part, changes in the molecular conformation. In almost all cases, a relatively small masking peptide is split off from the zymogen, which results in a catalytically active species of proteolytic enzyme.
- Protein digestion can be divided into gastric, pancreatic, and intestinal phases, depending on the tissue source of the enzymes.

29.5.1 Gastric phase

The digestion begins in the stomach where protein is denatured by low pH and is exposed to the action of proteolytic enzymes. The acidic environment also provides the optimum pH for pepsin activity. The zymogen pepsinogen, which is secreted by chief cells, is converted to pepsin in the acid medium (autoactivation) or by active pepsin (autocatalysis) by removal of a peptide consisting of 44 amino acids from N-terminus. Although pepsin has a broad specificity, it attacks primarily peptide linkages in which the carboxyl group is donated by aromatic amino acid residues. Pepsin is an endopeptidase and the products of its action consist of a mixture of oligopeptides.

29.5.2 Pancreatic phase

The proteolytic enzymes are synthesized in the acinar cells of the pancreas and secreted in pancreatic juice as zymogens. These include trypsinogen, chymotrypsinogen, proelastase, and the procarboxypeptidases. In the lumen of the small intestine, enteropeptidase (which used to be called enterokinase), a protease produced by duodenal epithelial cells, activates trypsinogen to trypsin (by scission of the hexapeptide). Trypsin, in turn, activates trypsinogen, chymotrypsinogen, proelastase, and the procarboxypeptidases to their respective active enzymes. Trypsin, chymotrypsin, and elastase are endopeptidases. Trypsin is specific for peptide linkages in which carboxyl is donated by arginine or lysine. The specificity of chymotrypsin is similar to pepsin. Elastase has a rather broad specificity in attacking bonds next to small amino acids such as glycine, alanine and serine. Carboxypeptidases A and B attack the carboxyl-terminal peptide bonds, thereby liberating single amino acids. The combined action of pancreatic peptidases results in the formation of free amino acids and small peptides of 2-8 amino acid residues.

29.5.3 Intestinal phase

The luminal surface of small intestinal epithelial cells contain amino peptidase and dipeptidases. The end products of cell surface digestion are amino acids and di- and tripeptides. These are absorbed by the epithelial cells via specific amino acid or peptide transport systems. The di- and tripeptides are hydrolyzed within the cytoplasmic components before they leave the cell. The hydrolysis of most proteins is thus complete to their constituent amino acids.

After active absorption by the intestinal mucosal cells, the amino acids are taken up primarily by the blood capillaries in the mucosa and are transported in the plasma to the liver and other tissues for metabolic use. A significant amount of the absorbed amino acids also appear in the lymph. The digestion and absorption of the majority of dietary proteins is about 95% complete in the normal human subject.

Lesson 30
MILK INTOLERANCE AND HYPERSENSITIVITY

30.1 Introduction

Among those over the age of five, approximately 90-95% of black individuals and 20-25% of white individuals throughout the world will have partial or complete lactose intolerance."There is a great deal of confusion between milk allergy and lactose intolerance, both adverse reactions attributable to milk. Different mechanisms cause different adverse reactions. The resulting symptoms may be quite different from or confusingly similar to each other.

30.2 Milk Intolerance

- Food intolerance is an adverse reaction to food that does not involve an immune response. Several basic mechanisms produce clinical manifestations of food intolerance. For example, the failure to digest lactose due to a deficiency of lactase leads not only to inefficient utilization of dietary lactose but also to a disordered gastrointestinal physiology.
- Metabolic or biochemical abnormalities can alter the intermediary metabolism of a substance. Inborn errors such as phenylketonuria and galactosemia have this effect and are described as follows.

30.2.1 Lactose intolerance

- Among many human populations, adults are unable to metabolize the milk sugar lactose and experience gastrointestinal disturbances if they drink milk. Lactose intolerance, or hypolactasia, is caused by a deficiency of the enzyme lactase, which cleaves lactose into glucose and galactose. (Fig. 30.1)

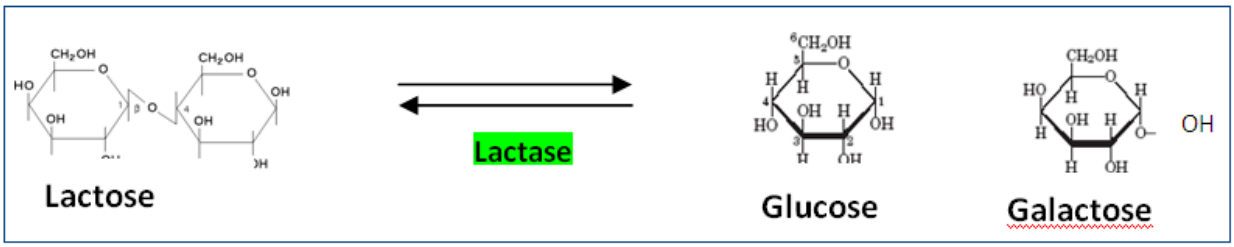

Fig 30.1 Hydrolysis of lactose

- Lactose cannot be completely digested and absorbed in the small intestine and passes into the large intestine, where bacteria convert it to toxic products that cause abdominal cramps and diarrhea.
- In lactase deficient, patients lactose gets accumulated which is a good energy source for microorganisms in the colon, and they ferment it to lactic acid and generate methane (CH_4) and hydrogen gas (H_2). The gas produced creates the uncomfortable feeling of gut distention and the annoying problem of flatulence.
- The lactic acid produced by the microorganisms is osmotically active and draws water into the intestine, as does any undigested lactose, resulting in diarrhea. If severe enough, the gas and diarrhea hinder the absorption of other nutrients such as fats and proteins.
- The problem is further complicated because undigested lactose and its metabolites increase the osmolarity of the intestinal contents, favoring the retention of water in the intestine.
- In most parts of the world where lactose intolerance is prevalent, milk is not used as a food by adults, although milk products predigested with lactase are commercially available in some countries.

30.2.2 Phenylketonuria (PKU)

- PKU is an autosomal recessive metabolic genetic disorder characterized by a deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH). This enzyme is necessary to metabolize the amino acid phenylalanine ('Phe') to the amino acid tyrosine. When PAH is deficient, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which is detected in the urine. If, however, the condition is left untreated, it can cause problems with brain development, leading to progressive mental retardation, brain damage, and seizures. In the past, PKU was treated with a low-phenylalanine diet.
- All PKU patients must adhere to a special diet low in phenylalanine. This requires severely restricting or eliminating foods high in phenylalanine, such as meat, chicken, fish, eggs, nuts, cheese, legumes, cow milk and other dairy products.

30.2.3 Galactosemia

- It is a rare genetic metabolic disorder that affects an individual's ability to metabolize the sugar galactose properly. Galactosemia is not related to and should not be confused with lactose intolerance.
- Galactosemia follows an autosomal recessive mode of inheritance that confers a deficiency in an enzyme responsible for adequate galactose degradation. Lactose in food (such as dairy products) is broken down by the enzyme lactase into glucose and galactose. In individuals with galactosemia, the enzymes needed for further metabolism of galactose are severely diminished or missing entirely, leading to toxic levels of galactose-1-phosphate in various tissues, as in the case of classic galactosemia, resulting in hepatomegaly (an enlarged liver), cirrhosis, renal failure, cataracts, brain damage, and ovarian failure. Without treatment, mortality in infants with galactosemia is about 75%.
- Galactose is converted into glucose by the action of three enzymes, known as the Leloir pathway. Accordingly, there are 3 known types of Galactosemia; type 1, 2 and 3

Type-I – deficiency of enzyme Glactose-1-phosphate uridlylyl transferase
Type II –defeciency of enzyme Glactokinase
Type III –defeciency of enzyme UDP galactose epimerase

30.3 Milk Hypersensitivity

- Allergy is a hypersentivity reaction and defined as any unusual or exaggerated response to a particular substance, called an allergen, in a person sensitive to that substance.
- Allergies are the result of the reactions of the body’s immunologic processes to “foreign” substances (chemical substances in such items as foods, drugs, insect venom) or to physical conditions. The reaction is caused by an allergen, either alone or coupled with a hapten, that stimulates the production of antibodies.
- Subsequent exposure to previously sensitized antibody-producing cells may precipitate an allergic reaction. The symptoms range from sneezing to vomiting, from headaches to hives, from edema to diarrhea, and many more, some minor and some quite serious.
- These effects are believed to be due to the release of histamine by an immunologic reaction.
- Many individuals are particularly sensitive to certain foods, just as others are to pollen or other particles in the air they breathe. Some foods are more likely to produce allergic reactions than others, but practically all foods can produce an allergic reaction in some people.
- Proteins are often considered the causative agents, and undoubtedly they are in most cases, but there are some assertions in the literature that fats and even carbohydrates can be responsible.
- Many different foods have been associated with allergies. They all contain proteins, one or more of which enter the body across the intestinal epithelium and elicit an immune response; however, any major alteration in the protein such as heat denaturation usually results in the loss of its allergenic properties. For example, raw or pasteurized milk may cause an allergic reaction, but if the same milk

is boiled - a process which denatures the proteins – the sensitive individual may be able to consume it without an allergic reaction.

- The foods which cause allergic reactions most frequently are milk, eggs, wheat, corn, legumes, nuts, and seafood. Also some people are allergic to strawberries and other berries, citrus fruits, tomatoes, and chocolate. Foods which rarely cause allergic reactions include rice, lamb, gelatin, peaches, pears, carrots, lettuce, and apples.
- The best treatment after the offending food or foods is identified, is to plan an adequate diet that does not contain the allergen.

30.3.1 Milk proteins as allergens

- Hypersensitivity to milk proteins is one of the main food allergies and affects mostly but not exclusively infants, while it may also persist through adulthood and can be very severe.
- Different clinical symptoms of milk allergy have been established. The diagnosis of milk allergies differs widely due to the multiplicity and degrees of symptoms, and can be achieved by skin or blood tests.
- Cow milk contains more than 20 protein allergens that can cause allergic reactions.
- Casein fractions and β-lactoglobulins (β-lg) are the most common cow milk allergens. Human milk is free of β-lg, similar to camel milk . On the contrary, β-lg is a major whey protein in cow, buffalo, sheep, goat, mare and donkey milk.
- Caseins in milk of the different species differ in fraction number, amino acid composition, and their peptide mappings. β -Casein is the major fraction in goat casein, which is similar to human casein and different from cow casein .
- Different procedures can reduce the allergenicity of cow milk proteins by heat or enzymatic treatments to some degree .
- Genetic polymorphism of milk proteins play an important role in eliciting different degree of allergic reactions. Goat milk is less allergenic than cow milk because of the absence of α-S1 casein which is typical for goat breeds.

30.3.2 Immunological mechanisms in milk allergy

- IgE-Mediated CMA (Immediate Hypersensitivity)

These are commonly mediated by a specific class of antibodies, known as immunoglobins (IgE), which are normally generated as part of immune reactions to parasitic infections, but for reasons that are partly understood can also be generated in following exposure to environmental agents, such as pollen, dusts, and foods. (Fig. 30.2)

- Non-IgE-Mediated CMA (Delayed Hypersensitivity)

These non-IgE-mediated reactions tend to be delayed, with the onset of symptoms occurring from 1 hour to several days after ingestion of milk. Hence, they are often referred to as "delayed hypersensitivity". A number of mechanisms have been implicated, including type-1 T helper cell (Th1) mediated reactions, the formation of immune complexes leading to the activation of Complement, or T-cell/mast cell/neuron interactions inducing functional changes in smooth muscle action and intestinal motility.

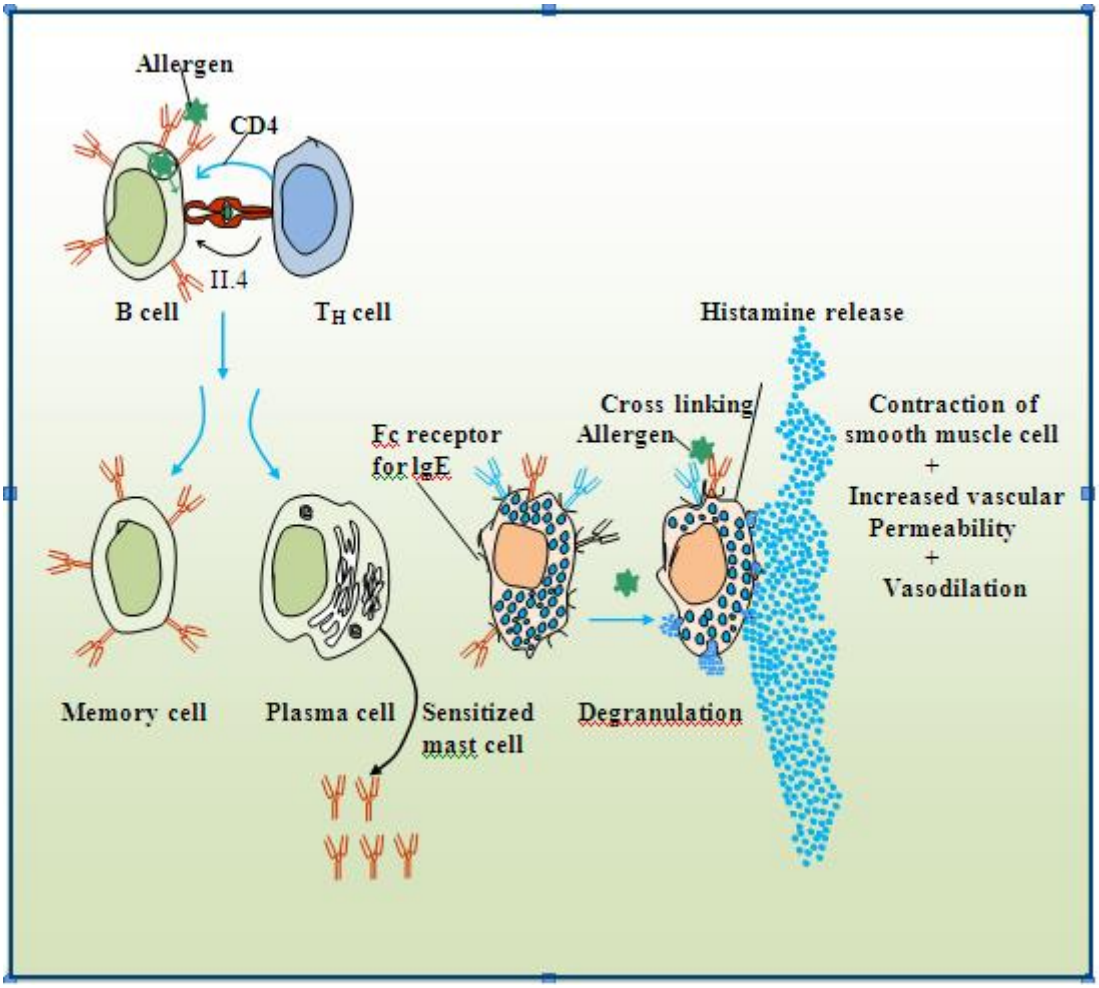

Fig 30.2 Ig-E Medicated milk allergy

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Lesson 31
ELEMENTARY KNOWLEDGE OF MILK SYNTHESIS IN MAMMARY GLAND

31.1 Introduction

- Precursors of milk come from the bloodstream. It is estimated that the production of 1 liter of milk requires 500 liter of blood moving through the mammary gland to provide the milk precursors.
- Some materials in the milk come unchanged from blood. These include minerals, some hormones and some proteins (e.g. immunoglobulins).
- Only precursors of milk protein and carbohydrates are present in blood. The primary substrates extracted from blood by the lactating mammary gland include glucose, amino acids, fatty acids, ß-hydroxybutyrate, and salt.

31.2 Milk Fat

- Cow's milk contains 3.5 to 5% fat.
- About 97 to 98% of the fat is triglycerides (also known as triacylglycerols or triacylglycerides) and phospholipids constitute about 1% (Table. 31.1). Palmitic (C16:0) and oleic (C18:1) acid are the main fatty acids in milk fat.
- Milk fat contains low levels of short chain fatty acid (C12 and less).

Table 31.1 Milk lipid composition of dairy cows

Lipid class	%of total lipids
Triglycerides	958
1,2 diglycerides	2.3
Phospholipids	1.1
Cholesterol	0.5
Free fatty acids	0.3

31.2.1 Biosynthesis of milk lipids (Triglycerides)

- Sources of Milk Fatty Acids

The fatty acids used to synthesize milk fat (triglycerides) come from two sources:

- Blood lipids: Derived from digestion and absorption of dietary fat and from mobilization of fatty acids from adipose tissue. Most of the fatty acids derived from blood plasma are of dietary origin (> 80%).
- De novo synthesis within the mammary epithelial cells (synthesis of new molecules of fatty acids from precursors absorbed from the blood) Acetate and ß-hydroxy-butyrate are the major carbon sources of fatty acid biosynthesis in the mammary gland. Almost all C4 to C14 fatty acids (short and medium-chain fatty acids) are synthesized de novo. Short chain fatty acids of various lengths are synthesized by the step-wise addition of acetate to ß-hydroxy-butyrate.

    - Milk fat triglycerides are synthesized in the cytoplasm surface of the smooth endoplasmic reticulum of mammary epithelial cells. Milk lipids (triglycerides) are synthesized from fatty acids and glycerol through the α-glycerol phosphate pathway

(Figure 31.1). Acetyl CoA carboxylase is the key milk biosynthesis enzyme and its activity increases considerably during lactogenesis (copious milk secretion).

- Two acyl CoA molecules react with α-glycerol-3-phosphate to form phosphatidic acid, which upon removal of the phosphate, leaves a 1,2 diacylglycerol. An additional long chain acyl CoA adds the final fatty acid, with the formation of triacylglycerol and CoA.

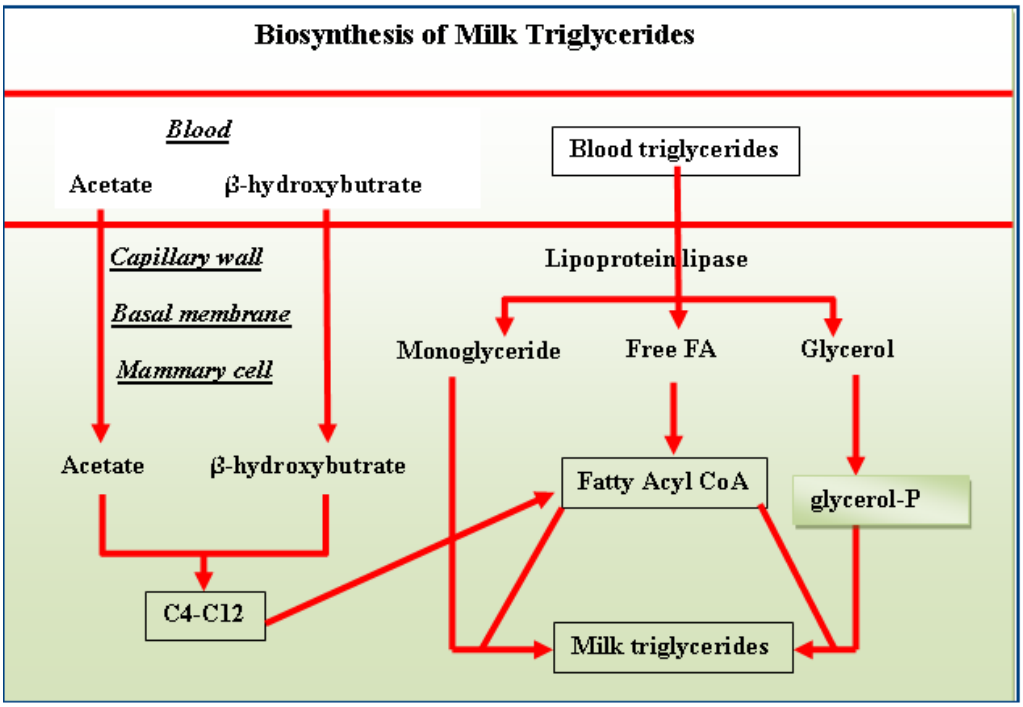

Fig 31.1 Biosynthesis of milk tryglycerides

31.3 Milk Proteins

- The nitrogen content of milk is distributed among three major groups:

• Caseins (76% of total milk nitrogen)
• Whey protein (18% of total milk nitrogen)
• Non-protein nitrogen (6% of total milk nitrogen)

- True proteins (i.e. excluding NPN) are classified into three fractions:

• Caseins present in micelles
• Whey proteins present in solution
• Fat globule membrane proteins on the surface of fat globules.

- Milk proteins contain more amino acids than any other natural food.

These include:
• Casein proteins; α-, β-, and k-casein
• Lactoglobulin; b-lactoglobulin (~50% of whey proteins)
• Lactalbumin. a-lactalbumin (~25% of whey protein)

- A second group blood proteins (e.g. immunoglobulins) and some proteins synthesized in the plasma cell adjacent to the secretory epithelium, enter the mammary gland and appear in the milk unchanged.

31.3.1 Biosynthesis of milk protein

- Milk proteins are synthesized from amino acids present in the mammary secretory cell. The biosynthesis takes place in the ribosome, which is attached to the rough endoplasmic reticulum. Steps of biosynthesis are similar to those of any other protein:

1. Transcription: A strand of messenger RNA (mRNA) is formed from DNA. It carries the code of a specific protein. The mRNA is located in the ribosome, which is attached to the rough endoplasmic reticulum.
2. Activation: Amino acids in the cytoplasm are activated by reaction with ATP and attachment to transfer RNA (tRNA). The tRNAs are specific for each amino acid.
3. Translation: Takes place in the ribosomes. The mRNA contains codes for amino acids. Each code consists of three nucleotides and is known as a codon. Located in the tRNA a trinucleotide anticodon that recognizes it. As each codon in the mRNA comes in position, the appropriate amino acid-tRNA complex moves in the amino acid joined the previous one in the chain.

31.3.2 Biosynthesis of milk carbohydrates (Lactose)

- Lactose is the most constant constituent in bovine milk (about 4.5%). The main function of lactose is to maintain the osmolality of milk during the formation and secretion process.
- Glucose is essential for lactose synthesis and cannot be replaced by any other sugar. About 45-60% of blood glucose in ruminants is synthesized in the liver from propionate by a process known as gluconeogenesis. Blood glucose levels in ruminants are about half those found in non-ruminants.

31.3.3 Lactose biosynthesis

- The site of lactose synthesis is the membranes of the Golgi apparatus. Glucose is the only precursor and two molecules of glucose are required for each molecule of lactose. One molecule of glucose is converted to galactose. The enzyme catalyzing this conversion appears just before parturition and its activity increase dramatically at the onset of milk synthesis in lactation (Lactogenesis).
- -lactalbumin) that must be together for lactose biosynthesis to take place. Therefore, the rate of lactose biosynthesis is greatly influenced by the availability of α-lactalbumin from the rough endoplasmic reticulum. (Fig. 31.2)αThe condensation of glucose and galactose involves the enzyme lactose synthase. The enzyme composed of two proteins (galactocyl trnasferase and
- Lactose is a nonpermeable disaccharide, which cannot diffuse out the Golgi membrane or out of the secretory vesicles' membrane. This is important for milk synthesis because it is the synthesis of the nondiffusible lactose, which results in water being drawn into the Golgi. Water is osmotically drawn into the Golgi to try to dilute the lactose.

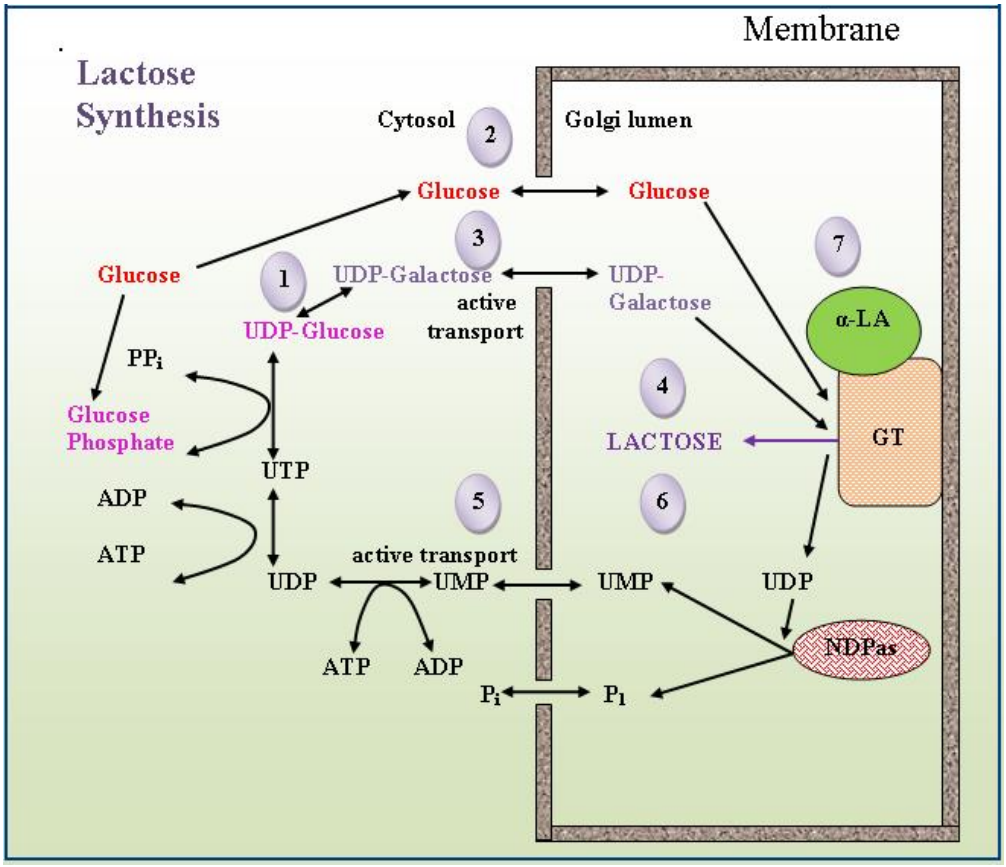

Fig 31.2 Lactose synthesis

31.4 Secretion of Milk Constituents

- The individual component of milk are kept separate inside the secretory cell and therefore, milk is not formed until the individual components reached the lumen where they are mixed together.
- Milk protein synthesized in the rough-endoplasmic reticulum where it is incorporated into the Golgi vesicles (vacuoles). Other non-fat components including lactose and salts are also incorporated into the Golgi vesicles. The secretory vesicles separate from the Golgi apparatus and move towards the apical region of the cell where the membrane surrounding each vesicle fuses with the plasma membrane and the content is discharged into the lumen.
- Milk fat or lipids take a separate secretion pathway than that taken by non-fat milk components (i.e. protein and lactose). Lipid molecules increase in size as they move from the endoplasmic reticulum towards the apical membrane where they push through and break away as globules engulfed in an envelope made of apical plasma membrane. The apical membrane is composed of lipids, which come from the walls of the secretory vesicles carrying the non-fat components of milk to the apical membrane. The milk fat globule is membrane-surrounded and has a number of membrane associated proteins, these proteins and others trapped during the process of cream separation are important for the whipping properties of cream.

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Lesson 32
PLANNING AND NUTRITIONAL POLICIES

32.1 Introduction

- Widespread poverty resulting in chronic and persistent hunger is the single biggest scourge of the developing world today.
- Under nutrition is a condition resulting from inadequate intake of food or more essential nutrient(s) resulting in deterioration of physical growth and health.
- The inadequacy is relative to the food and nutrients needed to maintain good health, provide for growth and allow a choice of physical activity levels, including work levels that are socially necessary.
- This condition of under-nutrition, therefore, reduces work capacity and productivity amongst adults and enhances morality and morbidity amongst children.
- Such reduced productivity translates into reduced earning capacity, leading to further poverty, and the vicious cycle goes on.
- The nutritional status of a population is therefore critical to the development and well being of a nation.

32.2 Need for a Nutrition Policy

- The need for a National Nutrition Policy is implicit in both the paramountcy of nutrition in development as well as in the complexity of the problem.
- The general problem of under-nutrition should be seen as a part of a larger set process that produces and consumes agricultural commodities on farms, transforms them into food in the marketing sector and sells the food to customers to satisfy nutritional, aesthetic and social needs.
- From being a deficit nation, depending on food imports in the sixties, to having become surplus in food grains in the eighties is a saga of concerted agricultural research, extension work and development, resulting in a dramatic productivity increase.
- Yet, from all accounts, endemic malnutrition and ill health resulting from malnutrition continue to stalk the country. It is this stark reality that underscores the need for a nutrition policy. Increased food production does not by itself necessarily ensure nutrition for all.

32.3 Nutrition Problems of India

The major nutrition problems of India can be classified as follows:-

- Under-nutrition resulting in:

(a) Protein Energy Malnutrition (PEM);
(b) Iron deficiency;
(c) Iodine deficiency;
(d) Vit, “A” deficiency;
(e) Low Birth Weight Children;

- Seasonal dimensions of Nutrition;

1. Natural calamities and the landless;
2. Market Distortion and Disinformation;
3. Urbanization;

4. Special Nutritional Problems of Hill people, Industrial Workers, Migrant Workers, and other special categories;
5. Problems of Overnutrition, overweight and obesity for a small section of urban population;

For India and much of the Third World nutrition status is characterized by varying degrees of undernutrition for women and children

- Protein Energy Malnutrition is the most widespread form of malnutrition among pre-school children of our country. A majority of them suffer from varying grades of malnutrition.
- Nutritional anaemia among the pre-school children and expectant and nursing mothers is one of the major preventable health problems in India. It has been estimated in various studies particularly those conducted by NIN that roughly 56 percent pre-school children and almost 50 percent of the expectant mothers in the third trimester of pregnancy suffer from iron deficiency, which is basically due to inadequate or poor absorption of iron from a predominantly cereal-based diet.
- In India, nearly 40 million persons are estimated to be suffering from goiter and 145 million are living in the known goiter endemic regions ranges from 1.5 per cent in Assam (Cachar Distt.) to 68.6% in Mizoram. It is estimated that iodine deficiency also accounts for 90,000 still births and neo-natal deaths every year.
- Nutritional blindness which affects over seven million children in India per year results mainly from the deficiency of vitamin A, coupled with protein energy malnutrition. In its several form, it often results in loss of vision and it has been estimated that around 60,000 children become blind every year.
- The prevalence of low birth weight children is still unacceptably high for India. The nutritional status of infants is closely related to the material nutritional status during pregnancy and infancy. In India 30% of all the infants born are low birth weight babies (Weight less than 2500 gms.)
- There are serious seasonal dimensions of the nutrition question. In large parts of India, the rainy months are the worst months for the rural, landless poor. This is when cultivation, deweeding, ploughing and other works demand maximum energy from them, while food stocks at home dwindle and market prices rise.
- These are again the months when water-borne diseases are so frequent. This condition goes on aggravating till late October or even November. This same group of rural landless poor is most vulnerable to droughts, floods and famines. As has been established in famine periods, worst affected groups are the landless agricultural labourers, artisans, craftsmen and non-agricultural labourers in that order.
- Under-nutrition in urban areas is a major area of concern. Studies by NNMB have actually shown that the nutritional status of urban slum dwellers in India is almost as bad as that of rural poor. The deleterious effects of rural urban movements on nutrition in much of the third world, is quite well known. The children of urban slum dwellers and of the urban informal sector are nutritionally the most fragile of all groups.
- Uncertainity of income and the absence of informal nutritional support system within society, so common to rural areas of India place many of these families on the very edge of survival.

32.4 Nutrition Policy Instruments

Nutrition is a multi-sectoral issue and needs to be tackled at various levels. Nutrition affects development as much as development affects nutrition. It is, therefore, important to tackle the problem of nutrition both through direct nutrition intervention for specially vulnerable groups as well as through various development policy instruments which will create conditions for improved nutrition.

- Direct Intervention

Nutrition Intervention for especially vulnerable groups:

Expanding the Safety Net The Universal Immunization Programme, oral Rehydration Therapy and the Integrated Child Development Services (ICDS) have had a considerable impact on child survival

- Reaching the Adolescent Girls
- Awarness among mothers regarding nutritional status of babies
- Ensuring better coverage of expectant women
- Fortification of Essential Foods
- Popularization of Low Cost Nutrition Food
- Control of Micro-Nutrient Deficiencies amongst vulnerable Groups

- Indirect Policy Instruments

  - Food Security
  - Improvement of Dietary pattern through Production and Demonstration
  - Improving the purchasing power: Poverty alleviation programmes, like the Integrated Rurat Development Programme (IRDP) and employment generation schemes like Jawahar Rozgar Yojana, Nehru Rozgar Yojana and DWCRA are to be re-oriented and restricted to make a forceful dent on the purchasing power of the lowest economic segments of the population. In all poverty alleviation programmes nutritional objectives shall be incorporated explicitly and the nutritional benefits of income generation shall be taken for granted.
  - Public Distribution System
  - Land Reforms
  - Basic Health and Nutrition Knowledge
  - Prevention of Food Adulteration
  - Nutrition Surveillance: Nutrition surveillance is another weak area requiring immediate attention. The NNMB/NIN of ICMR needs to be strengthened so that periodical monitoring of the nutrition status of children, adolescent girls and pregnant and lactating mothers below the poverty line values place through representative samples and results are transmitted to all agencies concerned
  - Monitoring of Nutrition Programmes: Monitoring of Nutrition Programmes (viz ICDS), and of Nutrition Education and Demonstration by the Food and Nutrition Board, through all its 67 centres 7 field units, should be continued.
  - Communication: Communication through established media is one of the most important strategies to be adopted for the effective implementation of the Nutrition Policy.
  - Minimum Wage Administration

32.4 Intervention Programmes to Combat Malnutrition

32.4.1 Integrated child development services (ICDS)

Integrated Child Development Services (ICDS) was launched in 1975. This programme is implemented by the Nodal Department i.e. the Department of Women and Child Development has been expended to 2765 projects up to December 1992. The package of services provided to the beneficiaries of the programme are supplementary nutrition, Immunization, Health check-up, Referral services, Non-formal pre-school education and nutrition and health Education. Supplementary nutrition is one of the major components of the programmes. The beneficiaries of the programme are children below 6 years, pregnant and lactating mothers and women in the age group 15-44 years. This programme supplements the health, nutrition and family welfare activities with appropriate cooperation and coordination between functionaries of the Health Department and nodal department.

32.4.2 Special nutrition programme

The special Nutrition Programme (SNP) was launched in the country in 1970-71. It provides supplementary feeding to the extent of about 300 calories and 10 gm of proteins to pre-school children and about 500

calories and 20 gm of protein to expectant and nursing mothers for 300 days a year. At present SNP is operated as a part of the Minimum Needs Programme in the various states.

32.4.3 Balwadi nutrition programme

The Balwadi Nutrition Programme (BNP) is being implemented SINCE 1970-71 through five national level voluntary organisations. The Central grant is given for supplementary feeding of children. It consists of 300 calories and 10 gm of protein per child per day for 270 days a year.

32.4.4 Wheat based supplementary nutrition programme

A centrally sponsored scheme called Wheat-based Supplementary Nutrition Programme (WNP) was introduced in 1986. This programme follows the norms of SNP or of the nutrition component of the ICDs. Central assistance for the programme consists of supply of free wheat and supportive costs for other ingredients, cooking, transport etc. At present around 3 million children and expectant and nursing mothers are covered under this programme.

32.4.5 Mid day meal programme

In 1956 the erstwhile Madras State launched a Prophylaxis programme of providing free meal to the elementary school children with a view to (a) enrolling poor children who generally remain outside the school due to poverty; and (b) to attract children to enroll themselves into school and to encourage regular attendance by providing supplementary nutrition.

32.4.5 Nutritional anaemia prophylaxis programme

Taking cognizance of this problem, the Government of India launched a Prophylaxis programme in 1970 to prevent nutritional anemia in mothers and children. Under the Programme, the expectant and nursing mothers as well as women acceptors of family planning are given one tablet of iron and folic acid containing 60 mg elemental iron (9180 mg of ferrous sulphate) and 0.5 mg of folic acid and children in the age group 1-5 years are given one tablet of iron containing 20 mg elemental iron (60 mg of ferrous sulphate and 0.1 mg folic acid) daily for a period of 100 days. This programme covered children and pregnant women with haemoglobin level less than 8 gm per cent and 10 gm per cent respectively.

32.4.6 Prophylaxis programme against blindness due to vitamin a deficiency

The programme was initiated by the Government in 1970. Under this programme children in age group 1-5 years are given an oral dose of 0.2 million I.U. of Vitamin A in oil every 6 months. During 1980, the Department of Food introduced a scheme of Fortification of Milk with Vitamin A to prevent nutritional blindness. At present there are 42 dairies in the country implementing this scheme.

32.4.7 Goitre control programme

A National Goitre Control Programme was initiated by the Government of India in 1962 to identify goitre endemic regions and to assess the impact of goitre control measure. The availability and production of iodized salt, strengthening of administrative machinery controlling the entry of non-iodized salt in the endemic regions have been recommended as measures to improve the implementation of the programme.

32.4.8 National diarrhoeal diseases control programme

The programme was launched in 1981 to reduce the mortality in children below five years due to diarrheal diseases through introduction of Oral Rehydration Therapy (ORT). The high priority accorded to the Programme is part of the package of services rendered under the MCH programme which was initiated during 1980-85 has now been strengthened extensively. The Anganwadi Centers of the ICDS Scheme have

served as nucleus for the propagation of Oral Rehydration Therapy (ORT) which has been found to be an effective measure of preventing dehydration caused by diarrhea.

32.5 Function of the Food and Nutrition Board

The Food and Nutrition Board as reconstituted on 26 July 1990, advises Government, coordinates and reviews the activities. In regards to food and nutrition extension/education; development, production and popularization of nutritious. Foods and Beverages; measures required to combat deficiency diseases; and conservation and efficient utilization as well as argumentation of food resources by way of food preservation and processing.

Lesson 33
SAFETY ASPECTS OF FOOD ADDITIVES, TOXIC ELEMENTS, RADIONUCLIDE IN MILK AND MILK PRODUCTS

33.1 Introduction

Though we have made rapid strides in the modern technology, maintaining and providing safe food still remains a global problem. Contamination of food remains a major risk and serves as source of disease and death in many of the developing countries. The food contaminants may generally be classified into two groups viz. chemical and biological. The reporting system for the food borne disease is not well organized and most of the problems are of biological in nature.

33.2 What are Food Additives?

A food additive is defined as a substance or mixture of substances other than a base foodstuff, which is present in a food as a result of any aspect of production, processing, storage or packing. This definition includes both intentional and unintentional additives. The unintentional additives, which are not added to achieve an effect in the food but which may accidentally enter into foods as a result of their use in agricultural production, raising animals, food processing or packing, are not additives in the technical sense of the term, but they are 'food contaminants'.

An expert committee on Food Additives made up of representatives of FAO and WHO has defined food additives as non-nutritive substances added intentionally to food, generally in small quantities to improve its appearance, flavour, texture or storage properties. This definition excludes substances added primarily for their nutritive value, such as vitamins and minerals.

33.3 Need for Food Additives

- Additives have provided protection against food spoilage during storage, transportation, distribution or processing. Also, with the present degree of urbanization it would be impossible to maintain food distribution without the processing and packing with which many additives are involved.
- Many foods, particularly those with high moisture contents, do not keep well. All foods are subject to microbial attack. Fats or oily foods become rancid, particularly when exposed to humid air. The conservation of the quality of foods against agents causing such deterioration of foods requires the addition of preservatives.
- Additives are also used to color foods, add flavor, impact firmness and retard or hasten chemical reactions in food to enhance stability with resulting reduction in waste. Over 3000 different chemical compounds are used as food additives. They are categorized into different groups. A few types of additives are indicated below.

33.3.1 Antioxidants

- An antioxidant is a substance added to fats and fat containing substance to retard oxidation and thereby prolong their wholesomeness, palatability and sometimes keeping quality (time). Antioxidants function by interrupting the free radical chain mechanism involved in lipid oxidation. They are effective in small concentrations (0.01-0.02%)
- It should not contribute an objectionable odor, flavor or color to the fat or to the food in which it is present. It should be effective in low concentrations and be fat soluble.

- Also, it should not have harmful physiological effect.e.g. BHA (Butylated hydroxyl anisole), BHT (Butylated hydroxyl toluene), PG (propyl gallate) and TBHQ (Tertiary butyl hydroquinone) which are all phenolic substances.
- Naturally occurring substances that act as antioxidants are the tocopherols, but they are rarely used as they are more expensive than synthetic ones.

33.3.2 Chelating agents

- Chelating agents or sequestrates are compounds that form complexes with metal ions. Many metals exist in food in a naturally chelated form, such as, Mg in chlorophylls, Fe in ferreitin and hemoglobin and Cu, Zn and Mn in enzymes.
- When metallic ions are released due to hydrolytic or other degradative reactions, they are free to participate in reactions that lead to discoloration, oxidative rancidity, turbidity and flavor changes in foods.
- Addition of chelating agents results in the complexing of these metal ions and thereby the stabilization of foods.
- Chelating agents are not antioxidants; they serve as scavengers of metals which catalyze oxidation. They however, are antioxidant synergists.e.g. Citric acid and its derivatives, phosphates and salts of ethylene diamine tetra acetic acid (EDTA).

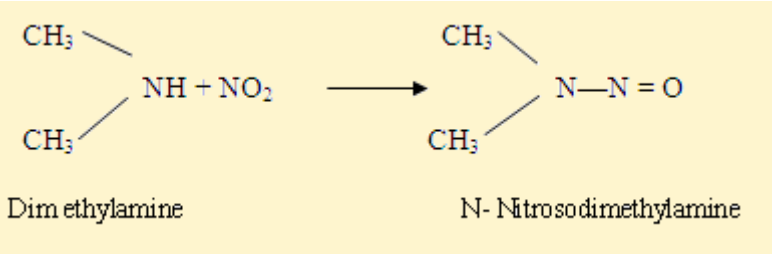
33.3.3 Colouring agents

- These include color stabilizers, color fixatives, color retention agents, etc. They consist of synthetic colors, synthesized colors that also occur naturally and other colours from natural sources.
- Colors add nothing to the nutritive value of foods, without certain colors most consumers will not take some foods. Thus, colors are frequently added to restore the natural ones lost in food processing or to give the preparations the natural color we expect.
- Originally, many color additives were natural pigments or dyes. E.g. spinach juice or grams, marigold flower, and cochineal (a natural red pigment obtained by extraction from the female insect Coccus cacti were used to obtain green, yellow and red color respectively.
- But synthetic colors generally excel in coloring power, color uniformity, color stability and cost. Furthermore, in many cases, natural coloring materials do not exist for a desired hue. Therefore, now synthetic ones are preferred.
- Carbonated beverages, gelatin dessert, candies and bakery goods are some foods that are colored with coaltar dyes. As a number of coaltar compounds have been shown to be potent carcinogens the use of coaltar dyes as food additives is restricted.
- A number of natural food colours extracted from seeds, flowers, insects and foods are also used as food additives. One of the best known and most widespread red pigment is bixin, derived from the seed coat of Bixa orellana, the lipstick plant of South American origin. Bixin is not considered to be carcinogenic. The major use of this plant on a worldwide basis is for the annatto dye, a yellow to red coloring material extracted from the orange-red pulp of the seeds. Ammato has been used as coloring matter in butter, cheese, margarine and other foods. Another yellow color, a carotene derived from carrot, is used in margarine. Saffron has both flavoring and coloring properties and has been used for coloring foods.

33.3.4 Curing agents

- These are added to preserve (cure) meats, give them desirable color and flavor, discourage growth of microorganisms and prevent toxin formation.
- Sodium nitrate has been used for centuries as a preservative and color stabilizer in meat and fish products. The nitrite, when added to meat, gets converted to nitric oxide which combines with myoglobin to form nitric oxide myoglobin (nitrosylmyoglobin) which is a heat stable pigment. Nitrite curing inhibits the growth of Clostridium and Streptococcus and also lowers the temperature required to kill Clostridium botulinum.

- It has been discovered that cooking nitrite cured meat products results in the formation of small amounts of N-nitrosamines, which are potent carcinogens. The nitrosamines are formed by the reaction of secondary and tertiary amines, through the following type of reaction.



Addition of ascorbates and isoascorbates reduce the formation of toxin during curing.

33.3.5 Flavors and flavor enhancers

- Flavouring additives are the ingredients both naturally occurring and added, which give the characteristic flavor to almost all the foods in our diet. Flavor enhancers are not flavors themselves but they amplify the flavors of other substances through a synergetic effect. Flavor and flavor enhancers constitute the largest class of food additives. There are about 2,100 approved natural and synthetic flavors of which more than 1,600 are synthetic ones.
- Natural flavor substances, such as spices, herbs, roots, essences and essential oils have been used in the past as flavor additives. The flavors of such materials are not uniform. They vary with the season and area of production. In addition, it would take about a ton of many spices to produce 1g of the flavor substances and in some cases even less than that. Natural food flavors are thus being replaced by synthetic flavor materials.
- The agents responsible for flavor are esters, aldehydes, ketones, alcohols and ethers. Typical of the synthetic flavor additives are amyl acetate for banana, methyl antheanilate for grapes, ethyl butyrate for pineapple etc. Generally, most synthetic flavors are mixtures of a no of different substances e.g. one imitation cherry flavor contains 15 different esters, alcohols and aldehydes.
- One of the best known, most widely used and somewhat controversial flavors enhancer is monosodium glutamate (MSG), the sodium salt of the naturally occurring amino acid glutamic acid. It is largely added to meat and soup to produce enhanced flavor. MSG enhances the desirable flavor while minimizing the undesirable ones, like the sharpeners of raw onions, vegetables etc. the 5'-nucleosides are a group of substances obtained by the enzyme hydrolysis of ribonucleic acid having the same flavor enhancing properties as MSG, but are more powerful. Both of these substances appear to affect all the four kinds of taste buds in the mouth.

33.3.6 Preservatives

- A Preservative is defined as any substance which is capable of inhibiting, retarding or arresting the growth of micro organisms, or any deterioration of food due to micro-organisms, or of making the effect evidence of any such deterioration.
- Chemical preservatives interfere with cell membrane of micro-organisms, their enzymes or their genetic mechanisms. The compounds used as preservatives include natural preservatives such as sugar, salt, acids, etc., as well as synthetic preservatives. Chemical preservatives are generally added after the foods are processed.

33.3.7 Artificial sweetness

- Some synthetic or naturally occurring compounds are used as artificial sweetners, which are used as a substitute for sugar.
- Most commonly used artificial sweetners are aspartame, saccharine (both synthetic), steroids (naturally occurring).

- Ministry of Health and Family Welfare has issued a notification for use of artificial sweetners. (Table 33.1)

Table 33.1 Notification issued by Ministry of Health and Family Welfare on 25th June, 2004

Name of artificial Sweetener	Article of food	Maximum limit PPM
Saccharin Sodium	Sweet (Carbohydic based milk products bazol)	500
Aspartane (methyl ester)		200
Acesulfame potassium		500
Sucralise (chlorinated sucrose) 500 to 600 the sweetner than sucrose		750

33.4 Food Toxins

Foods contain not only the nutrient we need but also a large number of chemicals some of which are toxic.

- Plants synthesize toxic chemicals apparently as a primary defense against attacks by bacteria fungi, insects and other animal predators.
- Some toxic chemicals may enter the food supply by fortuitous natural mechanisms. These may be of microbial origin and environmental pollutants (including heavy metals)
- Chemicals sprayed on plants in the form of pesticides may be present in foods of plant origin and may be transmitted through food grains to animals.
- There are also substances added to foods for functional purposes such as preservatives, antioxidants and calorie reducing agents.
- Natural toxicants in some common foods and their actions are given below. (Table 33.2)

Table 33.2 Natural toxicants in some common foods

Food	Toxicants	Biological action
Potato	Solanine	Interfers with transmission of nerve impulses
Lima beans, Cassava, almonds	Cyanide	Inhibits cytochrome oxidase
Spinach, Shubarb	Oxalate	Interferes with Ca absorption, amy form kidney stones
Cabbage, Cauliflower	Goitrogens	Interferes with Iodine utilisation
Cereal, legumes Vegetables	Phytate	Binds Ca, Fe and Zn and prevents their absorption
Apple and pea seeds	amygdaline	Cyanide instand dis fatal
Orange peal	Citral	Vit A antagonist
Linseed meal	Linetin	Vit B_6 antagonist
Sweet clover	Dicumarve	Vit K antagonist
Egg white	Avidin	Bioten antagonist

33.5 Radionuclide

- A radionuclide (radioactive nuclide) is a nuclide with an unbalanced and unstable nucleus.
- A nuclide is an atom with a defined atomic number and a defined neutron number. In any nuclide, the number of neutrons determines whether the nucleus is radioactive. For the nucleus to be stable, the number of neutrons should in most cases be a little higher than the number of protons. If the number of neutrons is out of balance, the nucleus has excess energy and sooner or later will discharge the energy by decay processes, that is, by emitting rays or subatomic particles. A nuclide with such an unbalanced nucleus is unstable and is called a radioactive nuclide, or radionuclide.
- Radionuclides are often referred to by chemists and biologists as radioactive isotopes or radioisotopes, and play an important part in the technologies that provide us with food, water, and good health. Radionuclides may occur naturally, but they can also be artificially produced.
- Nuclear weapons tests have released large quantities of plutonium, 90Sr, and 137Cs throughout the Northern Hemisphere, with maximum levels found around 40°N to 50°N latitude .
- In Switzerland, particular attention has been paid to the highly radiotoxic 90Sr since the beginning of the nuclear era . As milk and dairy products constitute an important part of the diet of the Swiss population, it was recognized that 90Sr, an alkaline earth cation, follows the same metabolic pathways as calcium, and represents the main contributor to the internal dose by fission products. There was a large increase in 90Sr activity in milk samples during the 1960s, corresponding to nuclear testing in the atmosphere. The 90Sr activity profile in milk teeth matches that of milk, illustrating that 90 Sr present in the environment has been transferred to the food. After the Chernobyl accident, it was observed that the 90Sr activity of milk and dairy products in Switzerland doubled from 0.1 to 0.2 Bq/l during the first months after the accident.
- Discharge routes for radioactive waste from a nuclear site can be liquid, gaseous, and solid. The aquatic pathway covers potential contamination of oceans, rivers, and lakes due to liquid discharges. The terrestrial pathway deals with potential contamination of land predominately due to gaseous discharges to the atmosphere.
- For terrestrial radiological monitoring programs, cow's milk is often the predominant sample taken because it is readily available, consumed by a large number of people, consumed by children in relatively large quantities, and is a good indicator of radionuclides present in the environment. In the U.S., the Environmental Protection Agency (EPA) runs the Environmental Radiation Ambient

Monitoring System program, which covers air, drinking water, precipitation, and milk . Under Euratom Treaty, the European Union (EU) recommends that member states analyze 137 Cs and 90 Sr in milk from large milk processing sites .

- The maximum average value is the mean concentration at the farm or dairy with the highest individual result. For most foods, the maximum concentration can be selected for a dose assessment, as there is the possibility of storage of that food following harvesting, which could coincide with a peak level of activity in the food. Milk is generally not stored for long periods, so maximum averages may be used on the basis that the farm or milk production site where the highest value is found can supply milk to a consumer who consumes it in large quantities (a “high-rate” consumer). 14 C is a naturally occurring radionuclide, so some will be present in all milk samples. The U.K. uses a carbon content of 7% in milk, a background activity value of 250 Bq 14C/kg total carbon, and a subsequent background level of 18 Bq/l 14C for milk samples .

Lesson 34
ESTIMATION OF VITAMIN C AND CHOLESTROL

34.1 Introduction

- Vitamin C also called ascorbic acid is a water soluble vitamin, present in nearly all fresh fruits and vegetables.
- It is Heat labile i.e. destroyed on heating. It is present in all higher plants.
- Human beings cannot synthesize this vitamin and hence require it in their diet. Daily requirement is 40 mg. Inability to synthesize vitamin C is because they genetically lack the ability to synthesize the enzyme
- Gluconolactone oxidase is required for the formation of vitamin C.
- Vitamin C cannot be stored in the body; excessive intake causes it to be secreted in the urine.
- The vitamin is neither present nor is required by microorganisms.
- Starting compound for the synthesis of vitamin C is D- Glucose. The biologically active form is L-ascorbic acid. D- ascorbic acid does not possess any antiscorbutic activity.
- Ascorbic acid in solution is rapidly oxidized to dehydroascobic acid. The oxidation is catalysed by copper and silver ions. The oxidation is faster at higher temperatures e.g. during cooking of foods.

34.2 Significance

- Regular intake of C is required for healthy bones and teeth. Vitamin C is essential for the growth of subcutaneous tissue, cartilage, bone and teeth.
- In the absence of Vitamin C, the collagen that is formed is defective and weak. This is because vitamin C is required for activating the enzyme, Prolyl hydroxylase, that promotes the hydroxylation step in the formation of hydroxyproline, an integral constituent of collagen. Hydroxyproline is found only in collagen and no other animal protein.
- Collagen is a fibrous protein present in all multicellular organisms and is the most abundant protein in mammals, constituting a quarter of the total. Collagen is the major fibrous element of skin, bone, tendon, cartilage, blood vessels and teeth. It is present to some extent in nearly all organs and serves to hold cells together in discrete units.
- Deficiency of vitamin C causes a disease called Scurvy, which has got the following effects:

    - Failure of wounds to heal.
    - Bone growth stops. No new bone formation occurs. In already formed bone, fractures if any, do not heal.
    - Blood vessels become extremely fragile and rupture easily, especially capillaries.
    - In extreme scurvy, muscle cells fragment, lesion of gums with loosening of teeth occurs. Severe hemmorages can eventually lead to death.

34.3 Principle

- Ascorbic acid reduces the indicator dye to a colorless solution. At the end point of titrating an ascorbic acid containing sample with dye, excess unreduced dye is rose- pink color in acid solution. The titer of dye can be determined using standard ascorbic acid solution. Food samples in solution then can be titrated with the dye and the volume of titration used to calculate the ascorbic acid content.
- Ascorbic acid reduces the dye 2, 6 – dichlorophenol indophenols to colourless, and itself gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour for 5-10 seconds. The dye is pink in acid medium. A mixture of metaphosphoric and acetic acid is used as the titrating medium.

34.3.1 Reagents

1. Metaphosphoric acetic acid mixture: Dissolve 15 g of metaphosphoric acid in a mixture containing 40 ml glacial acetic acid and 450 ml distilled water. Filter and store in refrigerator. Stable for 10 days.
2. Dye solution: Dissolve 42 mg sodium bicarbonate and 50 mg 2, 6 – dichlorophenol indophenol dye in 50 ml of distilled water. Make up the volume to 200 ml. Filter and store in the refrigerator
3. Standard Stock solution: 1 mg/ ml. Dissolve 100 mg of ascorbic acid in 100 ml of 3% metaphosphoric acetic acid mixture.
4. Working Standard solution: 0.1mg/ ml. Dilute 10 ml of the stock to 100 ml with 3% metaphosphoric acetic acid mixture.

34.3.2 Protocol

- Pipette out 5 ml of the working standard solution into a 100 ml conical flask. Add 10 ml of acetic metaphosphoric acid mixture and titrate with dye. End point is the appearance of pink colour which persists for 5-10 seconds.
- Extract the sample (0.5 – 5 g depending upon the nature of the sample) in acetic metaphosphoric acid mixture and make up to a known volume e.g. 100 ml. Filter. Take 5 ml of the filterate, add 10 ml of the acid mixture and titrate against dye.

34.3.3 Observations

Volume of dye used in titration of standard in ml
Volume of dye used in titration of sample in ml

34.3.4 Calculations

$$\frac{C_1}{C_2} = \frac{V_1}{V_2}$$

Where C_1 = Conc. of Standard

V_1= Vol. of dye used in titration of standard

C_2= Conc. of Sample

V_2 = Vol. of dye use in titration of sample.

or $C_1V_1 = C_2V_2$

$$C_2 = \frac{C_2V_2}{V_1}$$

Express results as concentration of vitamin C per ml of extract and per gm of sample.

34.3.5 Notes

1. 4% oxalic acid may be used in place of metaphosphoric: acetic acid mixture. The mixture is used as a titrating medium. It not only reduces the pH, thereby stabilizing vitamin C, but also forms complexes with metallic ions e.g. copper, thereby preventing the catalytic oxidation of the vitamin.
2. The titration is conducted in the presence of acetic and metaphosphoric acids in order to inhibit aerobic oxidation catalysed by certain metallic ions, to inactive the enzyme, ascorbic acid oxidase, to precipitate proteins and liberate protein bound ascorbic acid.
3. Certain batches of metaphosphoric acid may have impurities that reduce the dye. Hence, reagents used should always be checked to guard against this possibility. A reagent blank with addition of dye should always be run along with sample.

34.4 Estimation of Cholesterol by Colorimetric Method

34.4.1 Cholesterol

- Cholesterol is a member of a large group of substances called steroids, which include vitamin D and a number of steroid hormones, among them are the sex hormones of higher animals. (Fig 34.1)
- Cholesterol is an essential component of cell membranes, brain and nerve cells, and bile, which helps the body absorb fats and fat soluble vitamins.
- Normal level varies with age, diet and from one location to another, however the average is 150-250 mg/dl blood.
- Cholesterol may be elevated slightly in pregnancy

34.4.2 Principle

The reaction depend on the colorimetric determination of a green colored compound resulting from the reaction of cholesterol with acetic anhydride-sulphuric acid (strong dehydrating and oxidizing agent) the absorbance of the green compound is then measured at 610 nm.

34.4.3 Material

- Cholesterol reagent (Acetic anhydride + acetic acid)
- Sulphuric acid 95-97%
- Standard cholesterol (300 mgl /dl)
- Samples
- Test tupes
- Pipettes
- Cuvette
- Spectrophotometer
- Water path

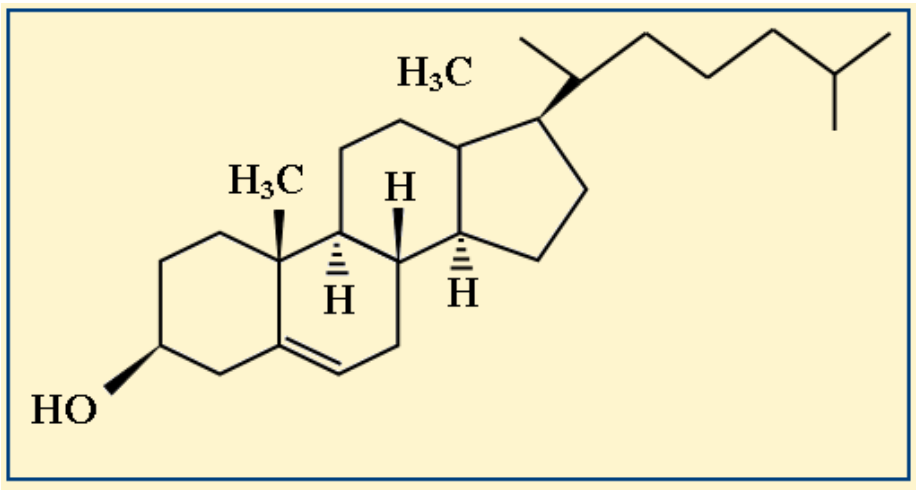


Fig 34.1 Chloestrol