NUTRTIONAL BIOCHEMISTRY

M.Sc. - 102

Directorate of Distance Education

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CARBOHYDRATES

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STRUCTURE

1.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

Understand the-meaning of nutritional biochemistry and discuss its evolution,

Classify carbohydrates,

Describe the chemical properties of carbohydrates,

Learn about mono, oligo and polysaccharides,

Discuss about importance of carbohydrates,

Learn importance of nutritional biochemistry,

1.2 INTRODUCTION

Nutrition is a relatively new science that evolved from disciplines such as Chemistry and Physiology. Thus, a student who intends to know and practice nutrition and dietetics should not only undertake intense leaming and training on the different techniques that relate nutrition with chemistry but they should also understand how nutritional processes are merged with chemistry. Further, it is important

to have a clear idea of the chemistry of nutrients for a better understanding of nutrition.

Do you have such knowledge? Ifthe answer is 'No', then it is absolutely necessary that you should learn intensely this unit and you will find that the contents provided in it will serve as the foundation for the other courses such as the Advanced Nutrition, Principles of Food Science and Public Nutrition courses. If your answer is 'Yes', then too you must go through the contents of this unit Lo assess if you have the correct understanding of the subject and to brush up your previous knowledge and at the same time, to gather any new knowledge that you may discover during study.

This unit will provide you with a broad introduction to metabolic and nutritional aspects of biochemistry. The major focus is on the structure, function and metabolism of carbohydrates.

1.3 INTRODUCTION TO NUTRITIONAL BIOCHEMISTRY

Being a student Of dietetics, a clear understanding of the term nutritional biochemistry, as well as, its deVelopment is pertinent to your knowledge.

Equally important is the applicability of nutritional techniques at the advanced level.

This sub-section, therefore, intends to:

make the meaning of nutritional biochemistry clear to you, and

inform you about the evolution of this science over the centuries.

1.3.1 Meaning and Importance of Nutritional Biochemistry

Nutrition is a science that cuts across several scientific disciplines, of which one is 'biochemistry'. It is the science that relates food to the functioning of the living organism. Nutrition includes the intake of food, digestion and absorption of food, transport and assimilation of nutrients, metabolism, liberation of energy and elimination of wastes. It, in fact, encompasses all the synthesis essential for growth, maintenance and reproduction.

Biochemistry, as per definition, is the chemistry of living organisms that covers all the chemical reactions occurring in our body. Biochemistry explores the functioning of living organisms from a molecular and cellular perspective.

As implied by the course, it emphasizes the problems of nutrition and the mechanisms of biochemistry. The reason for this is quite understandable as nutrients are basically chemicals and our body is a chemical machine composed and built up of chemicals. Therefore, the biological function of a nutrient is readily explained in terms of its chemical interaction at the molecular level with the body's endogenous chemicals.

A good understanding of the biochemical basis of nutrient function and of the consequence of nutrient deficiency or excess is important in the clinical and - laboratory diagnosis of nutritional diseases, clinical management of the same and in the control of endemic nutritional inadequacies. You, as a student of dietetics, should realize the importance of biochemistry in order to:

a) understand nutrition properly, and

b) utilize biochemical tools in solving nutrition-related health problems.

1.3.2 Development of Nutritional Biochemistry

Nutrition, in general, and particularly nutritional biochemistry, has traveled a long way since 1926, when nutrition was first recognized as an independent field of study with the appointment of Mary Swartz Rose as Professor of Nutrition at Columbia University.

This was actually the culmination of a developmental period stemming from Antoine Lavoisier 's experiments about 200 years back that formed the basis for the studies on respiratory exchange and calorimetry, the beginning of a new science - Nutrition.

About 100 years elapsed before carbohydrates, fats and proteins were identified as the source of energy fdr animal body. By. the end of the nineteenth century, the necessity for certain minerals in the diet was established. But it was not until the decade between 1930 and 1940 that the majority of the vitamins, were identified, isolated from different foods, synthesized in the laboratory and received serious attention in order to understand their involvement in various biochemical reactions.

1.3.3 Contemporary Interests in Nutritional Biochemistry

As newer techniques in biochemistry have emerged from time to time, these were of immense help in the understanding of utilization of different nutrients by the body.

Biochemical basis of some of the dreaded nutritional diseases of the past, such as beri-beri, scurvy, pellagra and pernicious anaemia are well understood. Likewise, problems such as protein-energy malnutrition, exophthalmia, iron deficiency anaemia, iodine deficiency disorders though well understood are still prevalent. Added to these are contemporary problems like diabetes and cardiovascular diseases. Reliable biochemical indicators are also now available to detect the prevalence of sub-clinical deficiency of many nutrients in the population.

Once the generation of free radicals in the body was correlated with the development of different fatal or crippling diseases, the importance of antioxidants increased many folds. In recent times, some nutrients such as vitamin E or selenium are getting special attention from research point of view, as these are potent antioxidants.

Recent biochemical studies have not only revealed the importance of

polyunsaturated fatty acids in maintaining the normal lipid profile but these also went further to prove that a desirable ratio of n-3/n-6 fatty acids is essential for preventing dyslipidemia (a condition in which an abnormal amounts of lipids and lipoproteins accumulate in the blood).

Importance of nutritional interactions is now being realized. Some nutrients are antagonistic to each other whereas others act synergistically. Examples of uniquely related nutrients include folate and vitamin B_{12}, vitamin E and polyunsaturated fatty acids, vitamin D and calcium, zinc and copper etc. Biochemical research has revealed that certain drugs such as omiprazole, lovastatin, allupurinol, thiouracil and others influence nutrient metabolism.

Not only nutrients, but the importance of non-nutrients such as dietary fiber for healthy living is also understood. Very recently, a group of compounds known as 'phytoestrogens' has created great interest among nutritional biochemists. These substances are considered to protect cancer, besides having other positive roles. As a consequence, many popular, as well as, uncommon foods and beverages are constantly under study to explore the presence of these compounds, as well as, to observe their biochemical effects. Tea and soybean are two good examples.

Neutraceuticals are gaining popularity at an exponential rate. These are classified as foods that provide medical or health benefits. Spirulina is already available in the Indian market besides other countries with high claims. Few uncommon edible oils such as flaxseed oil, primrose oil etc. which are already in use in the western countries for their apparent beneficial effects, may at any time enter the market in India.

Though, many of these preparations have proven positive effects on health, a more in-depth biochemical research will definitely help to know about these new generation nutritional substances.

1.4 CHEMISTRY OF CARBOHYDRATES

This is absolutely essential for you, as a student of Dietetics, to have a clear concept on the nature and physico-chemical properties of different nutrients.

After studying this sub-section, you will be able to know the following important aspects of carbohydrates:

Their chemical nature

Their classification, and

Their physico-chemical properties

You would realize that the family name ending —ose indicates a carbohydrate, for e.g. glucose, fructose, sucrose etc. Carbohydrates are basically polyhydroxy aldehydes or ketones and their derivatives. What do we mean by polyhydroxy aldehydes or ketones? Go ahead, read on and you will find the answer to this question soon. Carbohydrates, as you may already know, are found abundantly in both animal and plant tissues in different forms. While plants can produce

carbohydrates by photosynthesis, animal cells cannot but are dependent on food for these important sources of energy. Some carbohydrates have highly specific functions in the body.

Let us learn about these different carbohydrates.

NOTES

Carbohydrates are classified into three major groups:

1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides

Monosaccharides and oligosaccharides are also referred to as 'sugars'.

1.5 MONOSACCHARIDES

Monosaccharides consist of a single polyhydroxy aldehyde or ketone unit and are commonly known as simple sugars. These cannot be hydrolyzed into simpler forms.

The general formula for monosaccharides is $(CH_2O)n$ where n is 3 to 7. In biological materials, monosaccharides with 5 and 6 carbon atoms are most common. All the carbon atoms of the monosaccharide contain one hydroxyl group (—0H) except one that contains a carbonyl oxygen (as in —CHO or >C=O). If it is present at the terminal position, the monosaccharide is an aldehyde derivative and is called as an 'aldose' sugar position, e.g. glucose. On the other hand, if it is present in any other position, the monosaccharide is a ketone derivative that is known as 'ketose' e.g. fructose. The carbon atoms are numbered as shown in the Figure 1.1. C-l atom is an aldehyde functional. group and C-2 atom is a ketone functional group as highlighted in Figure 1.1. So now you know hdw carbohydrates get classified as polyhydroxy aldehyde or ketone.

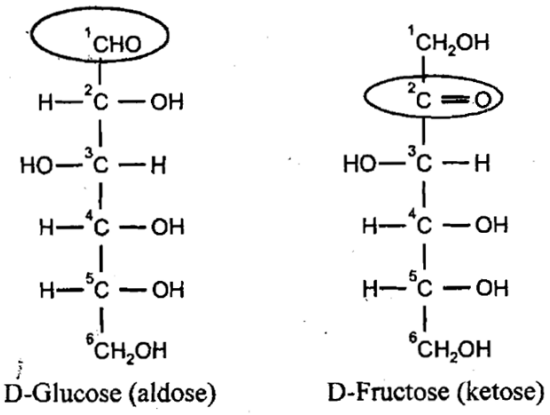

Figure 1.1: Numbering of carbon atoms in monosaccharides

Monosaccharides can further be divided on the basis of the number of carbon atoms they possess. This unit will be helpful to understand how the monosaccharides are classified, considering the number of carbon atoms in the molecule, as well as, the nature of the carbonyl oxygen with specific examples. The number of carbon atoms in an aldose or ketose may be specified by triose, tetrose, pentose, hexose or

heptose. For example, glucose with six carbon atoms is an aldohexose and fructose too with six carbon atoms is a ketohexose.

Table 1.1: Different types of monosaccharides found in foods

Monosaccharides		Specifi examples	
No. of carbon atoms	**Called as**	**Aldoses**	**Ketoses**
Three	Trioses	Glyceraldehyde	Dihydroxyacetone
Four	Tetroses	Erythrose	Erythrulose
Five	Pentoses	Ribose, Xylose, Arabinose	Ribulose, Xylulose
Six	Hexoses	Glucose, Galactose, Mannose	Fructose
Seven	Heptoses	Glucoheptose, Galactohe tose	Sedoheptulose

1.5.1 Isomerism of Monosaccharides

What do we mean by the term isomer? Existence of different compounds having same molecular form but different structural forms are isomers. Monosaccharides exhibit a variety of isomerism such as stereoisomerism, optical isomerism, aldose-ketose isomerism etc. These isomerisms are described as follows:

1. *Aldose-ketose isomerism*

We have already seen that in a monosaccharide either an aldehyde (-CHO) or a ketone (>C=O) group is present. The former is called as aldose while the latter is known as ketose. Glucose and fructose both have the formula $C_6H_{12}O_6$ but glucose is an aldohexose (aldehyde bearing hexose) and fructose is a ketohexose (ketone bearing hexose), so they are isomers to one another. Figure 1.2 graphically represents this isomerism.

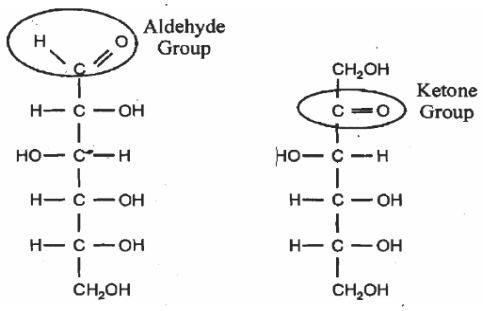

Figure 1.2: Aldose-Ketose isomerism

2. *Stereo isomerism*

Stereo isomerism occurs when the same compound due to different spatial arrangement of the groups attached to its asymmetric carbon atom exists in more

than one form.

What do we mean by an asymmetric carbon atom?

When any carbon atom of a compound is attached with four different groups or atoms, it is called as an asymmetric carbon atom. Look at Figure I .3. Why is the C-2 atom the determining factor in this configuration? The carbon atom C-2 (marked here with an *), is unique since it has four different groups (OH, CHO, H, CH_2OH) attached and is therefore an asymmetric carbon. The C-2 atom is also called the chiral carbon atom.

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According to this observation, monosaccharides may have two different forms namely 'D-sugar' and 'L-sugar', depending on their relation to the direction of the —OH group on the number 2 carbon atom as illustrated in Figure I .3. The D form will have the —OH group next to the bottom carbon atom (primary alcohol group) on the right side while the L form will have it on the left side. The simplest three carbon atoms naturally occurring glyceraldehydes (as indicated in Figure I .3) lack a plane of symmetry and exist as a pair of enantiomers (stereoisomers that are mirror image of one another) —D and L forms, which are the mirror images of each other. In this context, carbohydrates are chiral molecules since they have carbon atoms carrying four different groups. The majority of the monosaccharides occurring in mammalian metabolism are of D-configuration.

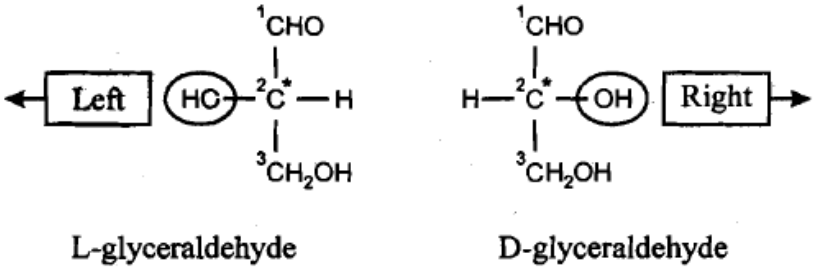

Figure 1.3: Structural isomerism of glyceraldehyde

Another type of stereo isomerism known as epimerism occurs with respect to a single asymmetric carbon atom of a monosaccharide possessing more than one asymmetric carbon atom. In Figure 1.4, there are three six carbon (hexose) sugars. Four carbon atoms (C-2, C-3, C-4 and C-5) are chiral atoms. These are structural isomers i.e. they have the same molecular formula. of $C_6H_{12}O_6$, but different structural formula, and consequently they differ in their physical and chemical properties.

Actually these isomers are formed as a result of interchange of the —OH and —H on carbon atoms 2, 3 and 4 of glucose. Glucose and galactose differ in the configuration of a single carbon atom (carbon atom 4) while glucose and mannose differ in C atom no. 2, as indicated in Figure 1.4. Compounds that differ in this manner are called as epimers.

In general, compounds with 'n' chiral carbon atoms have a maximum of 2n possible stereoisomers and half that many pair of enantiomers. For example,

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glucose an aldohexose has four chiral atoms and a total of 24=16 possible stereoisomers (8 pairs of enantiomers).

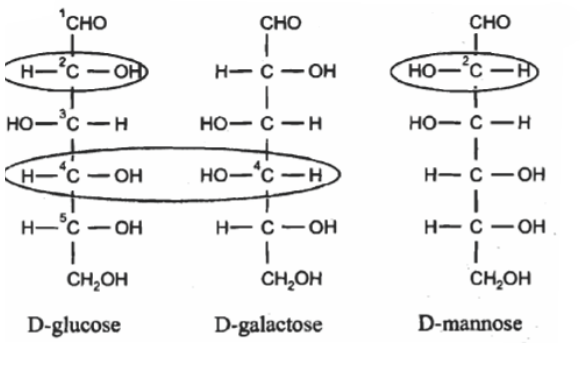

Figure 1.4: Epimerism of glucue, galactose and mannose

3. *Optical isomerism*

A compound is said to have optical activity when it rotates the plane of vibration of the rays of polarized light passing through it. This in fact results due to the presence of asymmetric carbon atoms (carbon atom in a compound attached to four different groups or atoms) in the molecule.

Two compounds having a similar molecular formula may have different optical activity. When a optically active substance rotates the plane of polarized light in a clockwise direction, it is called as dextrorotatory or 'd isomer' of the substance and when it rotates the plane of polarized light in the anticlockwise direction, it is laevorotatory or 'l isomer' of the substance.

The d and l isomers are also expressed as (+) and (—), respectively. If you look at Figure 1.3, the two forms of glyceraldehydes (D and L forms) rotate plane of polarized light in the opposite direction by the same amount. As it turns out, D-glyceraldehyde rotates the plane of polarized light to the right and is therefore, dextrorotatory, labeled as (+).

4. *Cyclic form of monosaccharides*

Aldohexoses (eg. glucose and galactose), ketohexoses (eg. fructose) and aldopentoses (eg. ribose) in solution undergo cyclization when treated with equivalent amounts of alcohol and form a hemiacetal or a hemiketal in the aldose or ketose, respectively, linking the carbonyl carbon atom with the other carbon atom by a C—O—C linkage.

This results in ring formation that is either a six membered ring with one oxygen atom (for aldohexoses) or five membered ring with one oxygen atom (for ketohexoses). The former is known as pyranose and the latter as furanose, as illustrated in Figure 1.5.

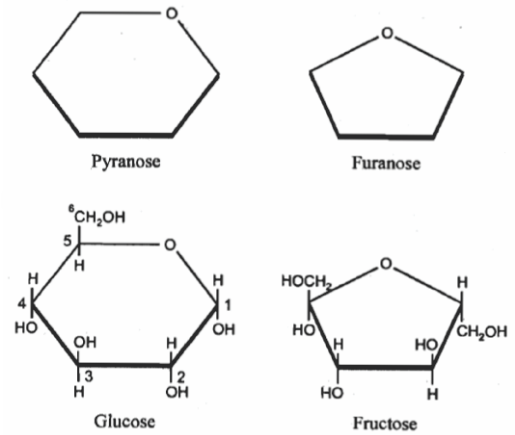

Figure 1.5: Ring structure of monosaccharides

Glucose, like all other monosaccharides, exists in two forms; the open chain form and the ring form, as illustrated in Figure I .6.

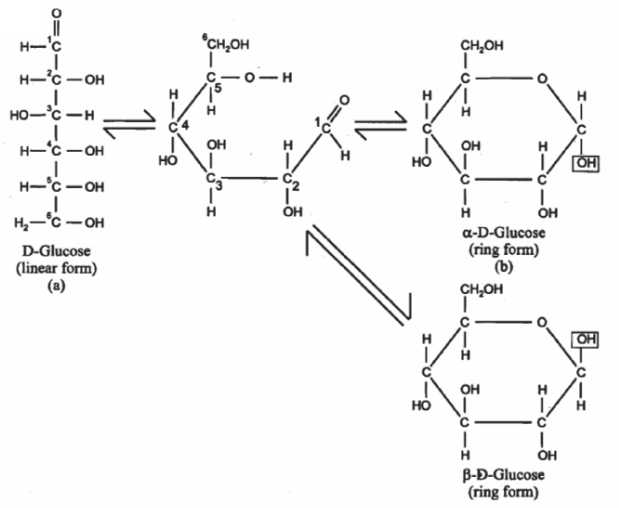

Figure 1.6: Open and ring form of glucose

The ring form has an additional asymmetric carbon atom called the 'anomeric carbon atom'. The asymmetry of C-1 makes possible the two ring forms, and p, with different optical rotations (mutarotation). Mutarotation is the process, whereby, the configuration of an anomeric carbon converts from a md and vice-versa. The a- form has the —OH group on the reverse side in comparison to ß•forrn at C-1, as highlighted in Figure 1.6.

Thus ring forrn of sugars (hemiacetals) containing an asymmetric carbon atom can exist in and ß anomers. Anomers are cyclic sugars that difer only in position of substituents at the hemiacetal cæbon; the a-forrn has the —OH group on the opposite side from the —CH_2OH; the -form has the —OH group on the same side as the —CH_2OH group, as is evident in Figures I .6 and 1.7. Glucose in its pyranose form is called as a-D Glucopyranose. Fructose in its furanose form is called "-D Fructofuranose as shown in Figure 1.8.

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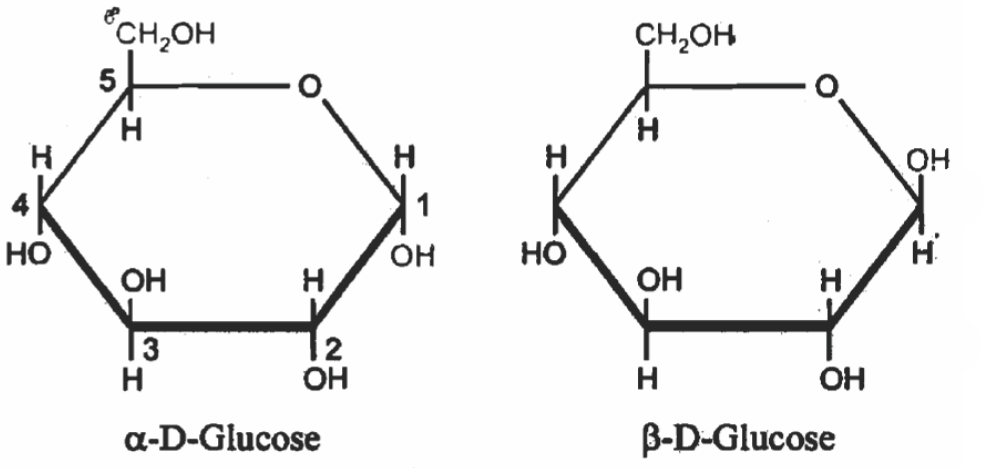

Figure 1.7: a and β anomers of D-glucose

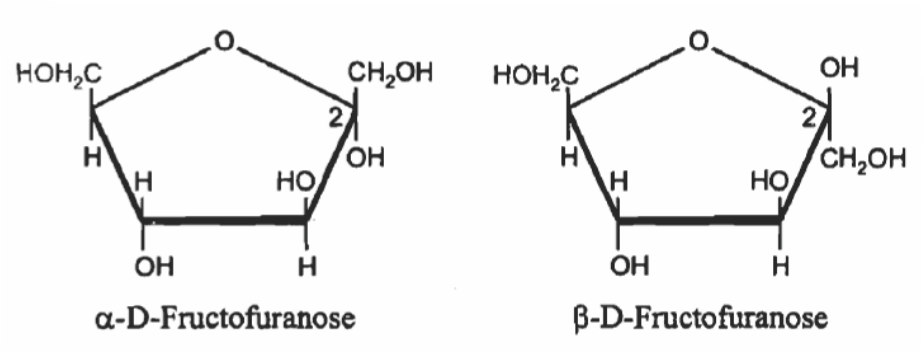

Figure 1.8: α and β anomers of Fructose

The structure or the form in which monosaccharide is present influences the properties of monosaccharides.

STUDENTS ACTIVITY - 1

1) Give examples of the following:

   a) Aldose-Ketose isomers

   .....

   .....

   b) Epimers

   .....

   .....

2) How would you classify a sugar as 'D' or 'L'?

   .....

1.5.2 Properties of Monosaccharides

Monosaccharides form water soluble, colourless, odourless, sweet crystals and show specific reactions of the aldehyde. or ketone and hydroxyl groups. which help to identify these compounds. .

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A. *Etherification*

As monosaccharides possess hydroxyl groups (—OH), they form esters with acids. Phosphoric acid esters of glucose and früctose have a great significance in nutritional biochemistry, as these are the metabolic intermediates of glucose or fructose. For example; Fructose-I, 6-bisphosphate.

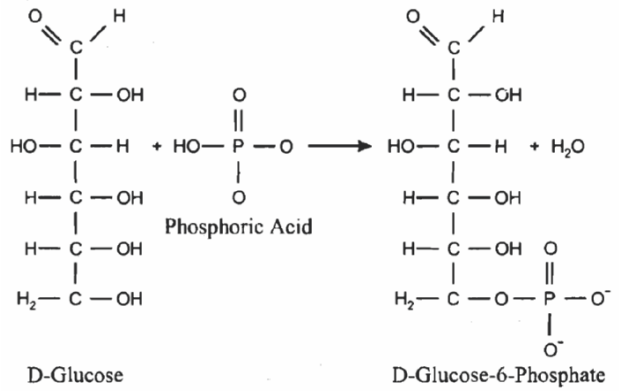

Figure 1.9: Esterificatio of glucose to Glucose-6-Phosphate

B. *Oxidation reactions*

Mild oxidizing agents such as sodium hypoiodite (NaOI) and bromine water oxidize aldoses to aldonic acids when —CHO (aldehyde group) of the aldose is converted to —COOH (carboxylic group). Glucose is oxidized to gluconic acid as shown in Figure 1.10.

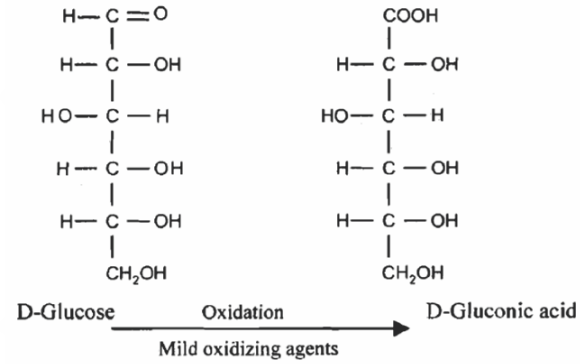

Figure 1.10: Oxidation ofglucose to gluconic acid

On the other hand, strong oxidizing agents,' such as .nitric acid oxidizes aldose to glucaric acid (aldaric acid). See Figure 1.11 for reaction. Here both —CHO and —CH_2 OH are oxidized to —COOH. Glucose is oxidized to glucaric acid. As ketoses

do not undergo oxidation, this reaction may be used to distinguish an aldose from a ketose.

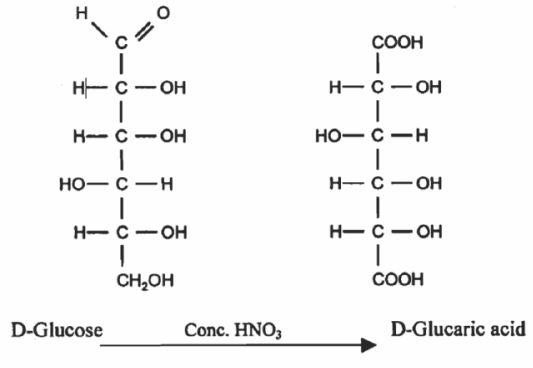

Figure 1.11: Nitric acid oxidation of glucose to glucaric acid

Dilute hydrochloric acid (HCI) oxidizes only the terminal —CH_2OH group with the formation of uronic acid. Glucose is thus oxidized to glucuronic acid.

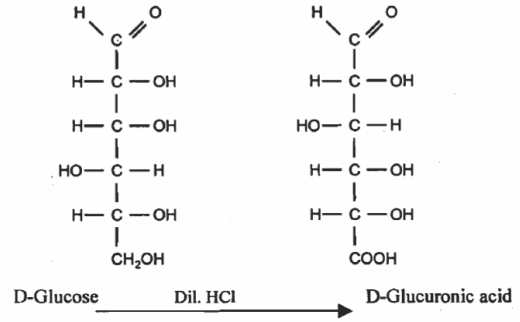

Figure 1.12: Oxidation of glucose to glucuronic acid

This is the basis of Fehling's test and explains the term reduciny sugar. In Fehling's test, the free sugar group (aldehyde or ketone) reduces the Cu + (cupric) ions in an alkal ine environment to form Cu_2O (cuprous oxide) and the sugar is itself oxidized to a carboxylic acid (mixture of sugar acids). The Cu_2O is red and precipitates. Carbohydrates that react in basic solution with oxidizing agents are classified as reducing sugars. In basic solution, all monosaccharides whether aldoses or ketoses, are reducing sugars. Simple sugars can act as reducing agents because the aldehyde or ketone group is readily oxidized to carboxylic acid.

C. *Reduction reaction*

Aldoses and ketoses are reduced to the corresponding polyhydroxy alcohols by sodium borohydride, sodium amalgam, etc. So, in this reaction, as given in Figure 1.13 glucose is reduced to glucitol (sorbitol).

Polyhydric alcohols are also called sugar alcohols and are the hydrogenated forms of the aldoses or ketoses. Glucitol, also known as sorbitol, has the same linear structure as the chain form of glucose, but the aldehyde (—CHO) group is replaced with a —CH_2OH group (at C-1).

Sugar alcohols have about half the calories of sugars and are frequently used in low-calorie or "sugar-free" products.

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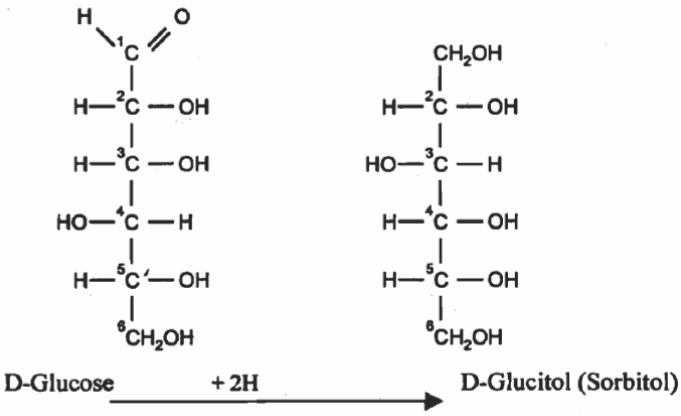


Fiiure 1.13: Reduction reaction of glucose

D. *Osazone reaction*

When a monosaccharide (or a reducing disaccharide) is heated with phenylhydrazine (a mild oxidizing agent) in acetic acid, the carbonyl group of the monosaccharide reacts with phenyl hydrazine to form a compound known as osazone. Osazones have characteristic yellow or orange microscopic crystals of specific melting points. This reaction can thus"be utilized to identi$' different sugars.

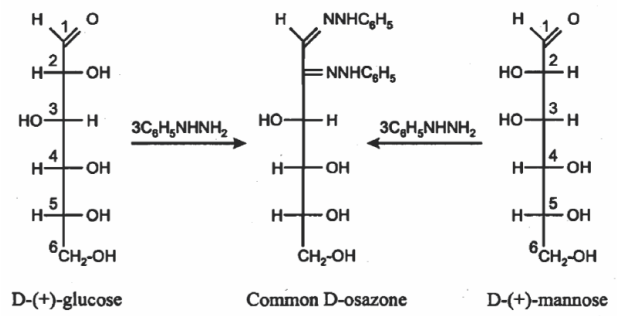


Figure 1.14: Formation of osazone

E. *Furfural formation*

When treated with concentrated mineral acids, monosaccharides are transformed to a class of compounds known as furfurals.

STUDENTS ACTIVITY - 2

1) Give any one test to distinguish a reducing sugar from a non-reducing sugar.

.....

.....

.....

1.6 OLIGOSACCHARIDES

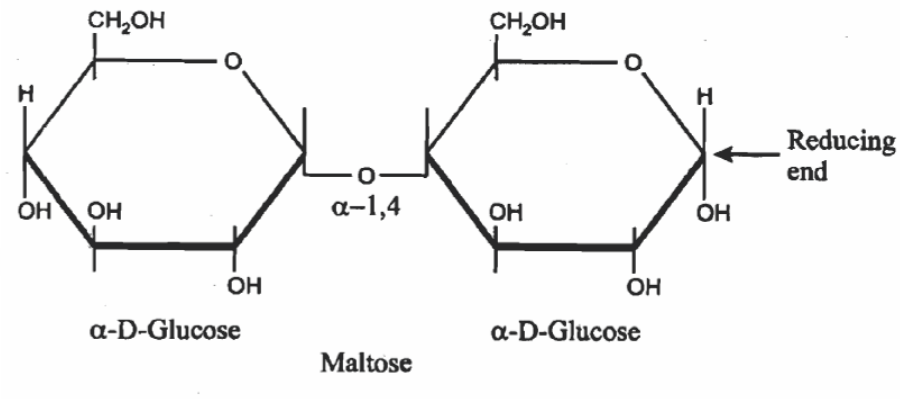
Oligosaccharides contain 2 to 6 monosaccharide units joined by a linkage knoum as glycosidic (acetal) linkage. On the basis of number Of monosaccharides present, these are divided into disaccharides, trisaccharides etc. Disaccharides are the most important compounds in this group. These consist of two monosaccharide units/molecules joined by a glycosidic linkage. Depending on the nature of monosaccharides present, different disaccharides are formed as highlighted in the Table 1.2.

Table 1.2: Constituents of disaccharides

Disaccharides	Monosaccharides resent
Maltose	Glucose (two molecules)W
Lactose	Glucose Galactose
Sucrose	Glucose Fructose

The glycosidic linkage or bond is formed through a reaction between a —OH group on the anomeric carbon of one unit and a —OH group contributed by the other unit. In sucrose, the second —OH group is also contributed by an anomeric carbon atom but in maltose and lactose, it is contributed by carbon atom 4. The configuration of the disaccharide glycosidic linkage depends on the anomeric form of the unit contributing the anomeric carbon atom. Thus exists a(l+2) glycosidic linkage in sucrose (carbon 1 of glucose in the alpha orientation to carbon 2 in the beta form of fructose), α (1+4) in maltose (2 glucose units joined from Cl to C4) and lactose.

The figures in the brackets indicate the position of the glycosidic linkage between the two monosaccharide units. The configuration of the glycosidic linkage affects the chemical properties of the disaccharide. Disaccharides with free anomeric carbon atoms like monosaccharides, exhibit the characteristic reduction reaction of carbonyl group with Fehling's reagent and Benedict's reagents (an alkaline solution containing a cupric citrate complex ion). Sugars which reduce these reagents are called as 'reducing sugars'. Sucrose, having no free anomeric carbon atom, does not respond to this reaction. Linkage of the disaccharides are shown in Figure 1.15.



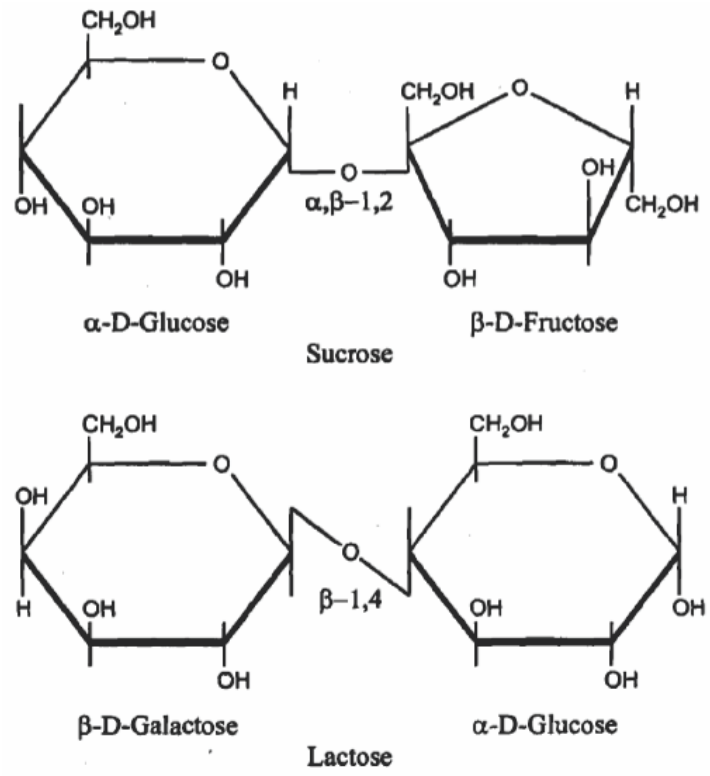


Figure 1.15: Structure of disaccharides

Besides disaccharides, some trisaccharides (possessing three monosaccharide units) are also freely available in nature. One important member of this group is raffnose, which is made up ofthe derivatives of galactose, glucose aid fructose.

Disaccharides consist of characteristic anomeric forms of monosaccharide units. Sucrose consists of α-D-glucose and ß-D-fructose, maltose consists of two α-D-glucose units while lactose consists of ß-D-galactose and a or ß-D-glucose units as shown in Figure 1.15.

Disaccharide molecules in which the second hexose unit has a free anomeric carbon atom may undergo mutarotation and the free anomeric carbon is capable of existing in a and ß forms. Sucrose, however, is an exception as it has no free anomeric carbon.

Next, let us leam about polysaccharides.

1.7 POLYSACCHARIDES

Polysaccharides are produced when many monosaccharide units are joined together by glycosidic linkage.

Chemically, polysaccharides are of two types:

a) Homopolysaccharides or homoglycans which possess only one type of monosaccharides, such as starch, cellulose etc., and

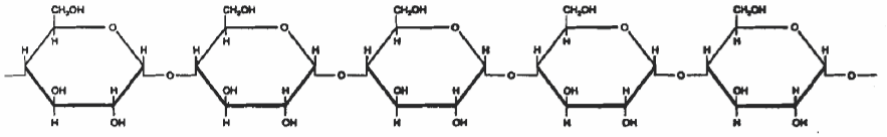
b) Heteropolysaccharides or heteroglycans which are formed by more than one type of monosaccharides, such as hyaluronic acid, heparin.

Polysaccharides		Monosaccharides present
Homopolysaccharides	Starch	Glucose
	Cellulose	Glucose
	Glycogen	Glucose
	Dextrins	Glucose
	Inulin	Fructose
	Pectin	Methyl D-galacturonate
Heteropolysaccharides	Hyaluronic acid	Glucuronic acid N-acetyl glucosamine
	Chondroitin sulphates	Glucuronic acid, 2-N-acetylamino galactose
	Heparin	Glucosamine lucuronic acid

A. *Starch*

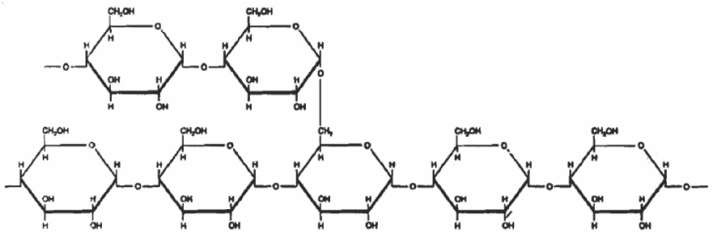
Starch is a plant polysaccharide synthesized by the plant through photosynthesis and stored mainly in grains, legumes, roots and tubers. Its molecular formula is $(C_6H_{10}O_5)n$. Starch consists of two forrns, amylose and amylopectin. Normally, 65-85% of the starch is amylopectin and only 15-35% is amylose. Amylose has a straight chain structure formed by 250-300 glucose residues linked together by glycosidic linkage as shown in Figure 1.16.

In aqueous medium, it assumes a folded conformation. Amylopectin, on the other hand, is a highly branched polymer of glucose. In amylopectin molecule, branching occurs at intervals of 24-30 glucose residues. Glucose units. of the main chain are joined by glycosidic linkage (similar to amylose) and the glucose units at the branch are joined by glycosidic linkages to the main chain (Figure 1.16). Amylopectin generally has about 3000-6000 glucose residues.



Amylose

(Glucose-a(l,4)-Glucose)



Amylopectin

(Glucose-α(l,4) and α(l,6)-Glucose)

Figure 1.16: Schematic diagram of amylose and amylopectin

Starch is not soluble in water and forms colloid in aqueous medium called as

micelles. Iodine reacts with both amylose and amylopectin, but the former produces a complex that gives an intense blue colour and masks the violet colour of the complex formed by the latter. As a result, we observe a blue colour when starch reacts with iodine. This colour complex dissociates on heating and reforms again after cooling.

Starch is a non-reducing substance, as except one carbonyl group, all other carbonyl groups are involved in glycosidic bond formation. It may be hydrolyzed by boiling with hydrochloric acid or by the action of enzyme amylase ultimately to free glucose.

B. *Cellulose*

It is the main constituent of plant cell walls and the most common and abundant of the D-glucose polymers. This does not occur in the animal body. It is a homopolymer of glucose like starch, except the linkages joining the glucose units are rather than α (1→4). Hence, cellulose is made up of ß-glucose molecules linked by ß (l,4) glycosidic linkage as shown in Figure I. 17.

Due to the difference in the chemical structure, it is not acted upon by the enzyme amylase of the digestive juice. Strong hydrochloric acid hydrolyzes cellulose to glucose.

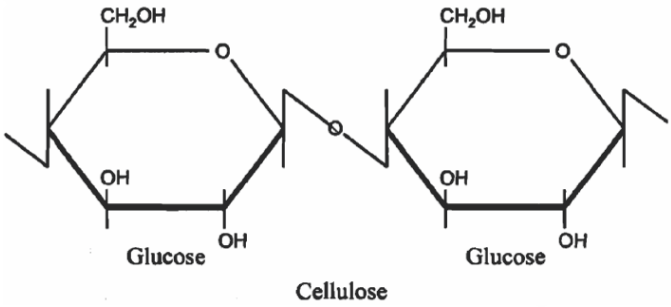


Figure 1.17: P(1,4) glycosidic linkage in cellulose

Glycogen is the storage polysaccharide found in the muscle and liver of animals and humans. It is a branched polymer having about 8 to 10 glucose units in each branch. Like amylopectin, its straight chains are formed by α (1,4) glycosidic linkages and α (1,6) glycosidic linkage exists at branch points. Thus, the chemical structure of glycogen and amylopectin are similar except that the former is more branched.

Each glycogen molecule may contain 5,000 to 10,000 glucose units. It is nonreducing, readily soluble in water and gives a red colour with iodine.

C. *Dextrins*

Dextrins are also polymers of D-glucose held by α(l, 4) glycosidic linkages. Dexfrins are, in fact, formed due to partial hydrolysis of starch by enzymes such as salivary amylase, dilute mineral Acids or heat.

Dextrins form sticky solutions in water and are frequently used as adhesives e.g. on postage stamps. They may have feeble reducing properties and when hydrolyzed,

NOTES

yield maltose and finally glucose.

D. *Inulin*

Inulin is a plant polysaccharide made up of fructose, soluble in warm water and does not give any colour with iodine. Inulins are polymers consisting of fructose units that typically have a terminal glucose. Inulins have a sweet taste and are present in many vegetables and fruits, including onions, leeks, garlié, bananas, asparagus, chicory etc.

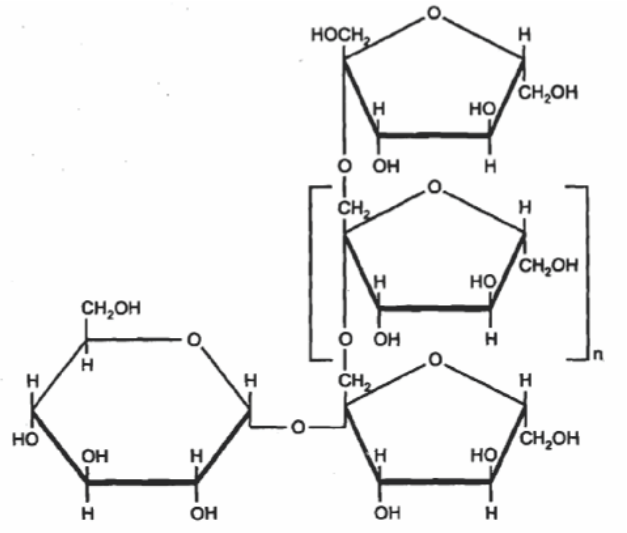


Figure 1.18: Inulin

STUDENTS ACTIVITY - 3

1) What bonds exist in sucrose, dextrins and cellulose?

……

……

2) What is a glycosidic. linkage?

……

……

1.8 LET US SUM UP

In this unit, we got introduced to biochemistry as a discipline, its scope and relevance in context to dietetics. We started our discussion on carbohydrates which form an essential part of our diet. This discussion, as you would now know, included definition and classification of carbohydrates along with their structure and properties.

We learnt about various monosaccharides, disaccharides, their structure and chemical properties. We saw how we can distinguish these through different

reactions. Finally, we discussed about various types of oligosaccharides and polysaccharides.

1.9 GLOSSARY

Asymmetric carbon atom : a carbon atom attached with four different groups or atoms.

Biochemistry : the chemistry of living organisms thåt covers all the chemical reactions occurring in our body.

Carbohydrates : polyhydroxy aldehydes or ketones and their derivatives.

Dextrin : polymers of D-glucose, formed due to partial hydrolysis of starch by enzymes.

Dextrorotatory : when an optically ,active substance rotates the plane of polarized light in a clockwise direction.

Inulin : plant polysaccharide of fructose units having a terminal glucose.

Isomer : existence of different compounds having same molecular form but different structural forms.

Monosaccharides : sugars consisting of a single polyhydroxy aldehyde or ketone unit.

Mutarotation : the process whereby the configuration of an anomeric carbon converts from a and ß and vice-versa.

Oligosaccharides : compounds containing 2 to 6 monosaccharides units joined by a glycosidic linkage.

Phytoestrogeas : group of compounds considered to offer protection against cancer.

1.10 CHECK YOUR PROGRESS

1) What do you understand by the term 'isomer'?.

2) What is meant by the following terms? Explain by giving examples.

   a) Chiral carbon atom

   b) Muta-rotation

3) What is the product formed by reduction of glucose? Also give the corresponding reaction?

4) What are polysaccharides? What are its types? Explain giving examples.

5) Differentiate between structural differences between amylose and amylopectin.

6) What is a glycosidic linkage ?

7) What bonds exist in sucrose, dextrins and cellulose?

NOTES

UNIT

2

LIPIDS AND PROTEINS

NOTES

STRUCTURE

2.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

describe the structure of lipids and classify them,

explain the chemical properties of fatty acids, neutral fats, phospholipids, steroids and eicosanoids,

illustrate the structure of amino acids, peptides, proteins and nucleic acids,

classify amino acids, peptides, proteins and nucleic acids, and

describe the chemistry of proteins and nucleic acids viz. amino acids, peptides, proteins, nucleotides and nucleic acids.

2.2 INTRODUCTION

In the last unit you were provided with a broad introduction to metabolic and nutritional aspects of biochemistry. The chemistry of carbohydrates, one of the physiologically important molecule, was focussed next. In this unit, we continue with our study of important molecules by focussing on lipids and proteins. Major

topics include structure, function and metabolism of amino acids and lipids. We would like to remind you once again that this unit is the basis for understanding the concepts related to lipids and amino acids/proteins as discussed in the Advance Nutrition and Food Science Courses. Hence, it would be a good idea to go through the units on Lipids and Proteins in these Courses together, as the information in each course would supplement each other.

2.3 CHEMISTRY OF LIPIDS - INTRODUCTION

Lipids are another important group of nutrients that should be looked into with interest by a learner of dietetics very seriously. This sub-section intends to focus on certain important aspects of lipid chemistry. These are as follows:

Chemical nature of lipids

Major classes of lipids

Important lipids of each class

Properties of fatty acids and neutral fats

At the outset, let us get introduced to lipids. Lipids are heterogeneous group of compounds occurring in both plants and animals. These are insoluble in water, but soluble in other solvents such as ether, chloroform and benzene. Because of heterogeneous nature, it is difficult to define these compounds in certain terms. However, neutral fats and oils derived from animal and plant sources are called neutral lipids, which on hydrolysis yield glycerol and fatty acid. .

2.4 LIPIDS - STRUCTURE AND CLASSIFICATION

Lipids are classified on the basis of their chemical structure as presented in Figure 2.1.

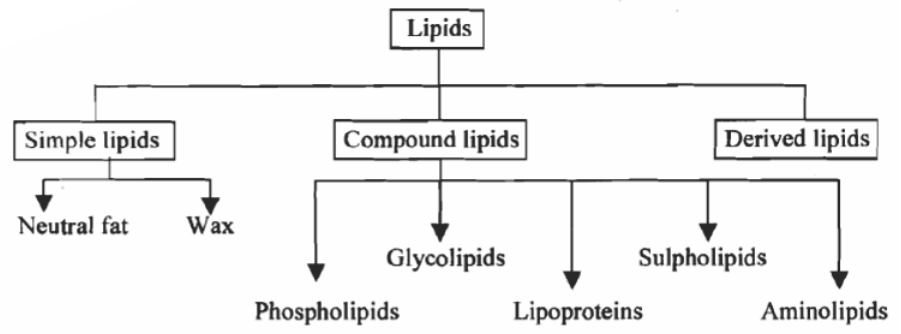


Figure 2.1: Classification of lipid

Simple lipids are esters of fatty acids with various alcohols. They include neutral fats, (which you learnt earlier, are esters of fatty acid with glycerol) and waxes (which are fatty acid with alcohol other than glycerol). Compound lipids are esters

of fatty acids containing groups in addition to an alcohol and a fatty acid. Examples include phospholipids (containing in addition to fatty acids and an alcohol, a phosphoric acid residue), glycolipids (compounds of fatty acids with carbohydrate; containing nitrogen but no phosphoric acid) etc. Derived lipids are substances derived from above groups by hydrolysis. This group includes fatty acids, glycerol, steroids, alcohols, sterols, fatty aldehydes and ketone bodies, vitamin A, D, E and K etc.

In the following section, we shall get to know a bit more about these lipids. A detailed discussion on all lipids is not included herewith, however, some important lipids are focussed. We start our discussion with the_chemistry of fatty acids.

2.4.1 Fatty Acids (Saturated and Unsaturated)

We saw earlier that fatty acids are obtained by the hydrolysis of neutral fats. Many different fatty acids occur naturally in foods. In their free form, the fatty acids have the configuration shown herewith

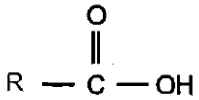


Figure 2.2: Fatty acids

where, R is a hydrocarbon chain (CH_2–CH_2–CH_2.....)

Fatty acids are grouped into two classes, saturated and unsaturated fatty acids. The terms 'saturated' and 'unsaturated' fatty acid may not be new to you. Every other day there is one report or another, highlighting the useful and/or harmful effects of these fatty acids on health.

What are these fatty acids? When a fatty acid contains one or more double bonds (-C=C-), it is said to be unsaturated, otherwise it is a saturated fatty acid (as highlighted in Figure 2.3).

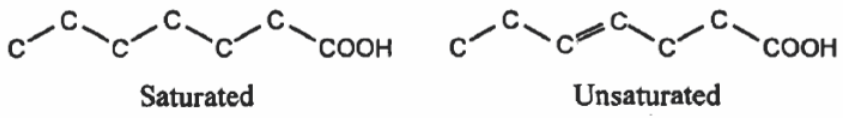


Figure 2.3: Saturated and unsaturated fatty acid

Presence of double bond in a fatty acid makes it relatively unstable to air and suitable reagents. Physical properties of the fat are also affected due to the presence of double bonds in the fatty acids. Fats rich in unsaturated fatty acids usually exist as liquid i.e. oil at room temperature while fats rich in saturated fatty acids exist in solid form. All food fats contain both saturated and unsaturated fatty acids in varying proportions. A detailed discussion on saturated and unsaturated fatty acids follows:

1. *Saturated fatty acids*

General formula of saturated fatty acids is $CH_3 (CH_2)_n$ COOH. It has a polar end with a free —COOH group and a non-polar end with a hydrocarbon chain, as can be seen in the structure of fatty acid illustrated in Figure 2.2. Fatty acids with less carbon atoms are readily miscible with water and the solubility decreases with an increase in carbon atoms. Saturated fatty? Cids with less than 10 carbon atoms are liquid at room temperature and those more are solids. Melting point of these fatty acids increases with increasing number of carbon atoms. Table 2.1 lists the common saturated fatty acids of food fats.

Table 2.1: Naturally occurring common saturated fatty acids

Common name	Molecular formula	Occurrence
Butyric acid	C_3H_7.COOH	Butter
Ca roic acid	C_5H_{11}.COOH	Butter
Ca lic acid	C_7H_{15}.COOH	Fats of plant origin
Ca ric acid	C_9H_{19}.COOH	Fats of plant origin
Lauric acid	$C_{11}H_{23}$.COOH	Palm kernel cinnamon, coconut oil
M iStic acid	$C_{13}H_{27}$.COOH	Nutme , Im kernel coconut oil
Palmitic acid	$C_{15}H_{31}$ COOH	Palm oil, animal and lant fats
Stearic acid	$C_{17}H_{35}$ .COOH	Animal fat and lant oil
Arachidic acid	$C_{19}H_{39}$.COOH	Groundnut oil
Li oceric acid	$C_{23}H_{47}$.COOH	Groundnut oil

2. *Unsaturated fatty acids*

Unsaturated fatty acids are liquid at room temperature and are characterized by having one or more double bonds. Fatty acids having two or more double bonds are called poly unsaturated fatty acih (PUFA) while those with a single double bond are called as mono unsaturated fatty acids (MUFA).

Unsaturated fatty acids show different types of isomerisms. You would realize that fatty acids with same molecular formula, as well as, same number of double bonds may differ in the location of double bonds and exhibit positional isomerism. Oleic acid has 15 different positional isomers. Again, the orientation of the two hydrogen atoms attached to the two carbon atoms joined by the double bonds may differ (geometric isomerism).

If both hydrogen atoms are placed on the same side of the double bond, cis-isomer results but if these are placed on either side of the double bond, it is a trans-isomer as shown in Figure 2.4 ("cis" comes from Latin word meaning "same" or same side; "trans" comes from Latin word meaning "across" or opposite sides). Examples of cis-trans isomer is •oleic acid and elaidic acid.

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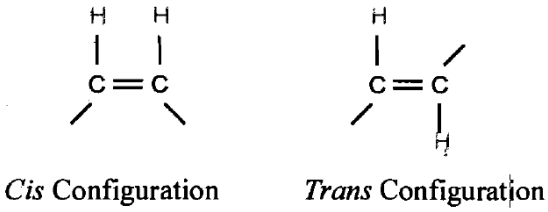

Cis Configuration *Trans* Configuration

Figure 2.4: cis and trans isomers

Carbon atoms of fatty acids are numbered from carboxyl carbon. Various conventions are used to indicate the position of double bonds in the fatty acid molecules. Like, $\triangle^9$ indicates a double bond between carbon atoms 9 and 10 of the fatty acid.

The Greek (α,P,ß, ..., 0) are used to identiö' the location of the double bonds. The "alpha" carbon is the carbon next to the carboxyl group, and the "omega" is the last carbon of the chain because omega is the last letter of the Greek alphabet. Linoleic acid is an omega-6 fatty acid because it has a double bond six carbons away from the "omega" carbon. Similarly, alpha-linolenic acid is an omega-3 fatty acid because it has a double bond three carbons away from the "omega" carbon. In other words, fatty acids known as 03 or n3 fatty acids have one double bond between carbon atoms 3 and 4, from the o carbon but no double bond between 6 and 7 carbon atoms. On the other hand, 06 or n6 fatty acids have double bonds between carbon atoms 6 and 7 from omega carbon. Table 2.2 presents the common unsaturated fatty acids found in nature.

Table 2.2: Naturally-occurring common unsaturated fatty acids

Common name	Chemical structure	Occurrence
Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	All fats
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	All fats, abundant in olive oil
Elaidic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Hydrogenated fat, margarine
Linoleic acid (LA)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7$ COOH	Mainly vegetable oils, also in some animal fats
Linolenic acid (LNA)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=$ CH- $(CH_2)_7COOH$	Mainly vegetable oils, particularly linseed oil
Arachidonic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2$- $CH=CHCH_2CH=CH(CH_2)_3COOH$	Peanut oil, groundnut oil, traces in some animal fats

EFA are essential to be provided in the diet. We must have them to live and to be healthy.

Our bodies cannot make them from other substances. We must obtain an adequate supply from external sources — from food or from supplements. Two fatty acids are essential to human health. The first is the omega 6 EFA, which is

called linoleic acid (LA). LA is abundant in polyunsaturated saffl01Åer, sunflower and corn oils. The second, known as the omega 3 EFA, is called alpha-linolenic acid (LNA) or ALNA. Sometimes referred to as super-unsaturated, LNA is found abundantly in flex and hemp seeds. Look at Table 2.2, for the structure of these two fatty acids.

LA and its derivatives belong to the omega 6 family of polyunsaturates. In addition to linoleic acid (LA), this family includes gamma-linoleic acid (GLA), dihomogamma- linolenic acid (DGLA) and arachidonic acid (AA).

LNA and its derivatives belong to an omega 3 family of superstaurates. Besides alpha-linolenic acid (LNA), this family includes stearidonic acid (SDA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). IfLNA is provided by foods, our cells make SDA, EPA and DHA.

STUDENT ACTIVITY - 1

1) Differentiate between saturated and unsaturated fatty acids. Give one example each of saturated, monounsaturated and poly unsaturated fatty acid.

.....

.....

2.4.2 Neutral fats

Neutral fats are esters of fatty acids with glycerol and found abundantly in nature. These are insoluble in water but readily soluble in ether, chloroform, benzene and carbon tetrachloride. They are bland, odourless substances and neutral in reaction. Neutral fats are good solvents themselves for other fats, fatty acids etc.

As glycerol has three hydroxyl groups, three molecules of fatty acids may get attached with it at the maximum. When one molecule of fatty acid binds with the glycerol, a monoacylglycerol (monoglyceride-old name) forms. Diacylglycerol and triacylglycerol form when two or three molecules of fatty acids combines with one molecule of glycerol, as indicated in Figure 2.5. Fatty acids reacting with the glycerol may be same or different.

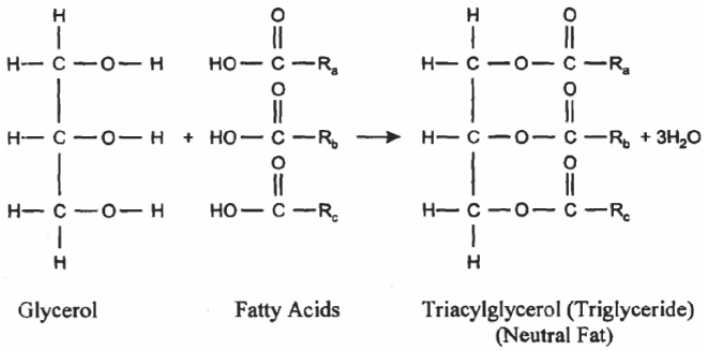

Figure 2.5: Triacylglycerol

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2.4.3 Phospholipids

Phospholipids are a heterogeneous group of compounds widely distributed in animal tissues particularly in the cell membranes.

Phospholipids are classified on the basis of alcohol present in the molecule. These are classified as:

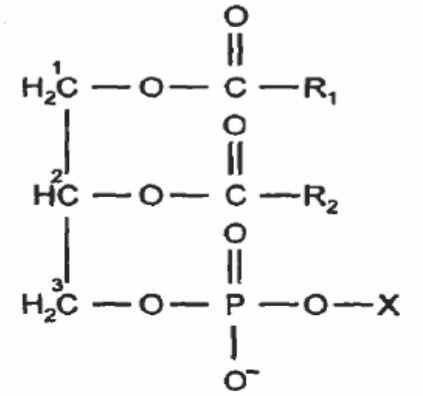
a) Glycerophosphatides, in which glycerol is the alcohol.

b) Phosphoinositides, in which inositol is the alcohol.

c) Phosphosphingosides, in which sphingosine is the alcohol.

Glycerophosphatides are esters of fatty acid with glycerol containing an esterified phosphoric acid and a nitrogen base. Brain and nervous tissues, liver, kidney, pancreas and heart contain large quantity of phospholipids.

In order to produce phospholipids (also called as phosphatides), first a phosphatidic acid is formed. In this compound, a phosphate group is present at the carbon atom 3, while two fatty acids are present in carbon atoms 1 and 2. This is, in fact, the simplest type of phosphoglyceride from which several other types are derived. The basic structure of phospolipids is very similar to that of the triacylglycerides except that C-3 (sn3) of the glycerol backbone is esterified to phosphoric acid as highlighted in the Figure 2.6.



X is usually a nitrogenous base.

Figure 2.6: Structure of phospholipids

Phospholipids exhibit important biological functions. They:

a) increase the rate of fatty acid oxidation

b) act as inorganic ion carrier across the membrane

c) help in blood clotting

d) act as prosthetic group for some enzymes, and

e) form the membrane structure.

Some important phospholipids are phosphatidylcholine (lecithin), phosphat idylserine,phosphatidylethanolamine (cephalin), phosphatidalethanolamine (plasmalogen), diphosphatidylglycerol (cardiolipin) and phosphatidylinositol. The chemical structure of phosphatidylcholine (lecithin) illustrated here in Figure 2.7, is typical of the phosphatides found in the brain, lung and spleen.

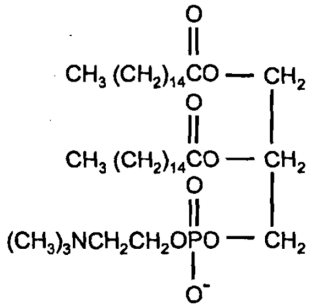


Figure 2.7: Structure of lecithin

We learnt earlier that fatty acids, steroids, hormones, vitamins A and D, etc. are categorized as derived lipids. You will find a detailed review of the chemistry of vitamins in unit 3. Hence, we shall not dwell on them at this stage. Steroids are important compounds. We shall get to know the chemistry of these compounds next, followed by eicosanoids.

2.4.4 Steroids

Steroids form a group of compounds which are often found in association with fats but structurally and functionally these are somewhat unrelated to most other lipids. Steroids are soluble in fat but resistant to sodium hydroxide i.e. these are unsaponifiable.

Basic structure of steroids consist of three cyclohexane rings (1, 2, 3 rings) and one cyclopentane ring (ring 4) as in the Figure 2.8. The parent compound of steroids is called as cyclopentano-per-hydrophenanthrene that has two methyl side chains typically at positions 10 and 13 constituting carbon atoms 18 and 19 in most steroids.

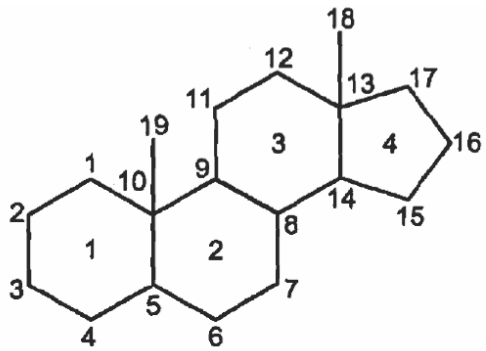


Figure 2.8: Basic structure of steroids

Steroids are of many types. These are given below:

a) Sterols : Cholesterol, ergosterol.

b) Bile acids : Glycocholic acid, taurocholic acid.

c) Hormones : Testosterone, estradiol, corticosterone.

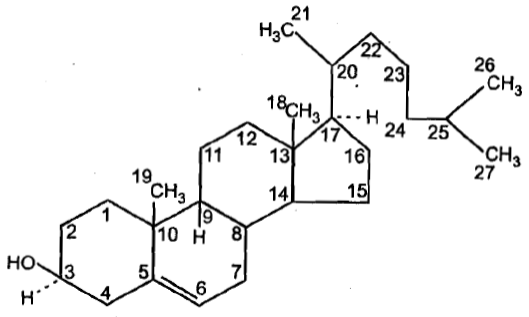
d) Vitamin : Vitamin D_2 and D_3

e) Cardiac glycosides : Stropanthin

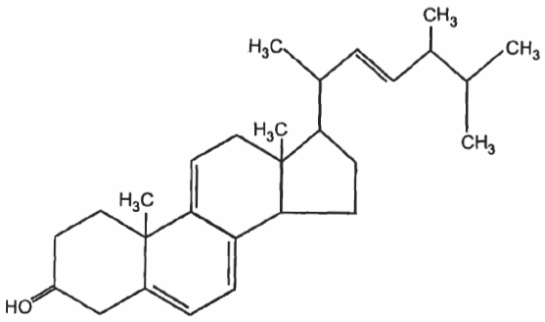
f) Saponins : Digitonin

Steroids containing an alcoholic group at C-3 position and a side chain of 8 to 10 Lipids and Proteins carbon atoms at C-17 are called as 'sterols'. The best known examples are cholesterol (yes, the much talked about compound because of its association with atherosclerosis) and ergosterol (which is a precursor of vitamin D) as illustrated in Figure 2.9 (a) and (b). Sterols, such as cholesterol, are alcohols with the cyclopentanophenanthrene ring system (atoms 1 through 17 in the Figure 2.9 (a)). This substructure is also found in steroid hormones such as testosterone (refer to Figure 2.10), progesterone and cortisol.

Cholesterol is considered an alcohol because it has a hydroxyl group (—OH) in position 3 of the ring system. While cholesterol is most abundant in the brain, nervous tissue, adrenals and skin, ergosterol is mainly found in yeasts, moulds etc. Sterols of vegetable origin are called "phytosterols". They have the same basic structure as cholesterol, but differ in the side chains attached to carbon 17. Phytosterols, such as stigmasterol from soybean oil, are of current interest because they lower blood cholesterol levels.



(a) Cholesterol ($C_{27}H_{45}OH$)



(b) Ergosterol

Figure 2.9: Structure of sterols

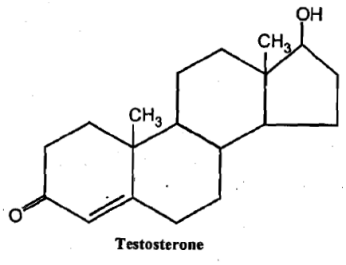


Figure 2.10: Structure of steroid hormone

2.4.5 Eicosanoids

Eicosanoids are the local hormones formed by body tissues during self-healing responses to stimuli. Eicosanoids are a family of compounds derived from poly unsaturated eicosanoic acids. They are produced from arachidonic acid, a 20-carbon poly unsaturated fatty acid. Eicosanoids comprise the prostanoids, leukotrienes (LTs) and lipoxins (LXs). Prostanoids include prostaglandins (PGs), prostacyclins and thromboxanes as shown in Figure 2.11. You will realize that prostaglandins and related compounds are collectively known as eicosanoids. Let us learn about these different eicosanoids.

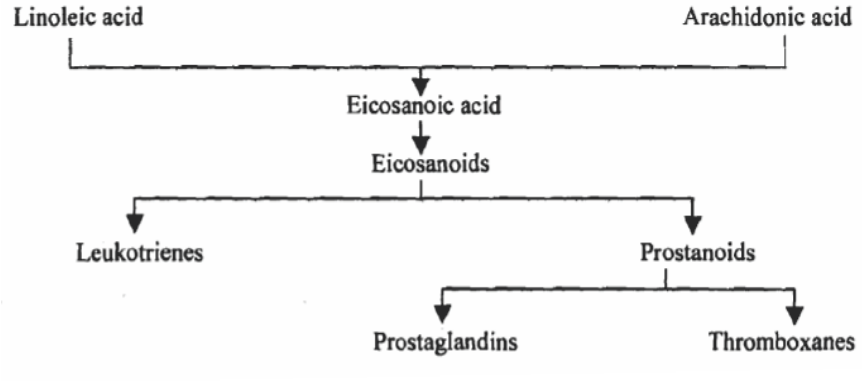


Figure 2.11: Classification of eicosanoids

1. *Prostaglandins*

Prostaglandins belong to a subclass of lipids known as the eicosanoids because of their structural similarities to the C-20 polyunsaturated fatty acids, the eicosanoic acids. Prostaglandins are known to occur in almost all tissues in very small quantities and have important physiologic and pharmacologic activities. These are synthesized in vivo by cyclization of arachidonic acid, which is either derived from dietary linoleic acid or ingested as such, to form a cyclopentane ring. There are three fatty acids known as eicosanoic acids (characterized by the number of double bonds present in the structure), which by joining with the cyclopentane ring, gives rise to three groups of prostaglandins viz. POI, PG2, PG3. These are listed in Table 2.3.

Table 2.3: Eicosanoic acids

Name	Molecular formula	Position of unsaturation	Food source
Timnoionic acid	$C_{19}H_{33}$ COOH	5,8,11,14,17	Fish oil
Clupanodonic acid	$C_{12}H_{32}$ COOH	7,10,13,l6,19	Fish oil
Cervonic acid	$C_{21}H_{31}$ COOH	4,7,1O,13,16,19	Fish oil

Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins. Of the six types of prostaglandins known, the primary types are prostaglandins E and F. All prostaglandin E types have a keto group in position 9 of the structure but all prostaglandin F types have a hydroxyl group in that position as highlighted in Figure 2.12.

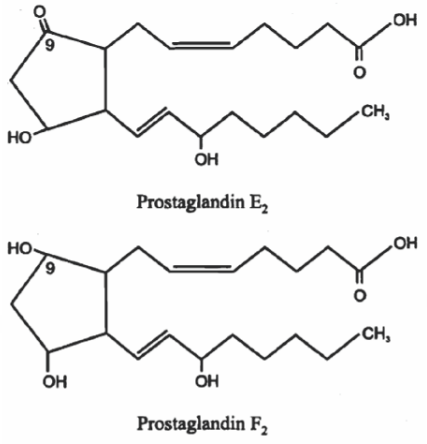


Figure 2.12: Prostaglandins E and F type

2. *Thromboxanes*

Thromboxanes are the second group of eicosanoids, first discovered in platelets and found to have a cyclopentane ring in the structure that is interrupted with an oxygen atom, as highlighted in the Figure 2.13.

Different types of thromboxanes (A, B, etc.) in each series (1,2,3) occur due to the variation of the substituent groups attached to the ring. coo-

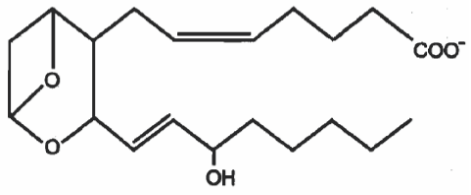


Figure 2.13: Thromboxane

3. *Leukotrienes*

Leukotrienes are the third group of eicosanoids, first discovered in leukocytes.

These are characterized by the presence of three conjugated double bonds. Figure 2.14 presents the structure of leukotriene.

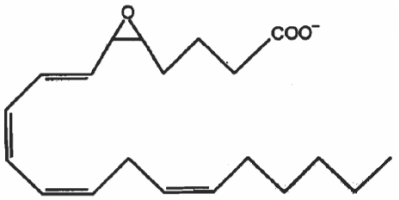


Figure 2.14: Leukotriene A_4

2.5 CHEMICAL PROPERTIES OF FATTY ACIDS AND NEUTRAL FATS

Fatty acids and neutral fats respond to different chemical reagents in different ways depending on the nature of these substances. Some of the important reactions of both of these groups of compounds are discussed briefly here. We start with the chemical properties of fatty acids.

2.5.1 Chemical Properties of Fatty Acids

This section is a review of the chemical properties of fatty acids, which include esterification, hydrogenation, halogenation etc.

Recognition of these properties is useful in understanding fatty acids. Let us learn about these properties then,

1. *Esterification*

Like any other organic acid, fatty acids also form esters with various alcohols. An alcohol such as glycerol is reacted with fat or oil to produce esters such as mono- and di-acylglycerols, Using the esterification process, edible qcids, fats and oils can be reacted with edibWalcohols to produce useful food ingredients that include many of the emulsifiers such as mono and diglycerides, lecithin etc

2. *Soap formation*

When fatty acids react with alkalies, metallic salts of fatty acids commonly called as 'soaps' are formed. Potassium soap of fatty acids is more water soluble than sodium soap.

3. *Hydrogenation*

When exposed to hydrogen at high pressure and temperature in presence of Ni or Pt catalyst, an unsaturated fatty acid (containing a double bond) accepts the hydrogen at the double bonds and is converted to a saturated fatty acid as shown herewith.

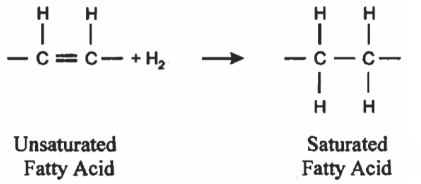


Figure 2.15: Hydrogenation reaction

Generally speaking, hydrogenation is used to change liquid oil into a semisolid or solid fat at ambient temperatures to enhance oxidative stability.

4. *Halogenation*

Fatty acids accept chlorine and iodine at the double bonds when treated with reagents such as iodine monochloride and a fatty halide results.

2.5.2 Chemical Properties of Neutral Fats

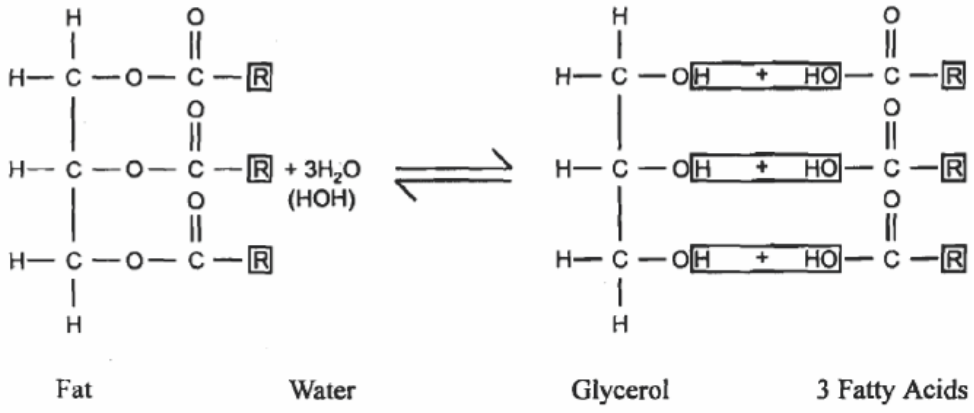
The chemical properties of neutral fats are highlighted in this section. These include:

1. *Sapon?fication*

Fats when boiled with alcoholic solution of NaOH or KOH undergo hydrolysis into glycerol and fatty acids and the latter form soaps with Na or K. The reaction is known as 'saponification'.

2. *Hydrolysis*

Fats when boiled with water at 220^{O}C under pressure in an autoclave, undergo hydrolysis to first form a.diglyceride and then ultimately glycerol and fatty acids are formed as illustrated in the Figure 2.16.



Side churns are represented by R

Figure 2.16: Hydrolysis of a fat molecule to yield glycerol and fatty acids

3. *Hydrogenation*

Hydrogenation, you learnt, is the process of turning liquid oil into solid fat. In presence of finely ground Ni or Pt catalyst and at 150-220^{o}C, glycerides of unsaturated fatty acids can be saturated by the action of hydrogen.

Partial hydrogenation produces margarines, shortenings, shonening oils, and partially hydrogenated vegetable oils. These products contain large quantities of trans-fatty acids and other altered fat substances, some of which are known to be detrimental to health because they interfere with the normal biochemical processes. Trans fatty acids are considered even more harmful than saturated fatty acids.

4. *Rancidity*

This results from the formation of aldehyde due to the oxidation of unsaturated glycerides or by the liberation of fatty acids due to hydrolysis.

In autoxidation, oxygen reacts with unsaturated fatty acids. Initially, peroxides are formed, which in turn, breakdown to hydrocarbons, ketones, aldehydes and smaller amounts of epoxides and alcohols.

The result of the autoxidation of fats and oils is the development of objectionable flavours and odours characteristic of the condition known as oxidative rancidity.

STUDENTS ACTIVITY - 2

1) What are neutral fats? Give its any two importânt properties.

.....

.....

2) What are the precursors of prostaglandins in the body?

.....

.....

3) Mention the significance/usefulness of the esterification property of fatty acids.

.....

.....

2.6 CHEMISTRY OF PROTEINS AND NUCLEIC ACIDS

Proteins are the third group of macronutrients and much attention should be given to these extremely important compounds. Nucleic acids are the main constituents

of gene and are responsible for protein synthesis. You being a student of dietetics should have some idea of such an important group of compounds that controls our activities.

NOTES

This sub-section will focus on the following aspects of protein and nucleic acid chemistry:

Definition of amino acids, peptides, proteins and nucleic acids

Classification of amino acids, peptides and protein

Structure of proteins

Physico-chemical properties of ahino acids and proteins

Biologically important peptides

Structure and classification of nucleic acid

Introduction to proteins and nucleic acids

Protein is derived from a Greek word "PROTOS" which means "Pre eminent' "first or foremost" or primary matter. This name was suggested by Berzilius to Dutch Scientist, Gerard Johanan Mulder, who coined the term protein in 1838. Earlier, protein was described as albuminous material, in that, it resembled albumin or the white of egg.

Proteins are compounds of carbon, hydrogen, oxygen and nitrogen. On an average, proteins contain 16% nitrogen. Most roteins also contain sulphur and some roteins contain iron, copper, hosphorus and zinc. roteins are, in fact, olymers consisting of chains of monomeric units. The chains are essentially linear and contain no branches. The monomeric units of roteins are amino acids (which you may already know are the building blocks of the body) and the linkage between two amino acids is called a eptide bond. We learn about the amino acids and the eptide bond in a little while from now.

A typical protein contains about 300 amino acids and proteins have a molecular weight ranging from to several million daltons. Proteins are essential constituents of living organisms and found in all plant and animal foods.

Next, let us get to know what are nucleic acids?

Nucleic acids, like proteins, are high molecular weight polymers present in all living cells. The two major classes of nucleic acids are DNA and RNA. Surely, you are aware of these nucleic acids. The monomeric units of nucleic acids are called as nucleotides. Complete hydrolysis of nucleic acids yields heterocyclic nitrogenous bases, five carbon sugars and phosphoric acid molecules. Their main function is storage and transmission of genetic information.

With a basic introduction to proteins and nucleic acids, let us get to know more about amino acids, proteins and nucleic acid with respect to their structure, classification and properties. We shall begin with amino acids

NOTES

2.7 AMINO ACIDS - STRUCTURE, CLASSIFICATION AND PROPERTIES

Amino acids are the building blocks of proteins. All proteins consist of a sequence of amino acids, which are compounds containing an amino group (—NH_2) and a carboxyl group (—COOH) as indicated in the general structural formula in the Figure 2.17. Normally 20 different 2-amino acids or a-amino acids (amino acids having —NH_2 group at carbon 2) are found in proteins, though very recently two more amino acids are also found to be present in some proteins. All the amino acids can be represented by a general structural formula,

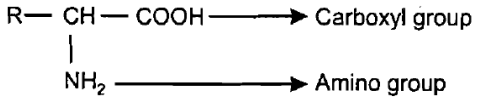


Figure 2.17: General structural formula of amino acids

in which R (the side chain) is a variable factor that changes from amino acid to amino acid, giving a character specific to a particular amino acid

2.7.1 Classification of Amino Acids

You would realize that amino acids are classified into four broad groups according to the nature of the side chain (R group). These are:

a) amino acids with non polar side chain

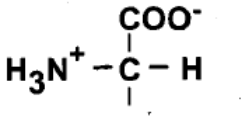
b) amino acids with polar but uncharged side chain

c) amino acids with positively charged side chain, and

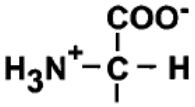
d) amino acids with negatively charged side chain.

1. *Amino acids With non polar or hydrophobic side chain*

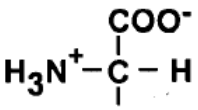
These are eight in number, as indicated in Figure 2.18, alanine, leucine, isoleucine, methionine, phenylalanine, .proline, tryptophan and valine (Ala, Ile, Leu, Met, Phe, Pro, Trp and Val). These are sparingly or less soluble in water than polar amino acids. Hydrophobicity increases with an increasing side chain length.



Alanine
Ala, A, (α-amino propionic acid)

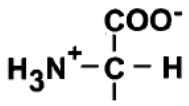


Leucine
Leu, L, (α-amino isocaproic acid)

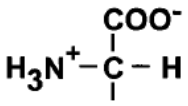


Isoleucine
Ile, I, (α-aminomethyl valeric acid)

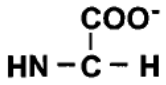
NOTES



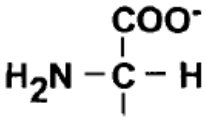
Methionine
Met, M, (a-amino-? methylthiol-n-butyric acid) Thioether gi-oup non polart



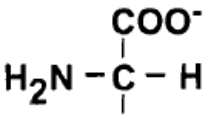
Phenylalanine
Phe, F, (a-amino-P-phenyl propionic acid)



Proline
Pro, P
(Pyrrolidone-2-carboxylic acid)



Tryptophan
Trp, W, (α-amino-ß or 3- indole propionic acid)



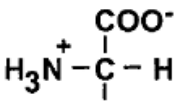
Valine
Val,V,(α-amino isovaleric acid)

Figure 2.18: Structures of amino acids with hydrophobic side chain

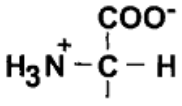
2. Amino acids with uncharged polar (hydrophilic) side chain

These have neutral polar functional groups and are able to establish hydrogen bonding with appropriate molecules, such as water. Polarity of serine and threonine is due to —OH group and of asparagine (Asn) and glutamine (Gln) is due to —$CONH_2$ (amide) groups. Sometimes glycine is also included in this group.

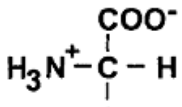
Cysteine and tyrosine possess most polar functional groups of this category, since both thiol and phenol groups may undergo partial ionization at pH values close to neutrality. In proteins, cysteine is often present in oxidized forms (i.e. cystine). Asparagine (Asn) and glutamine (Gln) also readily hydrolyze to form asparatic acid (Asp) and glutamic acid (Glu) in presence of an acid or alkali.



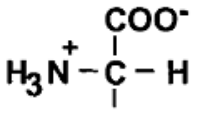
Serine
Ser, S, (α-amino β-hydroxy propionic acid)



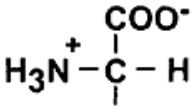
Threonine
Thr, T, (α-amino-β hydroxy Butyric acid)



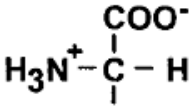
Glutamine
Gln, Q, (Amide of Glutamic acid)



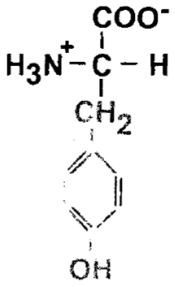
Asparagine
Asn, N, (Amide of aspartic acid)



Glycine
Gly, C, (α-amino acetic acid)



Cysteine
Cys, C, (α-amino β-mercapto propionic acid)

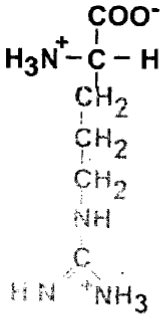


Tyrosine
Tyr, Y, [α-amino-β (p-hydroxyphenyl) propionic acid]

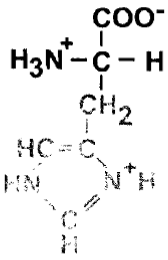
Figure 2.19: Structures of amino acids with hydrophilic side chains

3. *Amino acids with positively charged side chains (at pH close to neutral)*

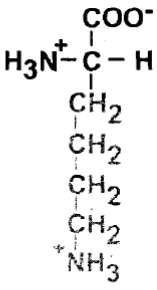
Lysine, histidine and arginine are the examples of this group. Amino group of lysine and guanidino group of arginine are responsible for +ve charges. Imidazole group of histidine is 10% protonated at pH 7.0 and 50% at pH 6.0.



Arginine
Arg, R (α-amino-δ-guanidino valeric acid)



Histidine
His, H (α-amino-β-imidazole propionic acid)



Lysine
Lys, K (α, ε diamino caproic acid)

Figure 2.20: Structures of amino acids with positively charged side chains

4. *Amino acids with negatively charged side chain*

NOTES

Aspartic acid (Asp) and glutamic acid (Glu) are the examples of this class.

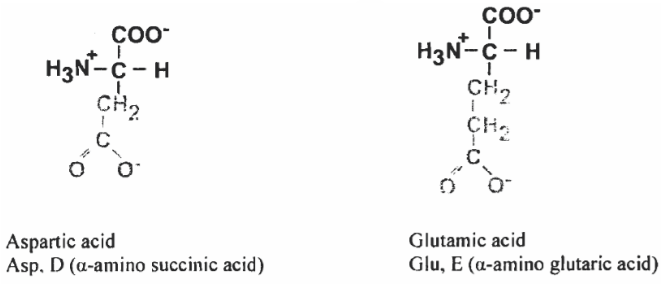


Figure 2.21: Structures of amino acids with negatively charged side chains

Complete list of these 20 amino acids is presented in Table 2.4.

Table 2.4: Amino acids commonly found in proteins

Amino acids with non polar side chain	**Amino acids with polar but unchaqed side chain**
Alanine	Glycine
Valine	Serine
Leucine	Threonine
Isoleucine	Cysteine
Proline	Tyrosine
Methionine	Asparagine
Phenylalanine	Glutamine
Tryptophan	
Amino acids both positively charged side	**Amino acids with negatively chain charged side chain**
Lysine	Aspartic acid
Arginine	Glutamic acid
Histidine	

STUDENT ACTIVITY - 3

1) What do you understand by the following terms? Explain giving examples.

   a) Proteins

   .....

   .....

   b) Nucleic acids

.....

.....

c) Nucleotides

.....

.....

2) How many amino acids are found in proteins? Name any five of them

.....

.....

.....

2.6.2 Properties and Chemical Reactions of Amino Acids

In this section we shall look at the physical and chemical properties of amino acids. We shall first study about the physical properties of amino acids

A) *Physical Properties*

1). Solubility: Amino acids are readily soluble in water, slightly soluble to insoluble in ethanol and insoluble in ether. Tyrosine is soluble in hot water but only sparingly soluble in cold water. Cysteine with difficulty is soluble in only hot water. Proline and hydroxy proline are soluble in alcohol and ether. Amino acids are generally soluble in acids and bases and form salts. Tyrosine is moderately soluble in acids and bases. Cysteine is soluble in strong mineral acids (HCI) but slightly soluble in acetic acid and dilute ammonia.

2). Melting Points: Amino acids have a high melting points in the range of 200 - 3000C.

3). Taste: Amino acids are usually sweet, tasteless or bitter. Eg. Gly, Ala, Val, Pro, hydroxy-Pro, Ser, TO' and His are sweet; Leu is tasteless, whereas, Ile and Arg are bitter in taste.

Sodium salt of glutamic acid (Monosodium glutamate, MSG, also known as Ajinomoto) is valuable as a flavouring agent for certain foods and sauces since it imparts and enhances the flavour (also for meat and meat products). MSG is being used since 1908 when a Japånese Chemist Kikunae Ikeda discovered that MSG could improve the flavour of the food products. However, tests on animals have shown that it can cause brain damage and cancer. It has not been proved to be dangerous to humans. However, food experts do not approve its use for infants and pregnant women. The acceptable daily intake of MSG for a person weighing about 60 Kg is approximately 7.2 gms.

4). Acidobasic Properties: Depending upon the pH of the solution, amino acids ionize and act as weak Bronsted acid or base (i.e. proton donor or acceptor). This

ionization facilitates their quantitative analysis. Thus, all the amino acids may be an acid, or a base.

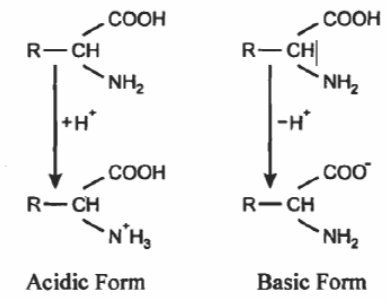


Figure 2.22: Acidobasic property of amino acids

Look at the structure of amino acid given in Figure 2.22. It is evident that amino acids contain both acidic (—COOH) and basic (—NH2) groups. Because of this unique structure, amino acids have the characteristics of an acid and a base and are capable of reacting chemically either as an acid or a base. Hence they are said to be amphoteric. They can donate or accept protons. However, when both the groups are ionized, the amino acid is said to be a zwitterion and behaves as a neutral compound. Among amino acids, neutral amino acids have a net zero charge because their structure is as illustrated in Figure 2.23. The positive and negative charge neutralizes each other. Any compound which has a net zero charge is called a 'zwitterion'.

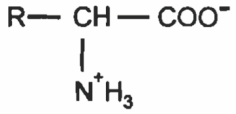


Figure 2.23: Zwitterion

Zwitterions are amphoteric molecules (no net charge on the molecule), which behave both as acid and base and the pH at which this form exists, is known as isoelectric point (pl).

Stereochemistry of Amino Acids: **Optical Properties** All amino acids (except glycine) rotate the plane of polarized light because of the presence of an asymmetric center at C-2. Both L and D enantiomers are possible for the amino acids. and L depends on the position of —NH_2 group on C-2. When the amino group is on the left of C2, it is called L form. Essentially, all the amino acids in the diet and in the body occur as the L isomer as shown in Figure 2.24.

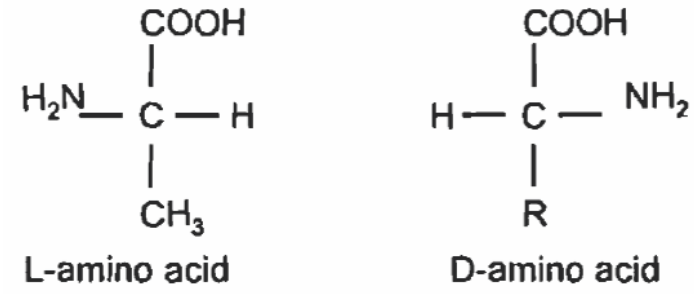


Figure 2.24: D- and G forms of a typical amino acid

Thr, hydroxyproline and hydroxylysine have 2nd asymmetric carbon also in their structure, thus four stereoisomers are possible for these amino acids. Optically active amino acids can be converted into racemic mixture by heating them with alkali.

Hydrophobicity ofAmino Acids

Hydrophobicity of amino acids and also of peptides and proteins may be determined by relative solubility of amino acid in water and in a less polar solvent (eg. ethanol) respectively. Amino acids are grouped as hydrophilic and hydrophobic depending on whether the side chains (R) like water (hydrophilic) or hate water (hydrophobic).

Ala, Arg, Asn (Asparagine), Asp, Cys, Glu, Gln (Glutamine), Gly, His, Lys, Pro, Ser, Thr are classified as hydrophilic. Sometimes, Tyr is included in this group though Tyr is more hydrophobic than hydrophilic. Amino acid side chains other than above are hydrophobic.

Absorption Spectra and Fluorescence Properties

Of all the amino acids, Try, Tyr and Phe absorb W light and have a maximum absorption at 280, 274 and 260 nm, respectively. Cysteine shows a slight absorption at 238 nm. All the amino acids absorb at wavelength near 210 nm.

Try, Tyr and Phe are the only amino acids, which show natural fluorescence. Try fluorescence remains even when it is bound in protein. Next, let us get to know about the chemical properties of amino acids.

B) *Chemical Reactions of Amino Acids*

The chemical reactions of amino acids can be divided into three classes:

i) Reactions of carboxylic group

ii) Reactions of amino group

iii) Reactions of the side chain

Here in this unit, we shall not go into each and every reaction specific to the three classes mentioned above, but few important reactions are described herewith.

i) *Reactions of Carboxylic Group*

a) Salt formation: In alkaline medium, —COOH group reacts with metal hydroxide to form amino acid salts as shown in Figure 2.25.

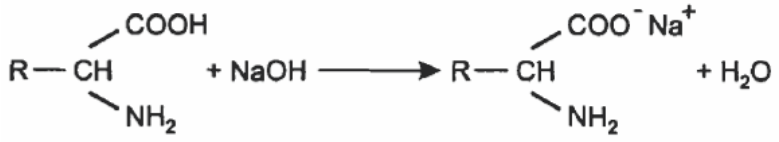


Figure 2.25: Salt formation

b) Ester formation: In presence of dry HCI, amino acids react with alcohol

to form esters. This is one of the ways of blocking —COOH group in the chemical synthesis of proteins. Figure 2.26 illustrates the ester formation.

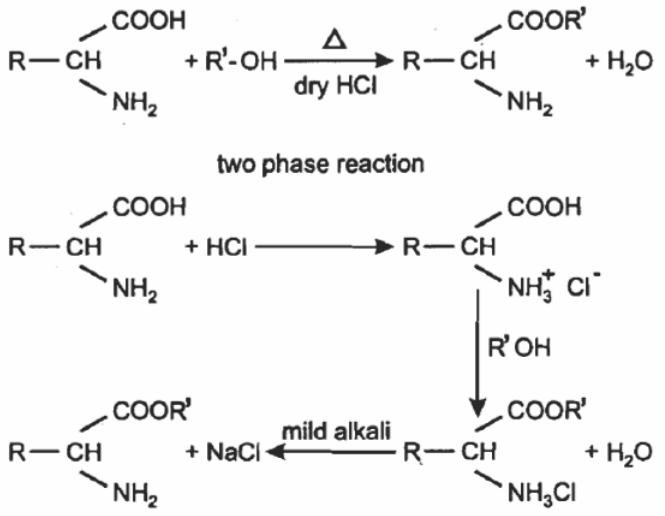
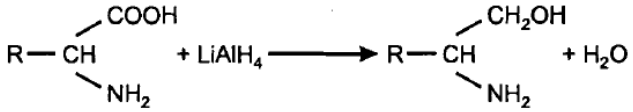


Figure 2.26: Two step reaction showing ester formation with alcohol

This ester as well as free amino acid can be reduced to corresponding alcohol with lithium aluminium hydride (LiAlh).



This is one of the ways of identifying C-terminal amino acid in the form of amino alcohol.

c) Decarboxylation: Amino acids undergo decarboxylation reaction, enzymatically or by treatment with heat, acid or alkali (barium hydroxide) to form the corresponding amines as shown in Figure 2.27.

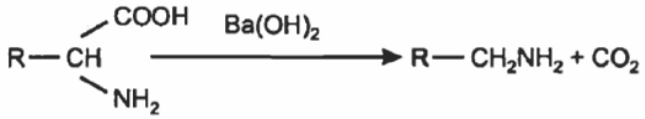


Figure 2.27: Decarboxylation reaction

In biological systems, a number of amines generated by this way have biological activity. For eg. histamine (decarboxylation product of histidine) mediates allergic, reactions, shock and stress. Similarly, histamine and tyramine (decarboxylation product of tyrosine), both possess pharmacological properties.

ii) Reactions of Amino Group

There are many reactions of amino group, namely methylation, reaction with nitrous acid, oxidative deamination of amino acids, reaction with aldehydes etc.

Some important reactions are described here.

Ninhydrin reaction: Ninhydrin is a powerful oxidizing agent. When it reacts with amino acids, oxidative decarboxylation results in the formation of $C0_2$, NH_3 and an aldehyde. The reduced ninhydrin subsequently reacts with liberated NH_3 forming a blue/purple complex which has maximum absorption at 570 nm. Proline forms a yellow colour in this reaction. This reaction is very useful for quantitative estimation of amino acids.

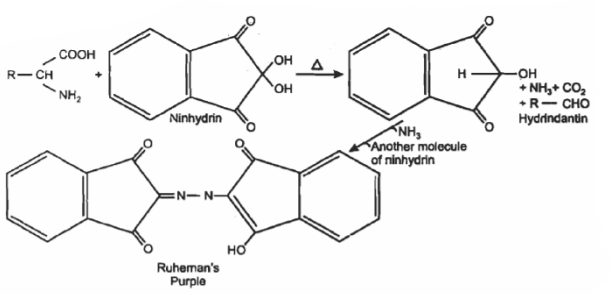


Figure 2.28: Ninhydrin reaction

Sanger 's reaction: This reaction is specific for the amino group of the amino acids or peptides. I-fluro-2,4-dinitrobenzene (FDNB) reacts with the sample resulting in the formation ofDNP- derivative, which is a yellow crystalline compound.

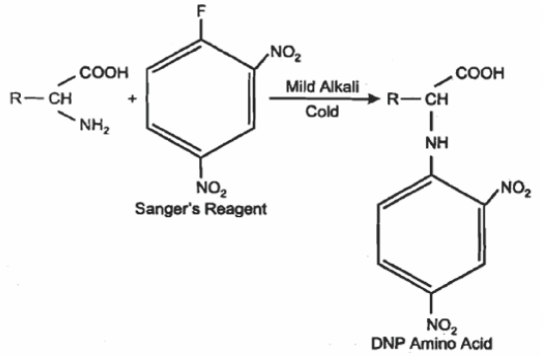


Figure 2.29: Sanger's reaction

Edman 's reaction: This is an important reaction for the identification of the amino acid at the N-terminal of the peptide. Phenylisothiocyanate reacts with the amino acid to yield a cyclic product, phenylthiohydantoin.

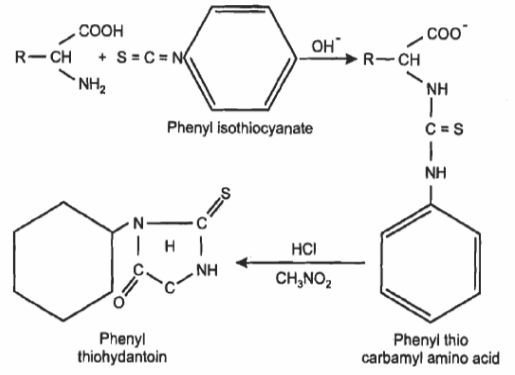


Figure 2.30: Edman's reaction

NOTES

iii) *Reactions of side chain*

The reactions of the side chain include, reaction of thiol group with iodoacetic acid, reaction of thioether group with formic acid (used for the determination of methionine as methionine sulphone) with p-chloro (p-hydroxy) mercuric benzoate (useful tool for the determination of cysteine), with Eihnan's reagent (reaction is used for the determination of cysteine) etc. These reactions are given in Figures 2.31 a,b,c,d.

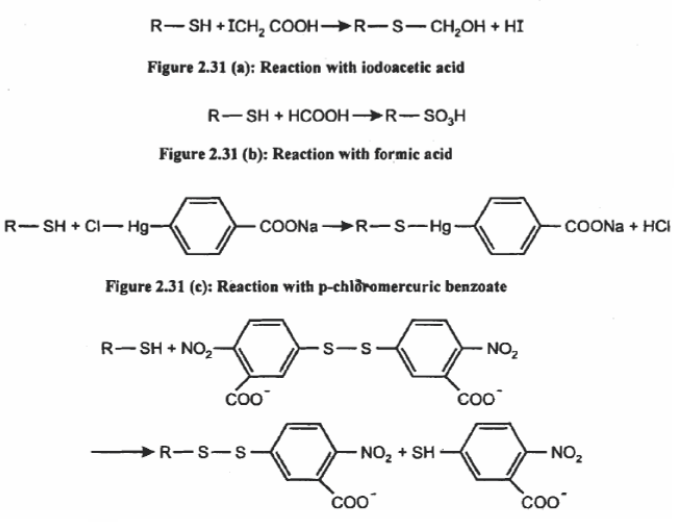


Figure 2.31 (a): Reaction with iodoacetic acid

Figure 2.31 (b): Reaction with formic acid

Figure 2.31 (c): Reaction with p-chloromercuric benzoate

Figure 2.31 (d): Reaction witbEllmanys reagent

2.6.3 Peptides — Biologically Important Peptides

What are peptides? Peptides, as you may already know, are formed by linking amino acids by peptide bond formation that involves a carboxylic group and an amino group with the elimination of water molecule as shown in Figure 2.32 (b). Figure 2.32 (a) shows the reaction of two amino acids, where R and R' are the side chains. The blue circle shows the water (H_2O) that is released, and the red circle shows the resulting peptide bond (–C (=0) NH-).

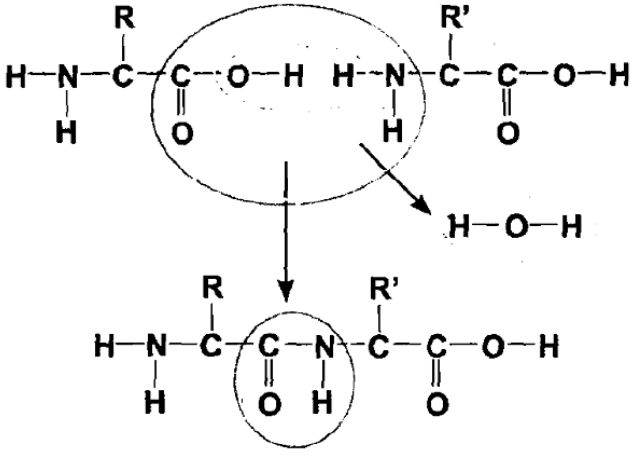


Figure 2.32(a): Peptide bond formation

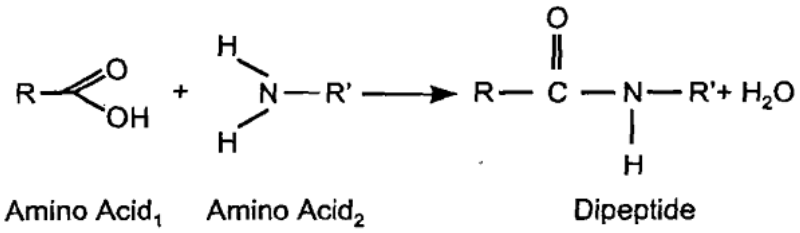


Figure 2.32(b): Peptide bond formation

Polypeptides and proteins are chains of amino acids held together by peptide bonds. Two amino acids link toform a dipeptide, three a tripeptide and so on. The peptide is called an oligopeptide when the number of amino acids in it is not more than 10. Beyond that, it is a polypeptide. Each peptide begins with an amino terminal and ends with a free carboxyl terminal.

Among the various peptides existing, some are especially important from the physiological point of view. These are:

Glutathione: It is a tripeptide made up of glutamic acid, cysteine and glycine. The compound is involved in oxidation-reduction rcactions.

Oxytocin and vasopressin: These cyclic peptide hormones of the pituitary gland consist of 8 amino acids. These aid in the ejection of milk and re-absorption in the kidney, respectively, besides other functions.

Angiotensin: Angiotensin I consisting of 10 amino acids, has slight effect on blood parameters. Angiotensin II, consisting of 8 amino acids, has significant effect on blood pressure.

Insulin: It consists of 51 amino acids, contains two polypeptide chains linked together by two —S—S— bridges. It helps in the utilization of sugar by the cells.

Having gone through section 2.6, we are now ready to embark on our journey to the world of proteins. Let's explore. But first rec pitulate what you have learnt so far. .

2.8 PROTEINS - STRUCTURE, CLASSIFICATION AND PROPERTIES

Proteins, as you may have realized by now, are the dehydration products of amino acids with each amino acid residue joined to its neighbour by a specific covalent bond. In this section, we shall unravel the mystery related to its structure, classification and properties.

2.8.1 Classification of Proteins

Proteins may be classified broadly into three groups: a) Simple proteins, b) Conjugated proteins, and c) Derived proteins. Let us get to know them better.

Simple preteins -contain only amino acids and do not have any non-protein part. Conjugated proteins have a non-protein part. Derived proteins are formed from either simple or conjugated proteins due to the unfolding of the tertiary structure or due to the cleavage of peptide bonds producing primary and secondary derivatives, respectively. These three main classes of' proteins can further be sub-classified according to their structure and properties. Table 2.4 depicts in detail the various classes of protein, their main. characteristics and few examples of such proteins.

Table 2.4: Classification bf protein

Class	**Characteristics**	**Examples**
Simple proteins		
a) Albumins	Soluble in water, coagulated by heat	Ovalbumin (egg), lactalbumin (milk)
b) Globulins	Insoluble in water, soluble in dilute salt solution	Vitellin (egg yolk), tuberin (potato) -
c) Glutelins	Insoluble in water, soluble in dilute acids and alkalis	Glutenin (wheat), oryzenin (rice)
d) Prolamins	Insoluble in water, soluble in 70% alcohol	Gliadin (wheat), Zein (maize)
e) Protamins	Soluble in water, dilute acids and alkalis	Salmine (salmon sperm)
f) Histones	Soluble in water	Globin of haemoglobin
Conjugated proteins		
a) Nucleoproteins	Consisting of simple proteins and nucleic acids, soluble in water	Nucleohistones
b) Lipoproteins	Consisting of proteins and lipids yolk, milk	Lipoproteins of egg
c) Phosphoproteins	Protein containing phosphate.	Caseinogen (milk), Ovovitellin (egg yolk)
d). Chromoproteins	Consisting of protein and the porphyrin	Haemoglobin, myoglobin
e) Flavoproteins	Protein containing riboflavin	Flavoproteins of liver and kidney
f) Metalloproteins	Proteins containing different metal ions	Ceruloplasmin (Cu), Ferritin (Fe)
g) Glycoproteins	Combination of proteins with carbohydrates	Ovomucoid (egg white), Mucin (saliva)

Derived proteins		
a) Primary derivatives		
i) Proteins	Derived by the action of dilute acids, alkalis or enzymes	Fibrin from fibrinoge
ii) Metaproteins	Derived by the action of slightly stronger acids and alkalis	Acid and alkali metaproteins
iii) Coagylated	Due to the action of heat, proteins x-rays, UV rays, etc.	Coagulated algumin
b) Secondary derivatives		
i) Proteoses	By the action of pepsin or trypsin, incoagulable by heat.	Albumose from albumin
ii) Peptones	Due to further cleavage of proteoses. Soluble in water, , incoagulable by heat	—
iii) Peptides	Compounds containing two or	Glycyl-alanine

2.8.2 Structure of Proteins

We have already seen that each molecule of protein composed of many molecules of amino acids joined by peptide bonds. But, protein is a complicated macromolecule and the complexity of its structure depends on its molecular size and shape. Considering the long peptide chains and variation in the structure of twenty different amino acids, biochemists have assigned four basic structural levels to — primary, secondary, tertiary and quaternary — as illustrated in Figure-2.33. LeLds get to know a.bit more about these structures.

i) Primary structure: It refers to the linear arrangement of amino acid residues in a given polypeptide chain linked through peptide bonds, as shown in Figure 2.33. The shortest peptide studied may have 20-100 amino acids (secretin and glucagons). Most of the proteins have 100-150 amino acids. Some rare chains may have up to 1000 amino acids. The number and sequence of amino acids determine the specificity of the protein and any disturbance in these would create a different protein. A good example is haemoglobin. A single replacement of glutamic acid with valine in position 6 of haemoglobin molecule changes the characteristics of haemoglobin resulting in the disease sickle cell anaemia.

ii) Secondary structure: It refers to a three dimensional arrangement of various atoms of the protein molecules. The polypeptide is folded systematically and the secondary conformation is stabilized due to binding forces between different segments of the peptide chain. The bonds that generally formed are hydrogen bonds, disulphide bonds, ionic bonds and hydrophobic bonds. Some proteins are found to be in a coiled form (a-helix) e.g. a-keratin and

some possess sheet like structure (P -pleated sheet) e.g. ß-keratin, collagen as illustrated in Figure 2.33.

iii) Tertiary structure: The tern refers to the tendency of the polypeptide chain {containing well defined (a-helix, ß-bends or sheets) or ill defined (random coil) secondary structure} to undergo extensive coiling or folding and produce a complex, somewhat rigid structure. Most native proteins have this kind of structure. The structure is stabilized by different types of intermolecular bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds. Due to the bonding, the distant regions of the chain are brought closer and the protein assumes a spherical, globular or ellipsoidal conformation.

iv) Quaternary structure: Many proteins, particularly enzymes, consist of several peptide chains linked by disulphide &nds. Such proteins are said to possess a quaternary structure. The most studied protein of this class is haemoglobin that consists four polypeptide chains, 2 α-chains and 2 ß-chains bound together.

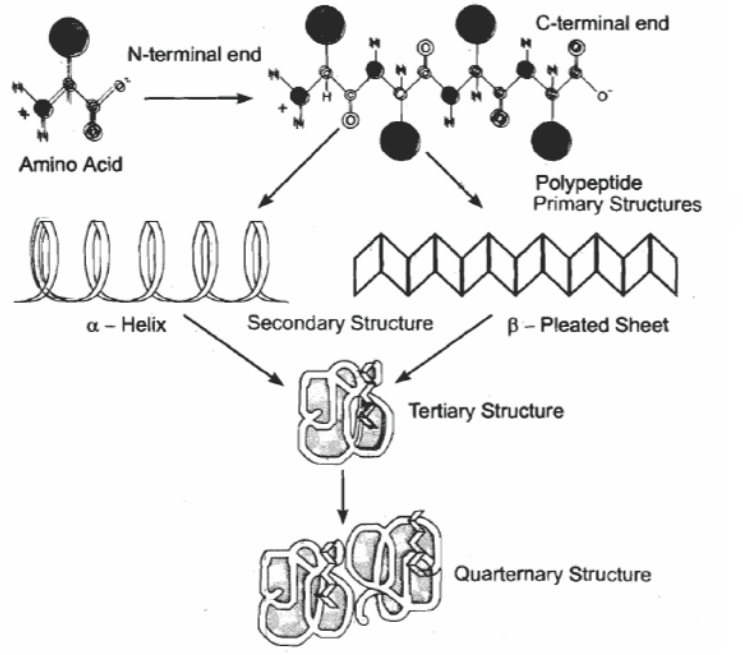


Figure 2.33: Structure of proteins

Having learnt about the structure, classification of proteins, let us finally study the properties of proteins.

2.8.3 Physico-Chemical Properties of Proteins

Proteins you learnt earlier, are high molecular compounds and exhibit characteristic properties in different environmental conditions. Some of these properties are described here.

a) Isoelectric pH: Many ionizable groups are present in a protein molecule. Depending on the pH of the medium, some of these groups act as proton

donors and some others act as proton acceptors. Thus, proteins are amphoteric compounds. At a specific pH, the protein exists as a dipolar ion (one positive and one negative ion) or zwitterion. So, at this pH the net charge of the protein becomes zero. This pH is known as isoelectric pH or pl of the protein. Having no net charge, protein does not move to either electrode in an electric field.

b) Solubility: Proteins behave differently in solution. Globular proteins are generally more soluble in aqueous medium in comparison to elongated fibrous proteins such as keratins. Solubility behaviour of proteins, however, is influenced by the nature of solvent, pH, temperature, etc

c) Precipitation: Proteins may be precipitated in different ways,

i) Isolectric precipitation: At isoelectric pH (pl), you already know, a protein does not have any net charge. So, they easily aggregate and precipitate without denaturation because of having minimum electrostatic repulsion.

ii) Salting out: Proteins in aqueous medium can be precipitated by Adding trichloroacetic acid (TCA) or perchloric acid (PCA). Salts of heavy metals, phosphomolybdic acid or phosphotungstic acid are commonly used for protein precipitation. This is known as salting out. Concentrated solutions of neutral mineral salts such as $MgSO_4$, Na_2SO_4 and $(NH_4)_2SO_4$ are also commonly used for precipitation of proteins.

iii) Action of non-polar organic solvents: A non-polar solvent like chloroform enhances the electrostatic attraction between the ions of proteins and thus facilitates their aggregation and precipitation.

The chemical properties of proteins are largely those of the side chains of the constituent amino acids. The arginine side chains, each containing a guanidine group can react with a-naphthol in the presence of an oxidizing agent such as sodium hypochlorite to produce red colour (Sakaguchi reaction). Similarly, tryptophan side chains, being indoles, can react with glyoxylic acid in the presence of concentrated sulphuric acid to produce a purple colour (Hopkins-Cole reaction). Tyrosine side chains, each possessing a phenolic group can undergo a variety of reactions. If treated with mercuric sulphate and sodium nitrate and then heated, a red complex is produced by the Millon reaction.

They also undergo the Folin-Ciocalteu reaction, if treated with tungstate and molybdate, a blue colour being formed. All of these procedures, particularly the last mentioned, can be used for the quantitative estimation of proteins, the intensity of the colour produced being dependent on the number of reacting groups present. The functional groups in amino acid side chains play an important role in the catalytic activity of enzymes: Many agents can inactivate enzymes by binding to these functional groups, for e.g. heavy metal ions bind strongly to the sulphydryl groups of cysteine residues and thus may act as poisons to a great many enzymes.

With the properties of proteins we come to the end of our journey through the world of proteins.

2.9 STRUCTURE AND CLASSIFICATION OF NUCLEIC ACIDS

NOTES

Nucleic acids are polymers of nucleotides. Nucleotides are nucleoside phosphates. Nucleosides are formed from a nitrogenous base and a pentose sugar. Thus, the nucleotides, as illustrated in the Figure 2.34, consist ofa nitrogenous base which may be either a purine (adenine or guanine) or a pyrimidine (cytosine or uracil or thymine) base, a pentose sugar (containing a five carbon atoms) which may be either ribose or deoxyribose and a phosphate. When seve neuclotides join, they form a polynucleotide.

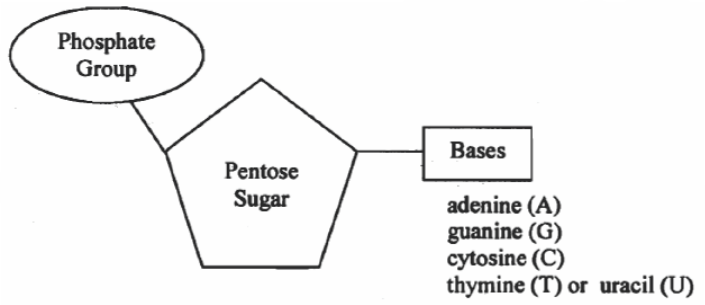


Figure 2.34: Nuclcotide structure

According to the presence of ribose or deoxyribose, they are called as ribonucleotides or deoxyribonucleotides. One of the most important naturally occurring nucloeotides is adenosine-5'-mOnophosphate (AMP). This compound together with two of its derivatives, adenosine-5'-diphosphate (ADP) and adenosine-5'-triphosphate (ATP), plays an extremely important role in the conservation and utilization of energy released during cellular metabolism. Other common nucleotides are guanosine-5'- monophosphate (GMP), cytidine-S'-monophosphate (CMP) and uridine-5'- monophosphate etc.Thus ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have ribose and deoxyribose as the pentose sugar, respectively. They also differ, to some extent, in possession of nitrogenous bases. DNA contains four types of nitrogenous bases adenine, thymine, cytosine and guanine, while RNA contains uracil in place of thymine and the three other bases as in DNA. Figure 2.35 illustrates the chemical structure of these nitrogenous bases.

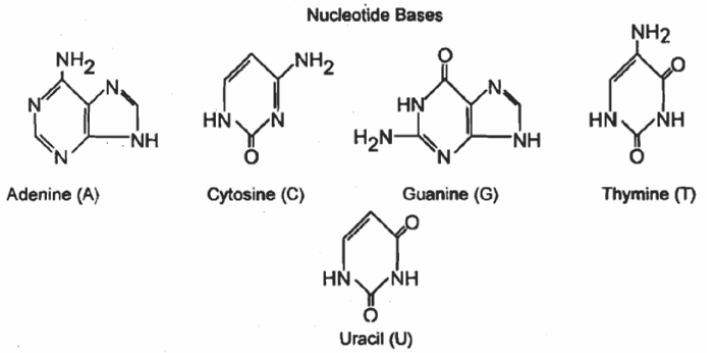


Figure 2.35: Chemical structure of nitrogenous bases

DNA is composed of two polynucleotide chains wound into a right handed helix that éoil around a central axis. The chains are held together by hydrogen bonds between the bases of two opposite chains. A critical feature of the double helix is the base- pairing relationship; adenine pairs with thymine and guanine pairs with cytosine. Thus, the base sequence of one chain matches with the sequence of the other. In simple terms, DNA forms a double helix (see Figure 2.36 (b) in which the nucleotide bases are attached to deoxyribose units linked through phosphate groups. The bases in the center of the DNA helix always oceur in complementary matched pairs, with cytosine linking to guanine and tKymine linking to adenine through hydrogen bonding (shown as dotted lines in Figure 2.36 a).

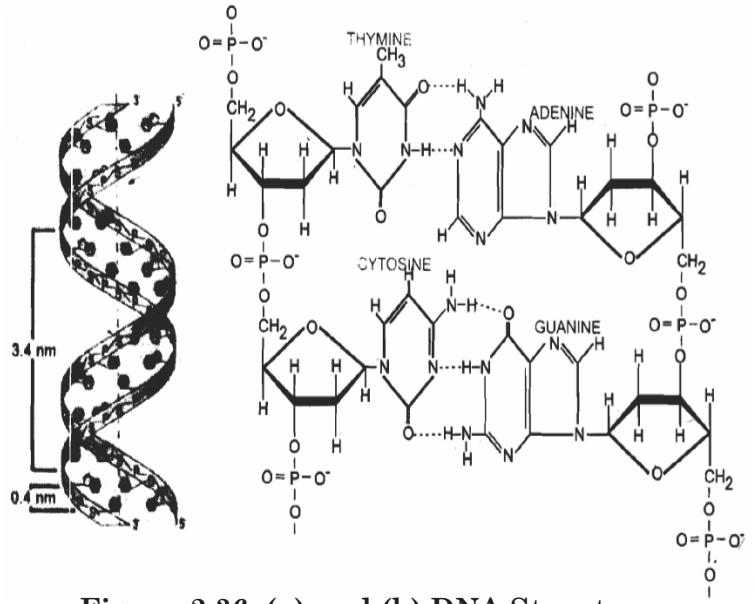


Figure 2.36: (a) and (b) DNA Structure

Structurally RNA differs significantly from DNA. Each molecule of RNA is single stranded and is made as a complementary strand of one of the two chains of DNA. RNA molecules are smaller than DNA molecules. There are three forms of RNA found in the cell, messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) having specific ftnctions during protein synthesis. You will learn about their functions in the Advance Nutrition course. .

2.10 LET US SUM UP

In this unit, we studied about chemistry of lipids, proteins and nucleic acids. We learnt the structure and classification of lipids — one of the physiologically important compounds. Then we had a look at the chemical properties of both fatty acids and neutral fats.

In the consequent sections, we learnt about proteins and amino acids, where we saw the structure and properties of amino acids and proteins. Finally, we dealt with nucleic acids and polynucleotides such as DNA and RNA, where we saw

how these essential polynucleotides are relevant to us and differ from each other structurally.

2.11 GLOSSARY

NOTES

Amino acids : building blocks of the body.

Amphoteric : amino acids having the characteristics of an acid and a base.

Cis-isomer : both hydrogen atoms located on the same side of the double bond.

Eicosanoids : local hormone formed by body tissues during self-healing responses to stimuli.

Hydrogenation : the process of turning liquid oil into solid fat.

Lipids : heterogenous group of compounds occurring in both plants and animals.

Monounsaturated : fatty acids having a single double bond.

Nucleic acids : high molecular weight polymers present in all living cells.

Neutral fats : esters of fatty acid with glycerol containing an esterified phosphoric acid and a nitrogen base.

Nucleosides : compounds formed from a nitrogenousbase and a penstose sugar.

Nucleotides : compounds consisting of a nitrogenous base, a pentose sugar and a phosphate group.

Oxidative : the development of objectionable flavours and odours

rancidity : characteristic of the autoxidation of fats and oils.

Peptide bond :linkage between carboxyl group and amino group.

Phytosterol : sterols of vegetable origin.

Polyunsaturated :fatty acid having two or morc double bonds. fatty acid

Proteins : compounds of carbon hydrogen, oxygen and nitrogen.

Rancidity : Formation of aldehyde due to oxidation of unsaturated glycerides.

Saponificatio : hydrolysis of fats when boiled with alcoholic solution of

NaOH/KOH into glycerol and salts of fatty acids (soaps).

Steroids : a group of compounds which are often found in association with fat.

Sterols : steroids containing an alcoholic group ate-3 position and a side chain of 8 to 10 carbon atoms at c-17.

Trans-isomer : both hydrogen atoms are placed on either side of the double bond.

Unsaturated fatty acid :a fatty acid containing one or more double bond.

Zwitterion : any compound which has a net zero charge.

Isoelectric pH : pH at which the net charge of the protein becomes zero.

2.12 CHECK YOUR PROGRESS

1) How are lipids classified? Give examples

2) What is the difference between the following:

   a) cis and trans fatty acids

   b) n-3 and n-6 fatty acids

3) What are essential fatty acids? Name 2 EFAs and where these are obtained from?

4) What is hydrogenation? What are the harmful health effects of partial hydrogenation of fats?

5) What do you understand by the following terms:

   a) Oxidative rancidity

   b) Saponificatio

UNIT

3

VITAMINS

STRUCTURE

3.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

Describe the chemical nature of vitamins,

Classify the different vitamins into classes to which they belong,

Illustrate their chemical structure,

Discuss the physico-chemical properties of vitamins, and

Relate the chemical structure and biochemical ülnction of the water-soluble vitamins and their metabolites and antagonists to their regulatory role in metabolism.

3.2 INTRODUCTION

Now, in this unit we shall focus on vitamins. Vitamins are another group of extremely important nutrients. These are the organic chemicals, other than essential amino acids and fatty acids, that must be supplied to the body in small amounts to maintain health.

Being a student of dietetics, it is absolutely important for you to have a comprehensive knowledge about these substances — their nature, structure and properties. You may wonder, why? Well, simply because from a nutritional

standpoint, the effect of cooking/processing on these substances will be best understood with the knowledge of the structure, properties etc. of these essential substances. In this Unit we will get to know about the structure, nature and properties of vitamins.

3.3 VITAMINS - INTRODUCTION AND THEIR CLASSIFICATION

Vitamins are organic compounds required in very small amounts by the body for growth, maintenance and sustenance of life. But, a prolonged absence of these substances from the diet leads to different generpl and specific disease symptoms about which you may already be aware of and we have also discussed about them in the Advance Nutrition Course in Units 7 and 8. Do look up these units as you read through this unit.

It was in the year 1880 when Sir Frederick Crowl and Hopkins first reported the necessity of certain accessory food factors besides the well known dietary substances for healthy living. However, it was Funk, who in 1912 first observed that diseases such as beriberi, scurvy and pellagra can be prevented by certain components of the food. He proposed the name vitamine for these food components considering that they are all amines. Afterwards, when it was realized that all such compounds do not possess nitrogen in their structures, the name was modified to vitamin.

The vitamins share a family of characteristics. They neither are catabolized to generate energy nor are they used for structural purposes. Many vitamins are, in fact, used as cofactors for enzymes and are called as coenzymes about which you will learn in detail in the next Unit. Two vitamins viz. vitamin A and D are converted to hormones. Vitamin A also functions as a cofactor in the visual cycle. These are a few functions of vitamins highlighted herewith. You will learn more about their role in Unit 10 later in this course.

Vitamins are generally synthesized by plants and found in animals as a result of food intake and also because of synthesis of some of the vitamins by gut microorganisms. All living beings not necessarily need the same number or kind of vitamins as some of these may be produced by the organism concerned. For instance, rat can synthesize vitamin C but a guinea pig or man cannot. We, human beings, in fact can synthesize only two vitamins — vitamin D and niacin.

Let us then learn about the chemistry of these vitamins. We start our discussion by first classifying them.

Classification of Vitamins

Vitamins widely vary from each other from the structural point of view. Conventionally, they are classified on the basis of their solubility in water or fat.

Accordingly, they are classified as:

Water-soluble vitamins, which include thiamin (vitamin Bl), riboflavin (vitamin B2), niacin and nicotinamide (vitamin B3), pantothenic acid (vitamin B5), pyridoxine and related compounds (vitamin B6), cyanocobalamin and related compounds (vitamin BID, ascorbic acid (vitamin C), biotin (vitamin H) and folic acid (vitamin M).

Fat-soluble vitamins, which include retinol (vitamin A), cholecalciferol (vitamin D), tocopherols (vitamin E) and phylloquinone and related compounds (vitamin K).

Based on this classification, we shall review the structure and properties of the two classes of vitamins, starting with water soluble vitamins.

3.4 STRUCTURE AND PROPERTIES OF WATER SOLUBLE VITAMINS

This section will focus only on the native structure and physico-chemical properties of some common water soluble vitamins. You will learn about their coenzyme (modified) forms in the next unit and biochemical role in Unit 10 of this Course. Food sources, daily requirements, physiology of digestion, functions, deficiency diseases, assessment procedures, concept of bioavailability etc. of these vitamins are discussed in the Advance Nutrition Course, Unit 8. Hence it is suggested that you go through the appropriate sections on Vitamins in the two courses together.

3.4.1 Thiamin (vitamin B_1)

Thiamin was first isolated in 1926 from rice polishing by Jansen and Donath. Its empirical formula was established in 1931 by Windaus and co-workers and the chemical structure of the compound was elucidated in 1936 by Williams and co-workers.

The chemical name for this water soluble vitamin is 3-[(4-amino-2-methyl-5-pyrimidinyl) methyl] - 5-(2-hydroxyethyl)-4-methylthiazolium. Do not get perturbed by this long name. You would not be expected to remember this chemical name for thiamin. However, the chemical name will help you understand the structure of thiamin. Look at the structure of thiamin given in Figure 3.1. As you would have noticed, thiamin possesses one pyrimidine and one thaizole ring that are connected by a methylene bridge (coloured red).

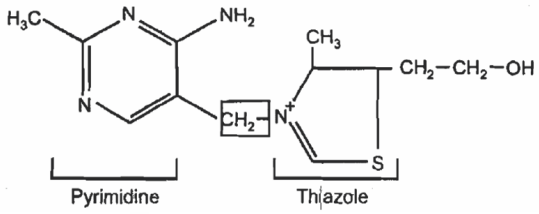


Figure 3.1: Structure of thiamin

Chemically, ring A is called as 2, 5-dimethyl-6-aminopyrimidine and ring B is called as 4-methyl-5-hydroxyethyl thiazole. The thiazole ring bears a primary alcohol side chain that becomes phosphorylated in vivo to give the thiamin phosphate esters that have the cofactor activity. Thiamin occurs in four forms: thiamin, thiamin inonophosphate (TMP), thiamin diphosphate (also known as thiamin pyrophosphate, TPP) and thiamin triphosphate (TTP), the later three of which have phosphate molecules attached to the side chain as shown in Figure 3.2, hence called the phosphorylated forms of thiamin. TTP is the most abundant form and constitutes almost 80% of total thiamin. We shall learn more about its activity in Unit 10 subsequently.

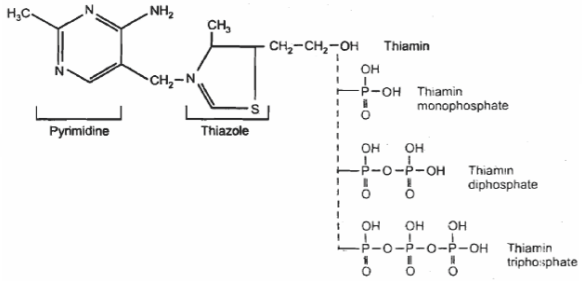


Figure 3.2: Chemical structures of thiamin, thiamin monophosphate, thiamin diphosphate and thianin triphosphate.

Properties

The important physico-chemical properties of thiamin are as follows:

a) Thiamin hydrochloride is a white, needle-shaped crystalline substance.

b) It has a characteristic smell like that of yeast. In fact, the characteristic smell of yeast is due to its content of thiamin. Thiamin has a sulfurours odour and a bitter taste. c

) The compound is readily soluble in water and slightly soluble in alcohol. This property makes it vulnerable, as thiamin in foods is easily lost during washing/soaking and other cooking procedures. Moisture greatly accelerates destruction and thus it is much less stable to heat in fresh foods than in dry foods.

d) It is stable in acid medium at room temperature but destroyed, if heated at 1200 C for 30 minutes.

e) Thiamin is readily destroyed by heat in neutral or alkaline medium. It is very sensitive to alkali and can be even destroyed at room temperature in an alkaline medium. This again is an important consideration during cooking procedures. In fact, after you read through the water soluble vitamins, you will realize that this is true in the case of most of the vitamins in this group.

f) The compound is converted to an inactive derivative-thiochrome by controlled oxidation (by the action of potassium ferricyanide in alkaline solution). Thiochrome has a strong fluorescence in UV rays

g) Thiamin, when dissolved in sodium bisulphate solution at pH 5.0 cleaves

into pyrimidine and thiazole. This property is utilized for the chemical estimation of the vitamin.

3.4.2 Riboflavin (vitamin B_2)

NOTES

Riboflavin was isolated in a crystalline form from milk by Kuhn and co-workers in 1933. Because of this, the early name of the vitamin was lactoflavin. It was synthesized by two independent groups of scientists working with Kuhn in 1935. Riboflavin has an isoalloxazine nucleus i.e. a pteridine ring with a benzene ring fused on to it. The side chain is a C5-polyhydroxy group, a derivative of ribitol, a pentahydroxy compound. Riboflavin is chemically known as 6,7-dimethyl-9-D-ribitylisoalloxazine. The structure of riboflavin is given in Figure 3.3. Once again, do not get intimidated by the chemical names of this compound. As a student of dietetics you may not be asked as such to illustrate the structure of these compounds, but surely knowledge of the structure will help you understand the compound apd its properties better.

Riboflavin has two major coenzyme derivatives, namely flavin mononucleotide (FMN) which is the active component of riboflavin and is formed by the addition of a phosphate group and flavin adenine dinucleotide (FAD) which is formed by the combination of FMN with one molecule of adenosine triphosphate (ATP). You will learn more about FMN and FAD in the next unit on enzymes and coenzymes.

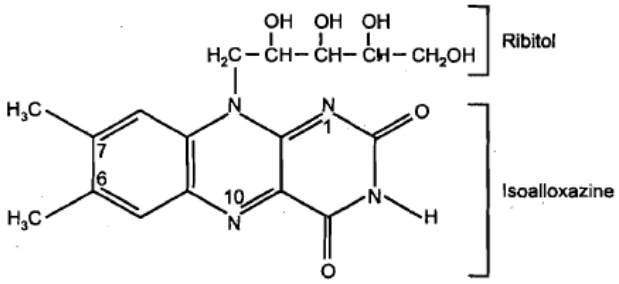


Figure 3.3: Chemical structure of riboflavi

Properties

Some of the important physico-chemical characteristics of riboflavin are as follows.

a) Riboflavin forms needle shaped orange crystals

b) It is sparingly soluble in water and ethanol but its solubility in water is much less than thiamin.

c) Aqueous solution of vitamin B2 emits a yellow-green fluorescence. To see this for yourself, empty the contents of a multivitamin capsule into a glass of water. Stir and observe the yellow-green fluorescence. Isn't it amazing

d) Though the compound is stable to boiling in acid medium, it is readily destroyed by heat in an alkaline medium. This becomes an important consideration during cooking procedure.

e) Riboflavin is sensitive to light and is destroyed if exposed to light for some time. Milk, which is rich in riboflavin, should not be exposed to sunlight for long. So next time when your milk man leaves the milk packet outside, surely you know what to do.

f) Reducing agents such as stannous chloride convert the vitamin to a colourless compound having no fluorescence

g) When an alkaline solution of riboflavin is exposed to ultra violet rays, it is converted to a compound lumiflavin which is soluble in chloroform and has a greenish yellow fluorescence in ultra violet light

h) When a neutral or acid solution of riboflavin is exposed to ultra violet rays, it is converted to lumichrome which has a slight blue fluorescence in ultra violet light.

The third vitamin in the list is vitamin B_3, i.e. Niacin. Let us get to know the chemistry of this chemical.

3.4.3 Niacin (vitamin B_3)

Though niacin or nicotinic acid has been known to the organic chemists since 1867 and Funk isolated it from yeast and rice polishing in order to identify the anti-beriberi vitamin in 1913, it was finally isolated as nicotinamide from liver by Elvehjem and his co-workers in 1937. This was actually after the discovery by Ruffin and Smith in 1934 that the crude extract of liver was effective against pellagra (you might know that pellagra is the disease condition caused due to the deficiency of niacin in the body).

Niacin or nicotinic acid is pyridine-3-carboxylic acid. It occurs naturally in the body as its amide, niacinamide or nicotinamide. Amino group substituted into carboxylic acid forms amide group. The chemical structures of niacin forms — nicotinic acid and nicotinamide is presented in Figure 3.4.

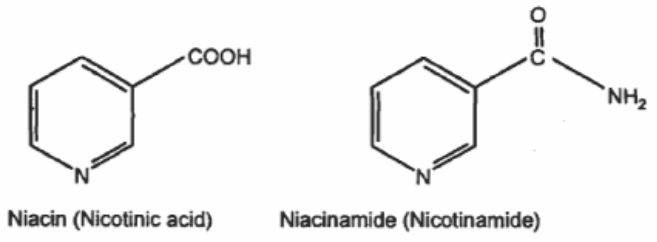


Figure 3.4: Niacin and its derivatives

Niacin is converted into the active forms nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) as shown in Figure 3.4. Both NAD+ and NADP+ function as cofactors for numerous enzymes about which you will learn in the next Unit and later in Unit 10.

What about the properties of niacin? Read and find out.

Properties

Important physico-chemical properties of niacin and niacinamide are as follows:

a) Niacin is a white crystalline solid.

b) While niacin is sparingly soluble in water, ethanol and glycerol, niacinamide is readily soluble in water.

c) Niacin is fairly heat stable and can withstand a temperature of 1200C for 20 minutes in acid or alkali. It is one of the most stable vitamins.

d) Niacinamide is converted into niacin if heated in strong acid or alkali. Next, let us get to know about pantothenic acid.

3.4.4 Pantothenic acid (vitamin B_5)

Pantothenic acid, also known as vitamin B5, is essential to all forms of life. Pure pantothenic acid was first isolated as its calcium salt from yeast by R J. Williams in 1939. Subsequently its synthesis was accomplished by several groups of workers.

The structure of pantothenic acid consists or ß-alanine and pantoic acid (dimethyl derivative of butyric acid) joined by a peptide bond (coloured red) as illustrated in Figure 3.5.

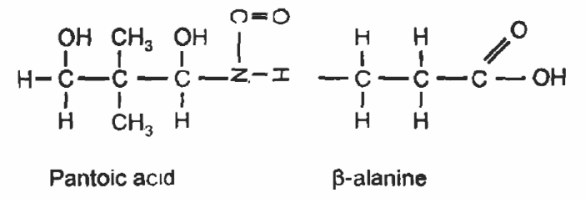


Figure 3.5: Pantothenic acid (a,pdihydroxy-B,P-dimerhylbugryl-PLalanide)

Pantothenic acid is found throughout living cells in the form of coenzyme A (CoA), a vital coenzyme in numerous chemical reactions. You will get to know about coenzynies in a little while from now in the next Unit. Let us get to learn about the properties of Vitamin B_5 now.

Properties

Imponant physico-chemical properties of pantothenic acid are as follows:

a) Pantothenic acid is a pale yellow oily liquid that can only be crystallized as its sodium, potassium or calcium salt. These are the forms in which it is generally available.

b) The compound is highly soluble in water.

c) It is stable at 120^{o}C for 30 minutes in neutral medium but is decomposed in acid or alkali solution.

d) It forms esters with alcohols.

NOTES

The next vitamin in the family of B-group vitamins is pyridoxine. Let us get to know this compound and its rerated compounds.

STUDENT ACTIVITY - 1

1) What are vitamins? List a few characteristics which all vitamins possess.

.....

.....

2) What are the two rings present in the structure of thiamin?

.....

.....

3.4.5 Pyridoxine (Vitamin B_6)

Pyridoxine, one of the B complex vitamins, was isolated in a pure form by five different groups of workers in 1938 and it was synthesized by two groups of workers independently in 1939. It was revealed that vitamin B6 activity was present in the alcohol derivative of pyridine, called as pyridoxine (3-hydroxy-4, 5-dihydroxymethyl-2-methyl pyridine), and also its aldehyde, pyridoxal and its amine, pyridoxamine as illustrated in Figure 3.6

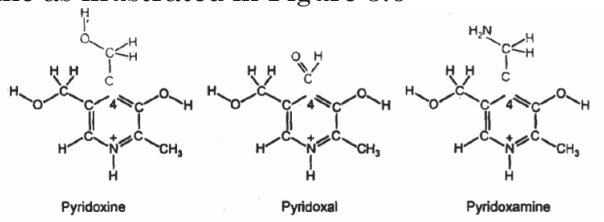


Figure 3.6: Structure of pyridoxine and related compounds

As can be seen in Figure 3.6, pyridoxine contains a pyridine nucleus, two primary alcoholic groups and one phenolic hydroxyl group. By replacing the —CH20H group on position 4 of the pyridoxine molecule with —CH2NH2 and —CHO respectively, two related compounds, pyridoxamine and pyridöxal can be formed as shown in Figure 3.6 which also have vitamin activity. These three compounds are interchangeable. The biologically active form or the so called coenzyme of pyridoxine is pyridoxal phosphate. This coenzyme is remarkably versatile, being involved in transaminations, decarboxylations, racemizations and numerous modifications of amino acid side chains. You shall learn more about it later in the next Unit on enzymes and coenzymes.

The properties of pyridoxine and its related compounds are discussed next.

Properties

Pyridoxine has the following physico-chemical characteristics:

a) It forms white, odourless crystals.

b) The compound is readily soluble in water.

c) When a neutral or alkaline solution of pyridoxine is autoclaved at 120°C for 30 minutes, partial destruction of the vitamin occurs.

d) When the alkaline solution of pyridoxine is exposed to light, it is slowly destroyed.

e) Pyridoxine produces a coloured complex by reacting with 2,6-dichloroquinone chlorimide.

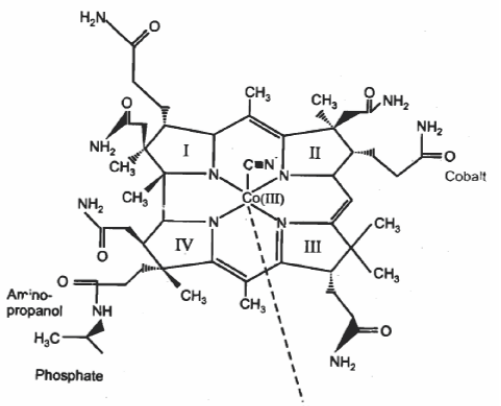
f) It forms salts with mineral acids and gives a violet colour with FeC_{13}.

3.4.6 Cyanocobalamin and Related Compounds (vitamin B_{12})

You might have heard of pernicious anaemia. Yes, it is a type of anaemia (low red blood cell count) caused by the body's inability to absorb vitamin Bn. Since Minor and Murphy discovered in 1926 that liver extract can cure pernicious anaemia, many attempts were undertaken over a period of 20 years to isolate the active principle from liver. Ultimately the isolation of crystalline vitamin was achieved by Smith and Parker, as well as, Rickes and co-workers, independently, in 1948.

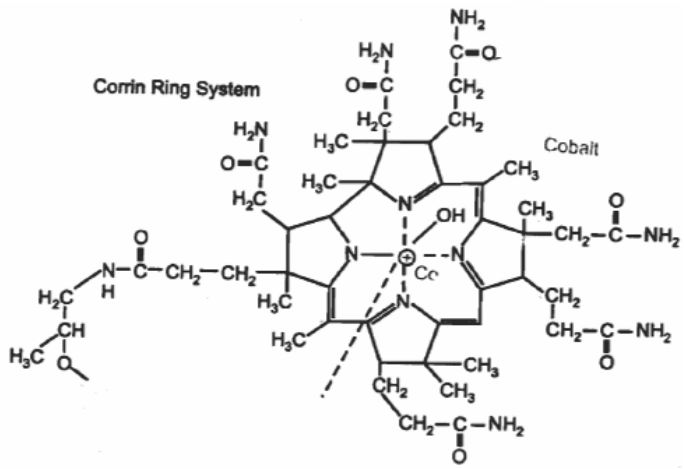
The chemical structure of cyanocobälamin was determined by Dorothy Crowfoot Hodgkin. Structure of cyanocobalamin is relatively large and complex in comparison to other vitamins as can be seen in Figure 3.7 (a). The central portion of the molecule consists of 4 reduced and substituted pyrrole rings (numbered I to IV) surrounding a cobalt (Co) atom. This central structure is known as corrin ring system. Below the system, there is a 5,6-dimethyl benzimidazole riboside that is connected at one end to the central cobalt atom and at the other end to the ribose moiety. The ribose moiety is connected to ring IV of the tetrapyrrole nucleus through phosphate and aminopropanol. Cyanide is attached to the cobalt atom and thus the name cyanocobalamin. The strueture of cyanocobalamin is presented below in Figure 3.7.

Do not get bogged down with this complex structure. It is meant more for your understanding rather than for reproduction.



5, 6-dimefiytbenzimidemle ribonucleotide

(a) Cyanocobalamin



5, 6-dimethylbeizimidazole ribonucleotide

(b) Cobalam in (vitamin B_{12})

Figure 3.7: Structure of vitamin B_{12}

Removal of cyanide results in the formation of 'cobalamin', as illustrated in Figure 3.7 (b). When the cyanide is substituted by other groups, different other derivatives result. For example, when methyl substitutes cyanide, methylcobalamin results. Let us now move on to the properties of cyanocobalamin.

Properties

The physico-chemical properties of cyanocobalamin are as follows:

a) The vitamin can be obtained in a crystalline form.

b) It is freely soluble in water.

c) Crystalline vitamin B_{12} is stable to heating at 100^{o}C for fairly long periods. Cyanocobalamin solution, when autoclaved, is converted partly to hydroxocobalamin. As this compound is heat labile, it is destroyed if heated at 120^{o}C for 30 minutes.

d) The compound is resistant to boiling in neutral or acid medium but readily destroyed in alkali.

e) On exposure to sunlight, the aqueous solution of vitamin B_{12} leads to destruction of the vitamin.

f) Vitamin C, when added to a solution of vitamin B_{12}, results in the reduction and subsequent destruction of the vitamin.

3.4.7 Biotin (vitamin H)

It was revealed that egg yolk could prevent dermatitis and emaciation in rats that were kept on raw egg white as the main protein source. The factor of the egg yolk was called as anti egg white injury factor. Szent Gyorui, in 1931, first recognized

it as a vitamin and named the factor as vitamin H. The vitamin was subsequently isolated in 1939 by Gyorui, Kuhn and Lederer. In 1942, Melville and co-workers isolated the vitamin from milk and named it as Biotin. It was first synthesized in 1943. The structure of the vitamin was established as hexahydro-2-oxo-1-thieno-3, 4-imidazole-4-valeric acid. Its structure is presented in Figure 3.8.

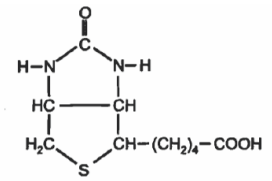


Figure 3.8: Structure of biotin

What are its properties? Let's read and find out.

Properties

Important physico-chemical properties of biotin include the following:

a) It forms colourless, needle shaped crystals.

b) The vitamin is sparingly soluble in cold water but highly soluble in hot water. The compound is sparingly soluble in alcohol.

c) Biotin forms salts with alkali hydroxides such as NaOH.

d) The compound is photostable.

e) Biotin is destroyed in acid solution.

3.4.8 Folic acid and Related Compounds (vitamin M)

Folic acid is a water soluble B-vitamin that helps build healthy cells. Along with iron, this vitamin is crucial during pregnancy as you may already know. Folic acid was isolated in a crystalline form from liver by Pifiner and co-workers in 1947 for the first time.

The vitamin that is also called as folacin or pteroylglutamic acid consists of a pteridine ring attached to a p-aminobenzoic acid and conjugated with one molecule of glutamic acid as presented in Figure 3.9. The molecule varies in structure by reduction of the pteridine moiety to dihydro folic acid (DHF) and tetrahydro folic acid (THF).

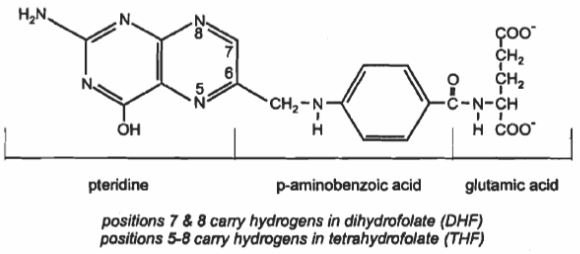


Figure 3.9: Structure of folate

There are at least three chemically related and nutritionally important compounds belonging to folic acid group that occur naturally. They only differ in the number of glutamic acid residues attached to the pteridine-aminobenzoic acid complex. Moreover, two reduced forms of folic acid, namely 7, 8-dihydrofolic acid (DHF) and 5, 6, 7, 8-tetrahydrofolic acid (THF) may be present in the tissues as discussed earlier. Let us now move on to the important properties of folic acid.

Properties

Folic acid has the following important physico-chemical properties:

a) It is a yellow, spear-shaped crystalline substance.

b) The compound is sparingly soluble in water.

c) It is stable in acid solution but when heated in alkali, it is readily destroyed.

d) The vitamin can withstand a temperature of 120^{O}C for 30 minutes at neutral pH but at pH 1.0, folic acid loses about 70-100% Of its activity when autoclaved at 120^{O}C for 30 minutes.

e) Riboflavin accelerates the photo-oxidation of folic acid

With folic acid, we come to an end of our study of the B-complex vitamins. Let us now look at the other water soluble vitamin i.e. vitamin C.

3.4.9 Ascorbic Acid (vitamin C)

Although the antiscorbutic (preventing the disease scurvy) effects of citrus fruits was known for a long time, the first important step towards its isolation was the discovery of Holst and Frolich in 1907. They reported that guinea pigs, like man and monkey, were also susceptible to scurvy. The isolation of vitamin C was carried out by Zilya during 1917-1927.

In 1928, while working in Hopkin's laboratory Szent Gyoroi isolated ascorbic acid from adrenal glands, oranges, as well as, from cabbage but he failed to recognize it as a vitamin. He called it as hexuronic acid. Afterwards, in 1932 Glen King isolated the vitamin in a crystalline form from lemon juice and identified it with Szent Gyorgyi's acid.

Within a few months of this the chemical structure of the vitamin was elucidated by Haworth and Hirst and its synthesis was also accomplished. In 1933, vitamin C was named as Ascorbic acid.

Ascorbic acid exists as L-ascorbic acid. Surely, by now you know the significance of L. L-ascorbic acid is a hexose sugar. In fact, ascorbic acid is a derivative of carbohydrate. It is closely related to the monosaccharide sugars in its structure as highlighted in Figure 3.10.

It is the most unstable of all vitamins and is readily oxidized to L-dehydroascorbic acid (DHA). DHA possesses the same vitamin activity as the L-ascorbic acid.

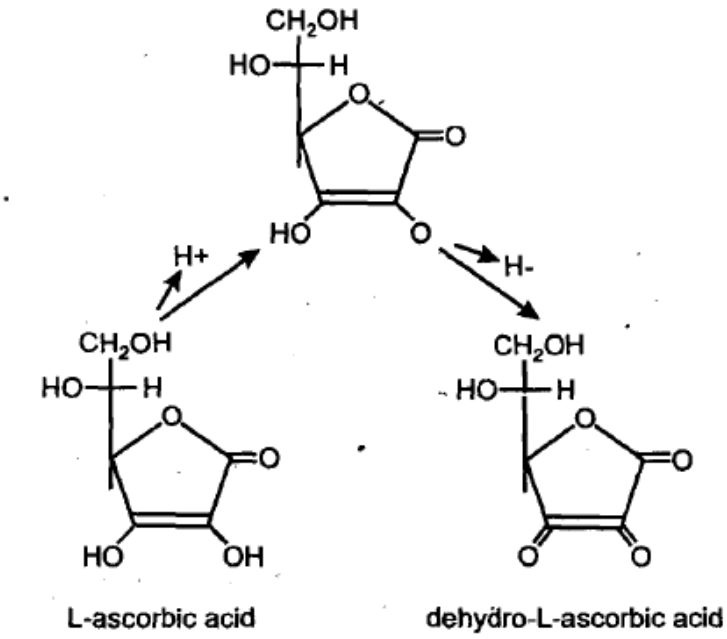


Figure 3.10: Structures Of different forms of ascorbic acid

Ascorbic acid has many vital functions to play. Let us read and find out.

Properties

Ascorbic acid possesses following important physico-chemical properties:

a) It is a white crystalline substance.

b) It is freely soluble in water.

c) Solution of the vitamin tastes sour.

d) In dry condition it is stable in air and light.

e) It is fairly stable in cold acid solution.

f) In an aqueous medium, particularly alkaline, it is easily oxidized on exposure to heat, light and traces of metals such as copper or silver.

g) It is easily destroyed during cooking.

h) The compound is a powerful reducing agent and can reduce fehling's solution. This is due to the presence of enol group (carbons 2 and 3) in its structure. (You have already learnt about the enol group in the carbohydrate Unit 1).

i) Vitamin C also reduces 2, 6-dichlorophenolindophenol to a colourless leuco compound. This reaction is very commonly utilized for chemical estimation of the vitamin.

j) Both the reduced form (L-ascorbic acid) and the oxidized form (L-dehydroascorbic acid) are biologically active.

STUDENT ACTIVITY - 2

1) What are the mo related compounds of pyridoxine? How are these different from pyridoxine?

.....

.....

2) Write down the Paine of the vitamin that contains a metal in the structure.

.....

.....

3.5 STRUCTURE AND PROPERTIES OF FAT SOLUBLE VITAMINS

After going through this section, you will be able to understand the structural and physico-chemical aspects of fat soluble vitamins, naively vitamin A, D, E and K. Biochemical roles of these vitamins will bc discussed in Unit 10 of this Course and their food sources, daily requirements, deficiency diseases, assessment procedures, etc. u ill be discussed in the Advance Nutrition Course, as informed earlier. Here, we begin our study of fat soluble vitamins with a general note i.e. all fat soluble vitamins have common features about their structure. They all contain an aromatic ring structure with an aliphatic side chain, one or more double bonds either in the ring or in lhc side chain and a functional group such as aldehyde, ketone, methyl or hydroxyl group. Let us take each vitamin one by one and understand its structure. We begin with vitamin A.

3.5.1 Vitamin A (Retinol and Related Compounds)

Hopkins, in his studies conducted between 1906 and 1912, observed that rats fed on a diet of casein, starch, sugar, lard and inorganic salts failed to grow and finally died. It was also observed that the rats could survive when a small amount of milk was added in their daily diet. With this finding, an accessory food factor in milk was established. In 191 3, two groups of workers isolated the factor from butter, egg yolk and cod liver oil and reported that the factor was fat soluble. McCollum and Davis in 1915 proposed the name 'fat-soluble A' for the factor. Rosenheim and Drummond first indicated the relationship of vitamin A to the plant pigment carotene in 1920. Subsequently, Moore in 1957 conclusively proved that carotene is the precursor of vitamin A.

The term "Vitamin A" is commonly used for those retinoids which exhibit the biological activity of retinol. The term retinoid is used to describe several related molecules — principally retinol, retinal, retinoic acid and retinyl palmitate, as well as, their numerous synthetic ana:ogs. What gre these retinoids? Let us get to know them.

Well, it is clear that Vitamin A is the collective name for a group of fat-soluble vitamins. There are two compounds possessing the activities of vitamin A. Can you name them? Yes, these are retinol (vitamin A1) and 3-dehvdroretinol (vitamin A2). Retinol or vitamin A1 is the main useable form of vitamin A in foods, often called preformed vüamin A as it is the active form in the body. Look at the structure of retinol given in Figure 3.1 1(a). You can see that it consists of a hydrocarbon chain with a ß-ionone ring at one end and an alcohol group (in red colour) at the other. Retinol is chemically a "pale yellow crystalline solid". The solid and its metabolites exist in nature as various isomers. The isomers of retinal a-e presented in Figure 3.11 (e).

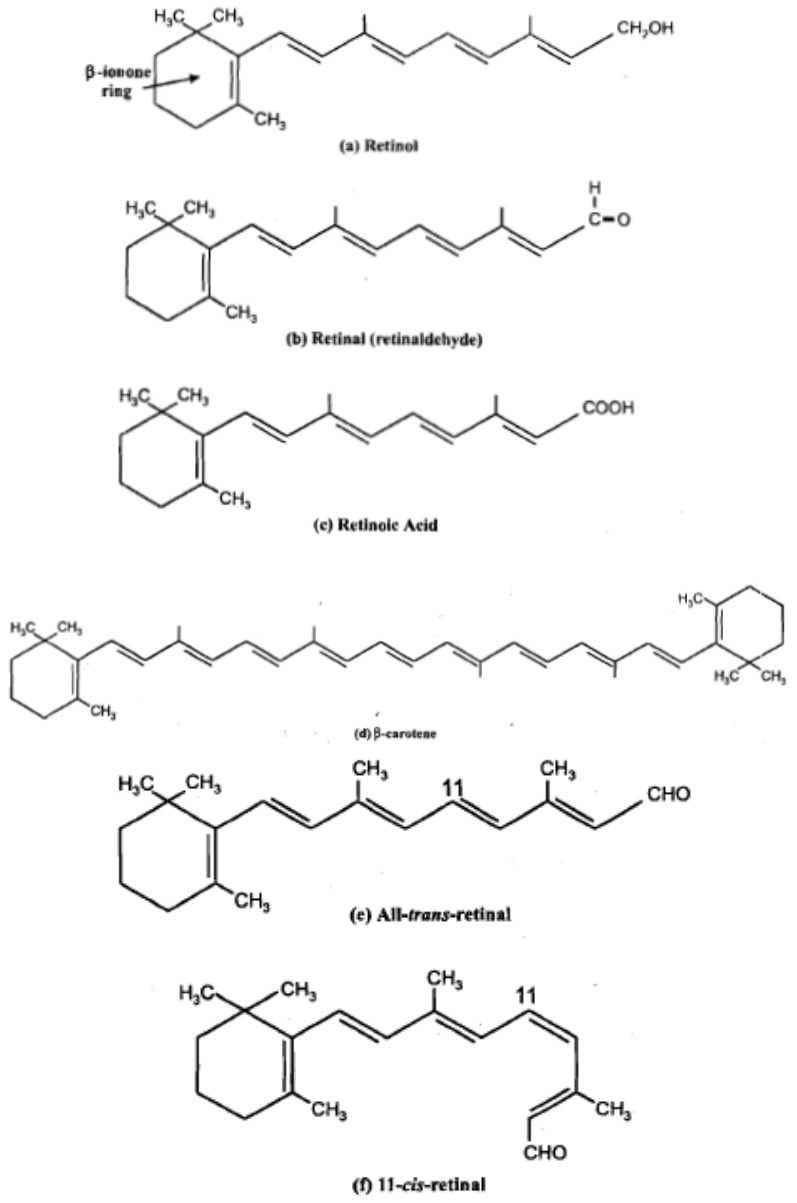


Figure 3.11: Structure of retinol, retinal, retinoic acid, β-carotene and isomers of retinal

The 3-dehydroætinol or vitamin A1 which occurs only in the liver of some Indian fish is of little nutritional importance. Moreover, it has only half the biological activity of retinol. It differs structurally from retinol by possessing an additional double bond between carbon 3 and 4 of the ß-ionone ring.

Retinol (an alcohol) can only be found in animal sources. It is the immediate precursor to two important active forms — retinal and retinoic acid. The terminal alcohol group (—OH) of retinol can be oxidized in the body to form an aldehyde (CHO), retinal or an acid (COOH), retinoic acid as can be seen in Figure 3.11 (b, c). Retinal (11-cis-retinal), plays a critical role in vision and retinoic acid, serves as an intracellular messenger that affects transcription of a number of genes. The primary storage forms of retinol in the body are retinyl esters, the most common of which is retinyl palmitate.

Vitamin A does not occur in plants, but many plants contain carotenoids such as beta-carotene that can be converted to vitamin A within the intestines and other tissues. Beta carotene, α hydrocarbon, is one of a family of dark pigments called provitamin A carotenoids. The most important of carotenes, Beta Carotene (ß-carotene) {refer to Figure 3.1 1(d)} which is widely distributed in plants can be split in the middle of its long hydrocarbon chain in the body to yield two molecules of retinol. It is the only carotenoid having a structural similarity with retinol in both halves of the molecule. Other carotenoids such as a-carotene, y-carotene, cryptoxanthine, etc. on cleavage yield only one molecule of retinol. Having understood the structure of vitamin A.

Properties

Physico-chemical properties ofvitamin A are as follows:

a) Retinol is a pale yellow, almost colourless liquid.

b) It is soluble in fats and fat solvents but not in water.

c) Vitamin A can withstand the ordinary cooking temperature (100°C) for a short period in absence of oxygen.

d) Fats containing retinol become rancid on oxidation which destroys the vitamin.

e) Retinol, on exposure to- sunlight, gets destroyed.

f) Oxidation and subsequent destruction of retinol is prevented in the presence of vitamin E.

Next, we move on to the study of vitamin D.

3.5.2 Vitamin D (Cholecalciferol and Related Compounds)

Nutritional value of cod liver oil was recognized by Hughes Bennett and it was used by Trousseau in the treatment of rickets. But, Mellanby in 1918, for the first time observed that rickets was responding to a fat-soluble vitamin present in cod liver oil. The vitamin was subsequently synthesized in 1931.

Vitamin D encompasses a group of sterols, of which only two have significant nutritional importance. These are ergocalciferol or activated ergosterol or vitamin D_2 and cholecalciferol or activated 7-dehydrocholesterol or vitamin D_3. Figure 3.12 illustrates the structures of vitamin D_2 and D_3. Both these compounds have identical biological activities in man. You may recall reading earlier in Unit 2, sub-section 2.3.4 that plants contain ergosterol, which is an unsaturated compound containing an extra methyl group in its side chain (refer to Figure 3.12). 7-dehydrocholesterol is found in animals and humans. Cholecalciferol, in fact, is a type of steroid hormone made in the skin from cholesterol when the skin is in direct sunlight. Let us learn about this now.

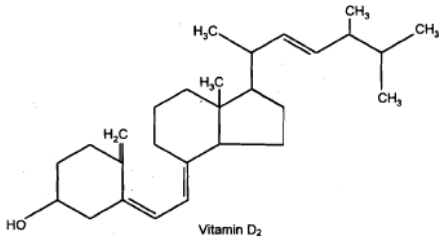


Figure 3.12: Structures of vitamin D compounds

Ultra violet (U V) irradiation cleaves B ring of both the compounds (ergosterol, 7—dehydrocholesterol) with the formation of ergocalciferol (vitamin D_2) in plants (by UV irradiation of ergosterol) and cholecalciferol (vitamin D_3) in animals. In the skin, 7—dehydrocholesterol is converted to cholecalciferol (vitamin 1)3) following UV irradiation. The Figure 3.13 shows the formation of vitamin D_3 from its precursor, 7-dehydrocholesterol.

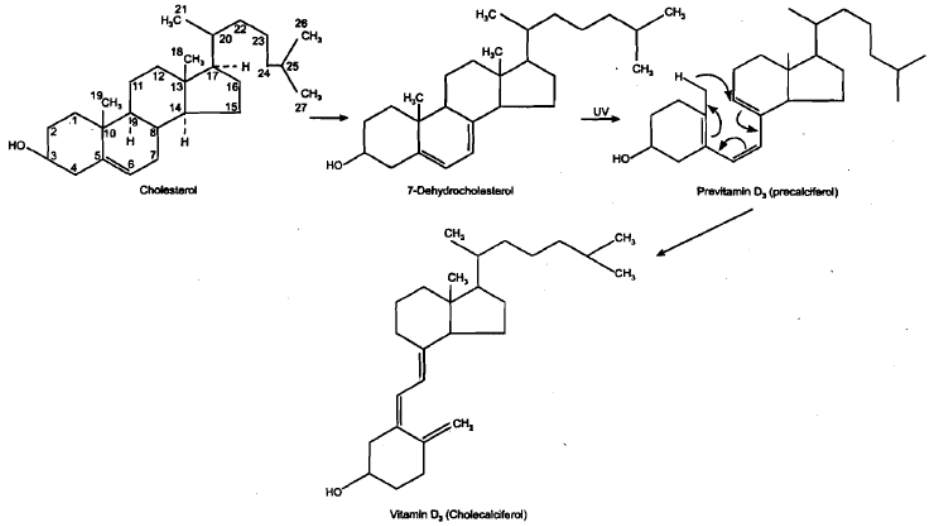


Figure 3.13: Steps in the formation of vitamin Ds in skin

The liver and other tissues metabolize vitamin D to 25-hydroxy vitamin D (25OH) D, the principal circulating form of vitamin D. 25(OH)D is then further metabolized to l, 25-dihydroxy vitamin principally in the kidney. 1, D is the principal hormonal form of vitamin D, responsible for most of its biologic actions. Figure 3.14 gives the structure of 25-hydroxyvitamin and 1, 25-dihydroxyvitamin D.

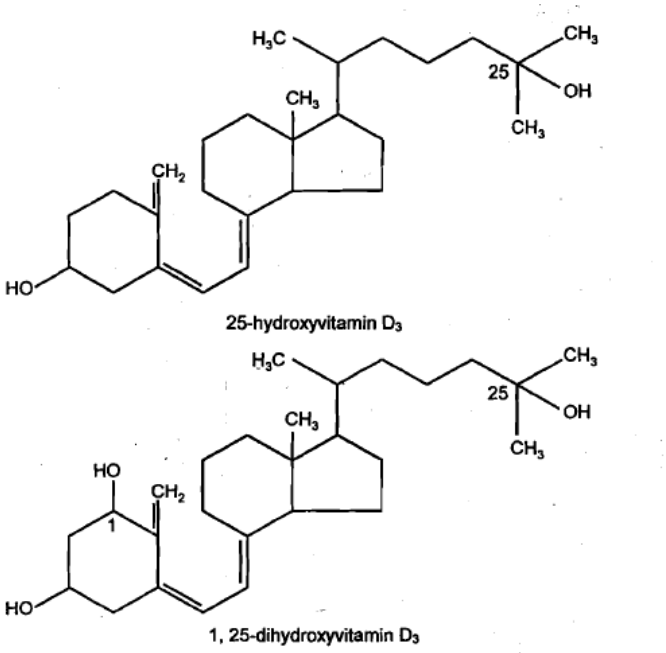


Figure 3.14: Structure of principal vitamin Ds forms

Properties

The physico-chemical properties of vitamin D are as follows:

a) Both vitamin D2 and D3 are soluble in fats and fat solvents.

b) They are not destroyed in presence of acid or alkali.

c) Both the compounds can withstand the normal cooking temperature (100^{o}C) and preserving processes.

d) They are fairly stable to oxidation.

e) In general, vitamin D is more stable than vitamin A.

STUDENT ACTIVITY - 3

l) Write down the chemical name of vitamin A. What factors lead to its destruction?

.....

.....

2) What are the two active forrns ofvitamin A? What is their physiological role

in the body?

.....

.....

NOTES

3): Name the compound that is structurally similar to retinol and can be easily converted to vitamin A.

.....

.....

3.5.3 Vitamin E (Tocopherols)

Vitamin E is an essential fat-soluble vitamin that functions, at least in part, as a lipid- soluble antioxidant. Presence of vitamin E was first revealed by Evans and Bishop in 1923. They observed that rats fed on a diet of corn starch, lard, casein, butter and yeast failed to reproduce. They also noticed that this was corrected by vegetable oil supplementation. The vitamin was isolated in 1936 by Evans and co-workers from wheat germ oil and was named as tocopherol. Subsequently, synthesis of the vitamin was accomplished by two independent groups of workers in 1938.

Vitamin E refers to a group of compounds known as tocopherols which are the derivatives of a parent compound called as 'tocol'. Four tocopherols namely α, ß, y and ö-tocopherols have been isolated. They differ from each other in the number and position of methyl groups attached to the aromatic nucleus. While and y-tocopherols have two methyl groups, α and ð-tocopherols have three and one methyl groups, respectively. All of these compounds possess comparable physiological properties, although a-tocopherol which is synthesized commercially is most potent. Some tocopherols are derivatives of tocotrienol, which has three double bonds in the aliphatic side chain. Structure ofa-tocopherol is shown in Figure 3.15:

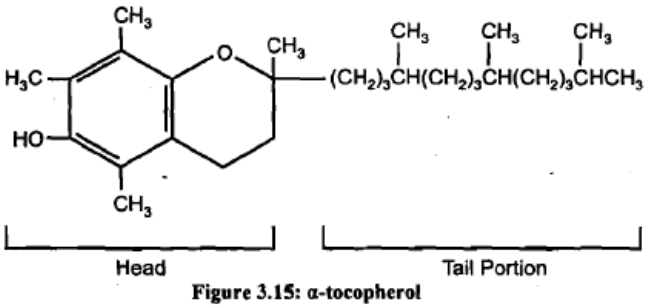


Figure 3.15: α-tocopherol

The tocopherols basically consist of a "ring7' portion called a chromane "head" or "ring" and a "tail" portion called a "phytyl" group. A chromane head has two rings which are essentially naphthalene with one carbon atom substituted with an oxygen atom, thus a cyclic ether and a pyhtyl group consists of a saturated 16-carbon isoprenoid. Isoprene is a branched chain unsaturated hydrocarbon of $ve carbon atoms. The tocotrienols are essentially identical to the tocopherols, except that they have three double bonds in the tail at 3', 7' and 11'. This can

"loosely" be called an unsaturated phytyl group or isoprenoid.

Properties

NOTES

Physico-chemical properties of vitamin E are as follows:

a) Tocopherols are yellow, oily liquids.

b) They are freely soluble in fat solvents.

c) The compounds are remarkably stable to heat. They can even withstand a temperature above 100°C.

d) Activity of vitamin E is destroyed in presence of oxidizing agents.

e) In alkaline medium, tocopherols are destroyed.

f) They are, however, stable to acidic medium.

g) When exposed to ultra violet light, the vitamin is destroyed.

h) All these compounds exhibit strong antioxidant properties.

Finally, let us learn about vitamin K.

3.5.4 Vitamin K

Vitamin K, as you may already know, is a fat-soluble vitamin that plays an important role in blood clotting. It was first observed in 1934 by Dam and Schonheyder that bleeding in chickens that was unrelated to vitamin C deficiency could he prevented by alfalfa or decayed fish meal effectively. It was possible to extract the active principle with ether and named as vitamin K by Dam in 1935. Dam, Karrer and co-workers isolated pure vitamin K_1 in 1939 and in the same year, Doisy and co-workers isolated pure vitamin K_2. Immediately after this, synthesis of the vitamin was accomplished by three different groups of workers.

Several coinpounds having vitamin K activity are known. These are basically derivatives of napthoquinone. The best known being vitamins K_1 (phylloquinone or phytalmenaquinone), K_2 (menaquinone or multiprenyl menaquinone) and K_3 (menadione).

Vitamin K or 2-methyl-3-phytyl-1,4-napthoquinone occurs naturally in plant foods, vitamin K_2 or 2-methyl-3-difarnesyl-l ,4-napthoquinone is synthesized by bacteria and vitamin K_3, popularly called as menadione, is 2-methyl-l ,4-napthoquinone which is a synthetic compound. While vitamin KI possesses a phytyl chain in position 3 of the aromatic nucleus, K_2 possesses a difarnesyl residue instead. Vitamin K_3 does not have any hydrocarbon chain attached to the aromatic nucleus. Among these three compounds, menadione is the most potent. Structures of vitamin K_1, K_2 and K_3 are presented here. Looking at Figure 3.16, you would have realized that the three structures differ only in side chain.

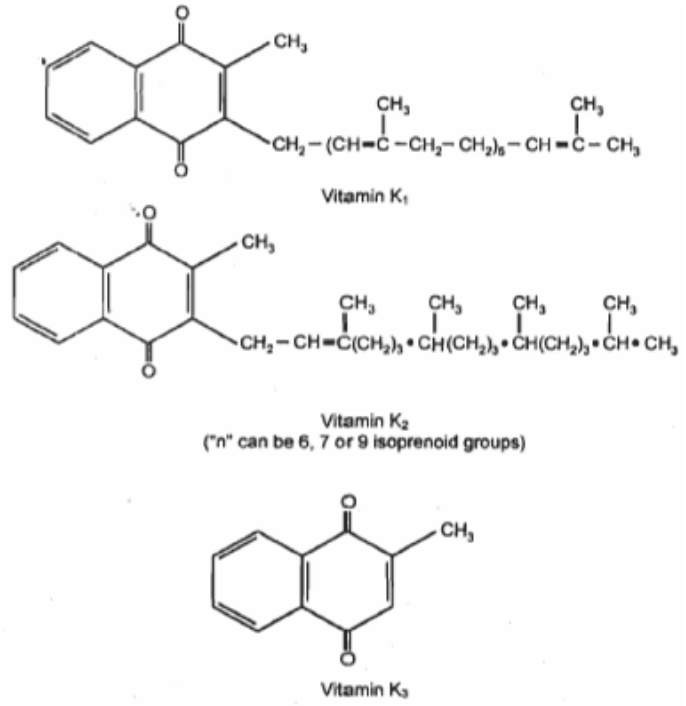


Figure 3.16: Structures of vitamin K and its forms

Properties

Physico-chemical properties of vitamin K are as follows.

a) Vitamin K_1 is yellow oil, whereas, vitamin K_2 is a yellow crystalline solid.

b) The natural vitamins K are only soluble in fat solvents.

c) Vitamin K_3 is slightly soluble in water for not having the long hydrocarbon chain.

d) Both vitamin K_1 and K_2 are alkali sensitive.

e) These compounds are also sensitive to light.

f) Both vitamin K_1 and K_2 are fairly stable to heat treatment.

After studying the structures of the fat soluble vitamins, you must have understood now that the chromane ring structure forms the basis for all fat soluble vitamins. Vitamin A consists of the ß-ionione ring.

The side chain consists of two isoprene units, four double bonds and one alcoholic group. Vitamin D consists of a sterane ring with differing side chain structures. Vitamin E has a chromane ring with an isoprenoid side chain and vitamin K, a quinine ring with isoprenoid side chain.

3.6 LET US SUM UP

In this unit we learnt the chemistry of a very important group of nutrients, vitamins. Besides learning the structure and physico-chemical properties of these substances, you also had an idea on what basis vitamins are classified and .also a

brief history of their discovery.

All the important vitamins namely, Vitamins Bl, B2, niacin, pantothenic acid, B6, B12, C, biotin and folic acid from the water soluble group and vitamins A, D, E and K from the fat soluble group were covered in the unit.

You not only visualized the structures of different vitamins but also got an idea how the structure of different members of same vitamin group differs. This difference sometimes results in difference in the activity of the vitamin. By leaming the physico-chemical properties of the vitamins, you not only had an idea about the stability of the particular vitamin against different environmental factors but you could also compare the vulnerability of the substance in comparison to other vitamins.

This unit actually acted as a foundation for further studies on vitamins, such as their biochemical role, assessment, deficiency and excess, food sources and daily requirements, etc. that you will leam in different units of the appropriate sections.

3.7 GLOSSARY

Alfalfa : a perennial hay crop of the highest quality.

Autoclaved : heated in an instrument called as autoclave in which the boiling point of water can be elevated by increasing pressure more than atmospheric pressure.

Beri-beri : disease occurs due to vitamin Bl deficiency.

Casein : the main protein of milk.

Coenzyme : a molecule that binds to an enzyme and is essential for its activity, but is not permanently altered by the reaction. Many coenzymes are derived from vitamins.

Emaciation : abnormal thinning

Fat solvents : solvents in which fats are soluble e.g. methanol, chloroform, acetone, etc.

Fehling's solution : alkaline copper sulphate reagent.

Isoprene : a branched chain unsaturated hydrocarbon of five carbon atoms.

Lard : the semisolid oil of hog's (full grown pig) fat.

Pellagra : disease occurs due to niacin deficiency.

Pernicious : a type of anaemia i.e. low red blood cell count caused by

anaemia the body's inability to absorb vitamin B_{12}.

Photo-oxidation : oxidation due to exposure to light. Scurvy disease occurs due to vitamin C deficiency.

Vitamins : the organic compounds required in very small amounts by the body for growth, maintenance and sustenance of life.

NOTES

3.8 CHECK YOUR PROGRESS

1) What are the two coenzyme derivatives of riboflavin
2) What is an important property of vitamins Bl and B2 which is crucial from the point of view of cooking?
3) What is a Corrin ring system?
4) Name a few compounds or conditions which can cause the destruction of vitamin B_{12}.
5) Give any four important physio-chemical properties of Vitamin C.
6) Indicate the steps involved in the formation of vitamin D_3
7) What is menadione? Is it available in the nature?.
8) Compare and contrast any two physio-chemical properties of vitamin K_1 and K_2.

NOTES

UNIT

4

ENZYMES AND COENZYMES

STRUCTURE

4.1 LEARNING OBJECTIVES

After studying this unit, you will be able to:

classify enzymes and coenzymes,
discuss their chemical nature and functions,
describe the factors on which enzyme activity depends, and
explain the diagnostic use of enzymes.

4.2 INTRODUCTION

Enzymes and coenzymes are two of the most important groups of biomolecules, without whose active participation, none of the nutrients can be utilized by the body. As a learner of dietetics, it is therefore essential for you to get the knowledge of different structural and functional aspects of enzymes and coenzymes. This unit focuses on these aspects which will enable you to understand the metabolism of

different nutrients which are to be discussed in subsequent units of this course.

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4.3 INTRODUCTION TO ENZYMES AND COENZYMES

Enzymes are the proteins that catalyze biochemical reactions. Study of these important biochemical reactions was started many years ago, from the time of Louis Pasteur, who for the first time demonstrated the fermentation of glucose by yeast. The catalytic agent of yeast cell was subsequently identified and named as ferment. At the end of nineteenth century, a cell-free extract of yeast was found to be capable of fermenting glucose by Buchner brothers. Considerable advances were made since then in order to properly know the nature of these agents named as enzymes and finally their true nature was revealed by James Summer in 1926 after the extraction and crystallization of the enzyme unease from jack beans. At present, no less than 150 enzymes have been prepared in crystalline form.

What is the chemical nature of enzymes? Let's find out. We all know that enzymes are proteins. Do you recall the strupture of proteins described in Unit 2 earlier? Enzymes are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds as shown in Figure 4.1.

An enzyme can be a large protein made up of several hundred amino acids, or several polypeptides that act together as a unit. Enzymes have molecular weights ranging from 10,000 to 2,000,0000.

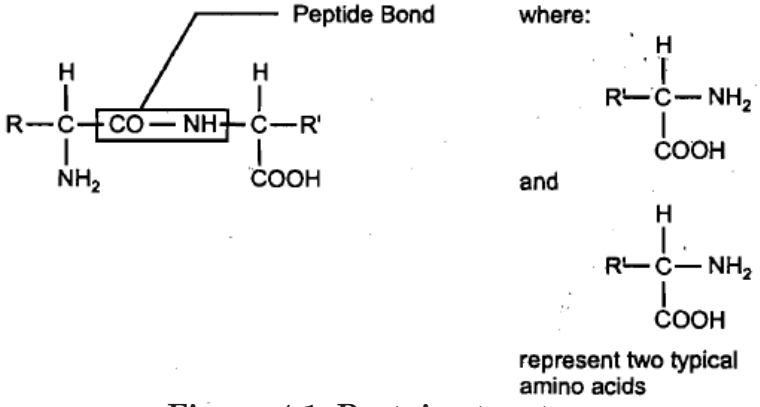

Figure 4.1: Protein structure

Until recently, it was understood that all enzymes are proteins. However, observations made in organelles from plants, yeast, viruses and higher eukaryotic cells, show that RNA can act as an enzyme. Such RNA molecule is called a ribozyme. Hence, ribozymes are RNA molecules with catalytic activity. These generally involve transesterification reactions, and most are concerned with RNA metabolism (splicing and endoribonuclease). Recently, a ribosomal RNA component was noted to hydrolyze an aminoacyl ester and thus to play a central role in peptide bond function i.e. having peptidyl transferase activity.

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Definitions of terms related to enzyme

Cofactor	Organic molecules or ions that assist many enzymes in their reactions.
Apoenzyme	The protein portion of an enzyme requiring a cofactor for its reaction.
Haloenzyme	A whole enzyme, as a complete and functional molecule. Generally, a holoenzyme consists of a polypeptide portion (an apoenzyme) and at least one cofactor or another coenzyme.
Enzyme	Proteins that act as catalysts, speeding the rate at which biochemical reactions proceed but not altering the direction or nature of the reactions.
Coenzyme	An organic, nonprotein molecule that binds with an apoenzyme (a protein molecule) to form an active enzyme. Coenzymes are often derived from vitamins.
Endoenzyme	An enzyme which is not secreted or exported out of the cell, but is kept and used by the cell which made it.
Substrate	The specific molecule an enzyme acts upon.
Metabolism	All of the organized chemical reactions in a cell which are under the control of enzymes.
Catabolic reactions	Reactions in which chemical compounds are broken down.
Anabolic reactions	Reactions in which chemical compounds are synthesized.
Enzyme immobilization	The attachment of an enzyme to a solid matrix so that it cannot escape but can still act on its substrate.
Enzyme inactivation	The disappearance of activity of an enzyme (*in vivo* or *in vitro*) due to presence of inhibitor molecules or inhibitory conditions (changes in pH, temperature, salt concentration etc.).

Enzymes are synthesized within the cells and sometimes can pass through cell membranes under certain conditions. Enzymes which act inside the cell are called as endoenzymes and those which are liberated outside the cell are called as exoenzymes. While enzymes like those involved in the production of energ.' belong to the first category, digestive enzymes belong to the second category.

Enzymes though basically are proteins may sometimes have a non-pr= component attached to the protein part. This may be an organic compound or a metal ion. While the first is called as coenzyme, the second is best known as cofactor. The protein part and non-protein part together form the holoenzyme, as illustrated in Figure 4.2. Sometimes, the non-protein part remains so tightly bound to the protein part that it cannot be dissociated. Such non-protein part is

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called as a prosthetic group. When the non-protein component dissociates from the protein part, the enzy me loses its catalytic function and is called an apoenzyme as can be seen in the Figure 4.2.

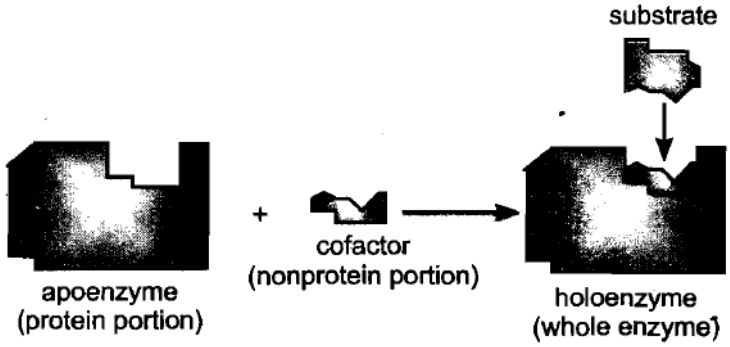

Figure 4.2: Holoenzyme

Thus, coenzymes which are usually vitamins, sometimes form an part of the active enzyme and make them indispensable for the enzyme to carry out the catalysis. You have already learnt about the classification of vitamins in the last Unit and know that these are broadly classified as water soluble and fat soluble. Remember that water soluble and not fat soluble vitamins constitute the precursors of coenzymes.

While participating in the mechanism of catalysis, a coenzyme undergoes alteration and its restoration requires the involvement of another enzyme. As coenzymes may participate in a variety of reactions catalyzed by different enzymes, it is always convenient to classify them on the basis of nature of element or group such as hydrogen, carboxyl, amino etc. they transfer in these reactions. We shall learn more about the classification of enzymes in the next section.

4.4 NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Enzymes are generally named by the addition of 'ase' to the root, indicating the substrate on which the enzyme acts. For instance, the enzyme which catalyzes the conversion of maltose into glucose is called as maltase. Many common names such as aldolase also persist which do not tell about the substrates, although the type of reaction can be recognized. Besides, trivial names of many enzymes are in use which do not follow this convention at all e.g. pepsin.

In order to follow a systematic nomenclature for all the enzymes and to classify them, the International Union of Biochemistry (IUB) has established a system, whereby, the enzymes are placed into one of the six major classes as summarized in Table 4.1. Each class is then subdivided into several classes, which are further subdivided.

The system for classification of enzymes also serves as a basis for assigning code numbers to them. The code numbers are prefixed by EC (Enzyme Commission) and contain four numbers separated by points, with the following meaning:

(a) the first number shows to which of the six main classes an enzyme belongs

(b) the second figure indicates the sub-class

(c) the third figure gives the sub-subclass, and

(d) the fourth figure is the serial number of the enzyme in its sub-class.

So you can see that a number is assigned to each class, sub-class Éind sub-subclass so that an enzyme gets a four digit number. The fourth digit, in fact, identifies a specific enzyme. For example, alcohol:NAD oxidoreductase is assigned the number 1.1.1.1 because it is an oxidoreductase, the electron donor is an alcohol and the acceptor is the coenzyme NAD. Thus, in naming an enzyme, the substrate is stated first followed by the reaction Wpe. The trivial name of the enzyme is alcohol dehydrogenase. Similarly, the EC number of catalase is EC 1.11.1.6. The first digit (1) indicates that the enzyme belongs to oxidoreductase (class 1). ECI.I indicated subsequent digits representing sub-classes and sub-subclasses.

Major classes of enzymes and the types of reaction catalyzed by them are summarized in Table 4.1.

Table 4.1: Major classes of enzymes and the types of reaction catalyzed by them

Enzyme class	General reaction catalyzed by the class	Specific reactions catalyzed by the member of the class determining the subclass
Oxido-reductases (EC1)	Oxidation and reduction of the substrate.	1. *Dehydrogenases:* Catalyze removal of two atoms of hydrogen. 2. *Oxidases:* Catalyze reduction of molecular oxygen. 3. *Oxygenases:* Catalyze incorporation of molecular oxygen into the substrate. 4. *Oxidative deaminases:* Catalyze the oxidation of amino compounds with the formation of ammonia. 5. *Hydroxylases:* Catalyze the introduction of hydroxyl radical into the substrate. 6. *Peroxidases:* Act on hydrogen peroxide.
Transferases (EC2)	Transfer of groups between two substrates	1. *Aminotransferases:* Catalyze exchange of amino and keto group between amino and keto acids. 2. *Kinases:* Catalyze the transfer of phosphate radical. 3. *Acyltransferases:* Catalyze the transfer of acyl/acetyl groups to an acceptor. 4. *Glycosyltransferases:* Catalyze the transfer of glycosyl groups.
Hydrolases (EC3)	Hydrolysis of the substrate	1. *Peptidases:* Catalyze hydrolysis of peptide bonds. 2. *Glycosidases:* Catalyze hydrolysis of glycosidic bonds. 3. *Esterases:* Catalyze hydrolysis of carboxylic acid esters.

		4. *Phophatases:* Catalyze hydrolysis of phosphoric acid esters. 5. *Phosphodiesterases:* Catalyze hydrolysis of phosphodiester bonds. 6. *Deaminases:* Catalyze hydrolysis of amines. 7. *Deamidases:* Catalyze hydrolysis of amides.
Lyases (EC4)	Removal of groups from substrates non-hydrolytically	1. *Decarboxylases:* Catalyze removal of carboxyl group from the substrate. 2. *Aldolases:* Catalyze removal of carbonyl group from the substrate.
Isomerases (EC5)	Isomerization of the substrate	1. *Racemases:* Catalyze the conversion of D-isomer to L-isomer and vice versa of a compound. 2. *Epimerases:* Catalyze the formation of an epimer of the substrate. 3. *Cis-trans isomerases:* Catalyze the inter-conversion of the *cis* and *trans* isomer of the substrate. 4. *Aldose-ketose isomerases:* Catalyze the conversion of aldose to ketose and vice versa. 5. *Mutases:* Catalyze the intramolecular transfer of a group.
Ligases (EC6)	Joining of two substrates at the expense of the breakdown of one pyro-phosphate bond of ATP	1. *Synthetases:* Catalyze the formation of C–O, C–S, C–N bonds at the expense of ATP. 2. *Carboxylases:* Catalyze the introduction of carboxyl group with a C–C bond formation at the expense of ATP.

Oxidoreductases

To this group (EC 1) belongs all enzymes catalyzing oxidation-reduction reactions. Common names include dehydrogenases, oxidases, reductases and catalases. The substrate (AH_2) that is oxidized is regarded as a hydrogen donor (AH_2 + B = A + BH_2). The recommended name is dehydrogenase but, as an alternative, the term reductase is used. The name oxidase is restricted to enzymes which exclusively use O_2 as the hydrogen acceptor. The second figure in the code number of oxidoreductases indicates the group in the hydrogen donor which undergoes oxidation e.g. CH-OH, CHO, CH—CH, $CHNH_2$, Therefore, EC 1.1 indicated acting on the CH—OH group of donors. EC 1.2 acting on the aldehyde or oxo group of donors etc. The third figure indicates the type of acceptor involved: 1 denotes NAD(P), 2 a cytochrome, 3 02, 4 a disulphide, 5 a quinine etc. So, EC t. 1. 1 states with NAD+ or NADP+ as acceptor, EC I. 1.2 with cytochrome as acceptor and so on, the list goes on.

Transferases

These are the enzymes (code EC 2) which catalyze the transfer of a group, e.g. a methyl or glycosyl group, from one compound to another. In many cases, the donor is a cofactor (coenzyme) carrying the group to be transferred. Common names include acetyltransferase, methylase, protein kinase and polymerase. The second figure (EC 2.1) in the code number of transferases indicates the group transferred: a carbon group (2.1), a carbonyl group (aldehyde or ketone) (2.2), a glycdsyl group

(2.3) and so on. The third figure informs of the group transferred: e.g. subclass 2.1 is subdivided into methyltransferases (2.1. l), hydroxymethyl and fomyltransferases (2.1.2) and so on.

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Hydrolyases

These enzymes (code EC 3) catalyze the hydrolytic cleavage of C—O, C—N, and some other bonds, including phosphoric anhydride bonds. Their trivial names are formed by adding the suffx . .. 'ase ' to the substrate which they hydrolyze. Examples include protease, nuclease, phosphatase. A number of hydrolases acting on ester, glycosyl, peptide, amide or other bonds are known to catalyze not only the hydrolytic removal of a particular group from their substrates, but also the transfer of this group to a suitable acceptor molecule. Yet, they are not grouped as transferases, because the transfer ofa specific group to water as the acceptor molecule is considered to be their main physiological function. The second figure in the code number of hydrolases indicates the nature of the bond hydrolyzed, e.g. esterases (3.1), glycosidases (3.2) and so on. The third figure generally specifies the nature of the substrate, e.g. carboxylic esters (3.1. l), thiol esters (3.1.2), phosphoric monoesters (3.1.3), O- glycosides (3.2. l), N-glycosides (3.2.2) and so on.

Lyases

These enzymes (code EC 4) cleave C—C, C—O, C-N and other bonds by elimination, forming double bonds or conversely adding groups to double bonds. Common names include decarboxylase, aldolase, dehydratase (if water is eliminated) or hydro-Iyase (if the reverse reaction is more important or the only one which can be demonstrated). Synthase, but not synthetase, may be used as in tryptophan synthase or cystathionine ß-synthase. The second figure in the EC number indicates the bond being cleaved for e.g. C—C-lyases (4.1), C—O-lyases (4.2) and so •on. The third figure informs about the group that is eliminated e.g. C02 (4.1.1) or H20 (4.2.1

Isomerases

These enzymes (code EC 5) catalyze geometric or structural changes within a molecule. According to the type of isomerism, they may be called racemases, epimerases, cis-trans isomerases (5.2), isomerases, tautomerases, mutases or cyclo- isomerases. EC 5.1 are racemases or epimerases, EC 5.1.1 indicates acting on amino acids and derivatives and so on.

Ligases (synthetases)

These enzymes (code EC 6) catalyze the linkage of two molecules coupled with the hydrolytic breakdown of a pyrophosphate bond in ATP or an analogous compound. The bonds formed are often high energy bonds. The second figure in the code number indicates the bond formed, e.g. C—O (6.1), C—S (6.2) etc. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

We hope the information given above may have helped you in understanding the

classification of enzymes. The system of the nomenclature and the classification of enzymes are based exclusively on the reaction that is catalyzed and does not consider their origin or multiplicity. Enzymes catalyzing the same reaction, but isolated from different species will have varying amino acid sequences so that they may be distinguished by electrophoretic methods. They may have different sizes and net negative charges and may even differ in their catalytic behavior.

4.5 SPECIFICITY OF ENZYMES

One of the major characteristics of enzymes is that they are highly specific. Enzymes, the organic catalysts, differ from the inorganic catalysts in their extraordinary specificity. Each of the enzymes that have been isolated and studied is found to possess different types of specificities. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In gyneral, there are four differenf types of specificity. These are as follows:

Reaction specificity

Bond specificity

Group specificity

Optical specificity

We shall look at each of these specificities one by one.

1) *Reaction specificity*

i) Some enzymes catalyze only one reaction acting on a specific substrate. Example: urease and catalase acts only on urea and hydrogen peroxide, respectively. This is also called absolute specificity

ii) Many enzymes can catalyze same type of reactions (phosphate transfer, oxidation-reduction, hydrolysis etc.) in several structurally-related compounds. Example: carboxypeptidase acts on protein chains and removes one amino acid at a time from the C-terminal, irrespective of the nature of amino acid.

iii) A substrate can undergo many reactions but in a specific reaction, an enzyme will catalyze only one of these reactions. Example: citrate synthase converts oxaloacetate to citrate in the presence of acetyl-CoA. But, in absence of acetyl- CoA, oxaloacetate is acted upon by a different enzyme malate dehydrogenase with the formation of malate.

2) *Bond specificity*

i) Some enzymes act on a particular bond (glycosidic, peptide, ester etc.). Examples: pepsin, trypsin, chymotrypsin etc. are all only acting on peptide

bonds, though the peptide bonds on which they will attack depend on the amino acids that have formed the bonds.

3) *Group specificity*

i) Some enzymes prefer a specific functional group to be present on the substrate molecules. Example: alcohol dehydrogenase acts on alcohols having —OH group. Some proteases act on the peptide bonds depending on the amino acids involved in the formation of the bonds. Example: chymotrypsin hydrolyzes only the peptide bond which is formed by the carboxyl group of phenylalanine, tyrosine or tryptophan.

4) *Optical specificity*

i) Some enzymes exhibit absolute optical specificity for at least a portion of the substrate molecule. For example, the enzyme maltase catalyzes the hydrolysis of a-glyeosidic bond between two glucose molecules but not the ß-glycosidic bond.

ii) Enzymes are able to discriminate between the optical isomers and act accordingly. Example: L-amino oxidase acts only on L-amino acids and not on D-amino acids.

STUDENTS ACTIVITY - 1

1) Define the following terms

a) Enzymes

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b) Holoenzyme

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c) Metabolism

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2) Differentiate between endoenzymes and exoenzymes, giving suitable examples.

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3) How are e4zymes classified? Explain giving an appropriate example

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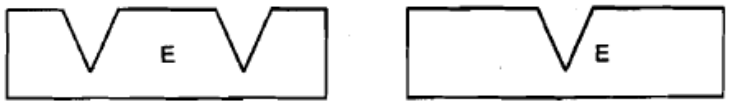
4.6 MECHANISM OF ENZYME ACTION

L. Michaelis and M. L. Menten developed a general theory of enzyme action in 1913. According to Michaelis and Menten, the enzyme (E) first binds the substrate (S) to form a transient enzyme-substrate complex (ES). This complex then dissociates into the product (P) and the unaltered enzyme (E).

$$E + S \longrightarrow \underset{\text{(Transient complex)}}{ES} \longrightarrow E + P$$

Let us understaiid how this ES complex forms.

The action of all enzyme is initiated when the reactants i.e. substrates bind at the catalytic sites or active sites on the enzyme molecule. The catalytic site of the enzylne molecule possesses a complex three-dimensional form and provides a cleft, which binds the substrate as shown herewith.



A change in the tertiary or quaternary structure of the enzyme may alter the three dimensional shape of the catalytic site and thus reducing its binding and catalytic activ~tiesT. he ES complex is formed mainly liy non-covalent bonds between specific groups of the substrate molecules and the specific amino acid side chains present at the catalytic site of the enzyme. Different models for enzyme-substrate complex formation exist. Let us look at these models next.

Modelsfor enzyme-substrate (ES) complex formation

There are two popular models to explain the enzyme-substrate interaction. These are:

Fischer's template or lock and key model, and

Koshland's induced fit model

The two models are described next.

Fischer 's template or lock and key model

According to this model, the catalytic site of the enzyme has a proper conformation compatible to a specific substrate even in the absence of the substrate molecule, as shown in Figure 4.3. The catalytic site binds the substrate and catalyzes the

reaction without any change in its own three dimensional conformation. It has become possible to explain the specificity of many enzymes for only one of the stereoisomers of the substrate by this model. This model, however, failed to explain the change in enzyme activity in presence of allosteric modulators (low molecular weight regulatory substances that bind at a specific site on the enzyme molecule other than the catalytic site and thereby enhance or inhibit the enzyme activity) or the action of the noncompetitive inhibitors

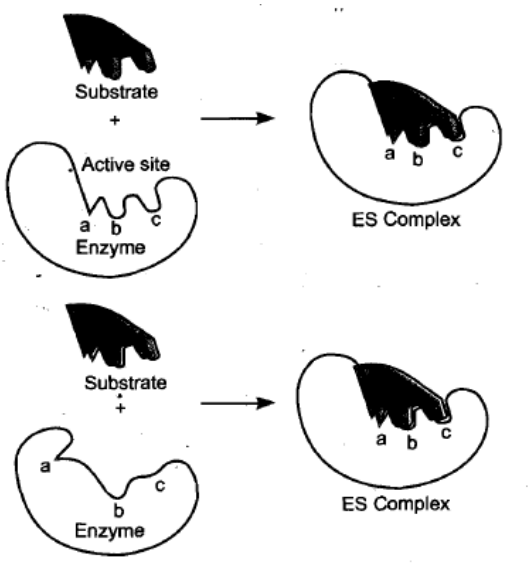


Figure 4.3: Fischer's template or lock and key model and Koshland's induced fit model

Koshland 's induced fit model

This model considers a flexibility in the three dimensional conformation of the catalytic site. According to this model, despite having the required amino acids, the catalytic site of the enzyme does not possess the conformation complementary to the substrate in absence of the substrate molecule. Only when the substrate approaches towards the enzyme or during its binding, the conformation of the catalytic site changes so that the enzyme can hold the substrate properly, as shown in Figure 4.4. This model, therefore, can suitably explain the noncompetitive inhibition and allosteric modulation of the enzyme. We will learn about the noncompetitive inhibition later in this Unit in section 4.8.

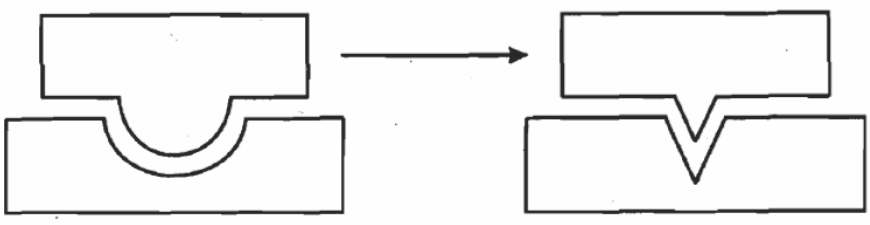


Figure 4.4: Koshland's induced fit mode

While on the topic of the mechanism of enzyme action, it is also important to know about the unit of enzyme activity i.e. how to express the activity of an enzyme. Have you heard of the term 'katal'? Recommended unit of enzyme activity is called as 'katal', which is the amount of an enzyme that transforms 1 mol of substrate into product in one second.

In fact, other than katal, the activity of an enzyme may be expressed in different other ways as highlighted herewith:

a) International enzyme unit (IU) is defined as the amount of enzyme that catalyzes the transformation of 1 g mol of substrate into product in one minute.

b) Specific activity of an enzyme preparation is expressed as kat/kg or IU/mg of protein.

c) Molar activity of an enzyme is kat/mol of the enzyme.

d) Turnover number of the enzy'me is the number of molecules of substrate transformed per catalytic site of the enzyme per minute.

After understanding the mechanism of enzyme action, we move on to enzyme kinetics. What is enzyme kinetics? Let's find out.

4.7 ENZYME KINETICS

The study of the rate at which an enzyme works is called enzyme kinetics. You have read in the last section that L. Michaelis and M. L. Menten developed a general theory of enzyme action and kinetics in 1913. According to this theory, we learnt that in an enzyme substrate reaction, the enzyme (E) first combines with the substrate (S) with the formation of an enzyme-substrate (ES) complex, which subsequently breaks down into the product (P) and the enzyme (E) is recovered. Thus, the enzyme catalyzed reaction proceeds in the following two steps:

$$\text{(a)}\quad E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \qquad \text{(b)}\quad ES \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P$$

The reactions are assumed to be reversible and KI, K2, K3 and K4 are the rate constants of each reaction.

Let us examine enzyme kinetics as a function of the concentration of substrate available to the enzyme. The enzyme substrate interaction may be studied with the help of Figure 4.5.

In this graph, substrate concentration and velocity of reaction of the enzyme catalysis has been shown on X and Y axis, respectively.

When the velocity of a reaction is plotted against different substrate concentrations, as it) the present graph, a hyperbolic curve is obtained.

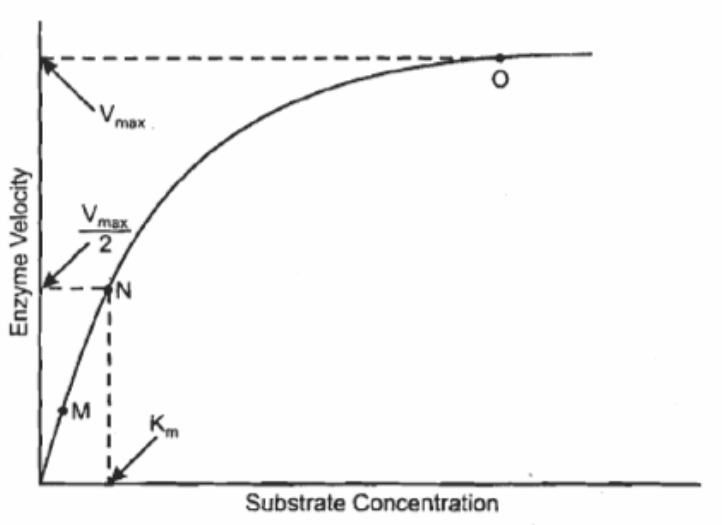


Figure 4.5: Effect of substrate concentration on the reactiop velocity of an enzyme catalyzed reaction

M, N and O are three points on the curve representing three stages of enzyme catalyzed reaction. While M represents that stage of the reaction when the substrate concentration is very low and the rate of reaction is directly proportional to the substrate concentration, O represents the stage when the reaction velocity reaches its maximum due to the gradual increase in substrate concentration resulting in a saturation of the active site (ff the enzyme. In between these two extremities lies the third point N which represents the reaction velocity of the enzyme catalyzed reaction that is half of the maximum velocity.

The mathematical relationship between the initial velocity of an enzyme catalyzed reaction, the concentration of the substrate and certain characteristics of the enzyme are expressed by the Michaelis-Menten equation, which was derived on the basis of Michaelis-Menten theory and assumptions of G. E. Briggs and J. B. S. Haldane and was proposed in 1925.

The Michaelis-Menten equation is:

$$v = \frac{V_{max} \times [S]}{K_m + [S]}$$

where, v is the velocity of the reaction at any stage,

V_{max} is the maximum velocity,

[S] is the substrate concentration and

Km is the Michaelis-Menten constant, usually expressed in moles per litre.

K_m represents the substrate concentration at which the velocity of the reaction is half of Vmx K_m is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the K_m the greater the affinity (so, lower is the concentration of substrate needed to achieve a given rate).

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This equation is widely used to describe the most enzyme catalyzed reactions. Now, we can verify the situations of the three points M, N and 0 in Figure 4.5 with the help of this equation.

i) Point M: At this stage of enzyme-substrate reaction, the substrate concentration is much less than K,,, and its value does not changes significantly with the increase in substrate concentration. Thus, [S] can be ignored in the denominator of the Michaelis-Menten equation. So it becomes,

$$v = \frac{V_{max} \times [S]}{K_m}$$

As V_{max} and K_m are both constants, V_{max} / K_m ratio may be replaced by a new constant, K. Thus, we have

$$v = K \times [S]$$

So, reaction velocity (v) of the enzyme-substrate reaction at this stage is directly proportional to substrate concentration [S].

ii) Point N: At this point, substrate concentration [S] is equal to Michaelis-Menten constant, K_m. Thus the Michaelis-Meriten equation becomes:

$$v = \frac{V_{max} \times [S]}{[S] + [S]}$$

$$v = \frac{V_{max} \times [S]}{2\,[S]}$$

$$v = \frac{V_{max}}{2}$$

This clearly established that K_m is the substrate concentration at which the reaction velocity of an enzyme catalyzed reaction is half of maximum velocity.

iii) Point O: At this point, the substrate concentration is much higher in comparison to K_m and so K_m may be ignored in the Michaelis-Menten equation. Thus it becomes,

$$v = \frac{V_{max} \times [S]}{[S]}$$

$$v = V_{max}$$

Plotting the reciprocals of the same data points yields a "double-reciprocal" or Lineweaver-Burkplot. This provides a more precise way tq determine Vmax and Km.

Lineweaver-Burk equation

Michaelis-Menten equation is often algebraically transformed to other forms for convenience in plotting experimental data. One such form is Lineweaver-Burk equation, which is derived by taking the reciprocal of both sides of Michaelis-Menten equation. Thus results:

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

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

after arrangements,we get

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

This is known as Lineweaver-Burk equation. Look at Figure 4.6. When 1/v is plotted against 1/[S], a straight line is obtained having a slope of K_m / V_{max}, an intercept of $1/V_{max}$ on the l/v axis and an intercept of— $1/K_m$ on the 1/[S] axis.

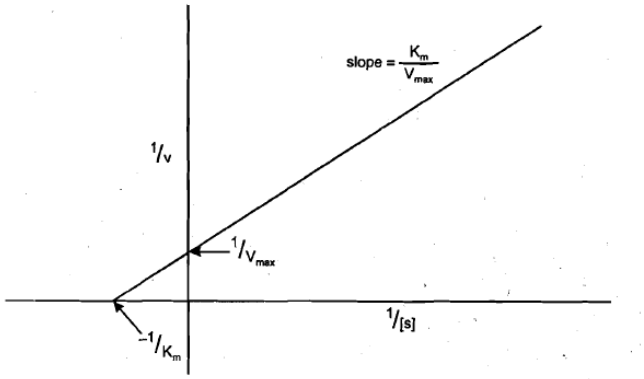

Figure 4.6: Lineweaver-Burk plot

Note that Km is not a fixed value but may vary with the structure of the substrate, pH and temperature. For enzymes having more than one substrate, each substrate has a characteristic Km. Affinity of an enzyme for a substrate inversely varies with the Km. For example, glucose can be acted upon by both hexokinase and glucokinase but hexokinase has lower Km value for glucose and therefore greater affinity in comparison to glucokinase.

At this stage, we suggest you take a break. Try to recapitulate what you have learnt so far. We agree this Section may have been a bit tough. Perhaps give this section another reading. Alternatively, try to attempt the check your progress exercises given next. This will help you clarify your doubts and facilitate your understanding about the topic.

NOTES

STUDENTS ACTIVITY - 2

1) Discuss the general theory of enzyme action.

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2) What is meant by lock and key model? What are its drawbacks?

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3) Name one model of enzyme-substrate interaction which can explain noncompetitive inhibition.

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4.8 FACTORS AFFECTING ENZYME ACTIVITY

Rate of an enzyme substrate reaction depends on several important factors. These include concentration of enzyme, concentration of substrate, pH, temperature etc. Without the optimum condition of these factors, the enzymes will be unable to exhibit its best activity. These factors are discussed briefly in this sub section.

1) *Concentration of enzyme*

Increase in enzyme concentration will increase the rate of an enzyme catalyzed reaction. This is because of the availability of additional catalytic sites to which the substrates can bind. Accordingly a straight line graph is obtained as can be seen in Figure 4.7. Since the concentration of substrate relative to that of enzyme is always very high, increase in enzyme concentration results in increased enzyme activity. Enzyme Concentration

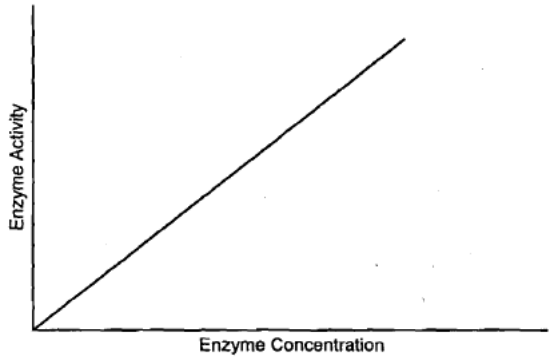

Figure 4.7: Effect of enzyme concentration on enzyme activity

2) *Concentration of substrate*

When the concentration of the substrate is low, the rate of enzyme catalyzed reaction also remains low, inspite of concentration of substrate being higher compared to enzyme concentration.

This is because at this stage all the catalytic sites of the enzyme are not occupied by the substrate molecules. So, with the increase in substrate concentration, the rate of the reaction also increases until all the catalytic sites of the enzyme are utilized.

Rate of the reaction becomes maximum at this point and beyond this, it remains constant as can be seen in Figure 4.8.

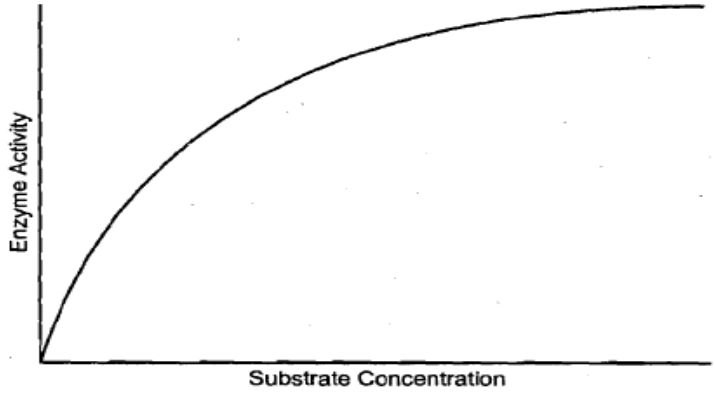

Figure 4.8: Effect of substrate concentration on enzyme activity

3) *Temperature*

The rise in temperature accelerates the rate of enzyme-catalyzed reaction up to a certain temperature known as optimum temperature for the enzyme.

At very high temperature, the enzyme undergoes denaturation and subsequent loss of activity. For most enzymes, the optimum temperatures are close to that of ambient temperature of the cell.

For human beings, the temperature is in the region of $37^{O}C$ while the optimum temperature of plant urease is $60^{O}C$. Certain microbial enzymes have a higher optimum temperature that enables them to adjust in a new higher ambient temperature of a new environment.

The effect of temperature on the reaction rate is shown in Figure 4.9. You can see that the rate of the reaction is almost zero at $O^{O}C$ and this gradually increases with the rise of temperature until the optimum point reaches. Beyond this point, the activity of the enzyme falls due to denaturation and the curve bends reaching ultimately the zero level.

It has been found that a rise of $10^{O}C$ will double the activity of the enzyme.

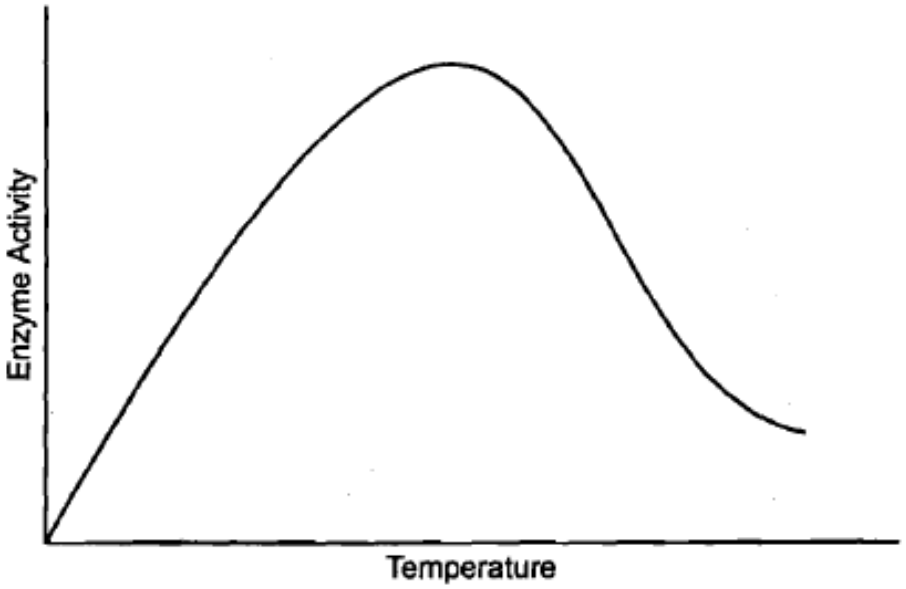

Figure 4.9: Effect of temperature on enzyme activity

4) *pH*

Enzymes are influenced by pH changes as they have a ionic character due to the presence of amino and carboxylic groups. All enzymes have an optimum pH for showing highest catalytic activity and a change of this affects their activity. However, within a narrow pH range, the changes in the reaction rate are reversible but if the pH becomes too low or high, denaturation of the enzyme may occur. Most enzymes have an optimum pH range between 5 and 8, although some enzymes like pepsin and topsin are most active at high acidic (pH 1.5) and high alkaline (pH 8) condition. A typical curve of enzyme activity against pH changes is presented in Figure 4.10.

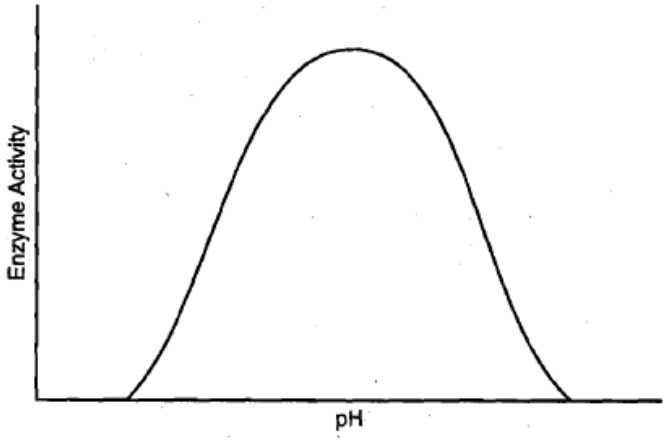

Figure 4.10: Effect of pH on enzyme activity

5) *Activators*

Activity of many enzymes is influenced by certain ions called as activators. Large

number of enzymes such as hexokinase that require ATP are also in need of divalent cations like Mg^{2+} or Mn^{2+}. Many enzymes such as ATPase require monovalent cations like Na^{+}, or NH^{4+} for maximum catalytic activity. Amylase requires cr. Generally, these ions interact with the substrates so that the substrates can bind with the catalytic sites of the enzyme properly. Thus, in absence of the activators, the enzymes become inactive or sluggish.

6) *Oxidation*

Some enzymes which have the sulfhydryl (—SH) group in the catalytic site are very sensitive to oxidation. Due to oxidation of the —SH group by aerial oxygen or oxidizing agents, a disulfide linkage (—S—S—) forms with the subsequent loss of enzyme activity. The enzyme activity can be restored by the reduction of the enzyme by some reducing agent such as cysteine or glutathione.

Having studied about the factors influencing the enzyme activity, we move on to factors which. inhibit the enzyme activity in the next section.

4.9 ENZYME INHIBITION

Enzymes are often inhibited by the presence of suitable inhibitors. Much of current drug therapy is based on this. Basically, there are three major classes of enzyme inhibition. These are:

Competitive inhibition, when the substrate and inhibitor compete for binding to the same active site

Noncompetitive inhibition, when the inhibitor binds somewhere else on the enzyme molecule reducing its efficiency, and

Uncompetitive inhibition

Let us learn about each of these classes of inhibition.

1) *Competitive inhibition*

This type of inhibition takes place when a compound having a strong structural resemblance to the substrate competes with it for the catalytic site of the enzyme. Once the compound binds, the enzyme cannot convert the inhibitor to products. Increasing substrate concentration, however, is capable of displacing the inhibitor. Thus this type of inhibition is reversible in nature. A good example of this is the reaction catalyzed by succinate dehydrogenase in the citric acid cycle. You will learn about the citric acid cycle in Unit 6, later in this Course. In this reaction, succinate is converted to fumarate with the aid of this enzyme. Now, the compound malonate is structurally similar to succinate and if present, it will compete with succinate for the catalytic site of succinate dehydrogenase and reduce the product formation. Thus, malonate is a competitive inhibitor for this particular reaction. The structure of succinate and malonate is given in Figure 4.11.

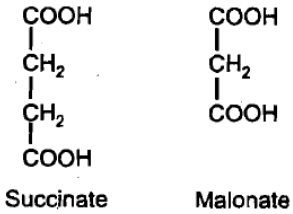

Figure 4. 11: Structure of succinate and malonate

In this type of inhibition, the K_m of the enzyme for the substrate shows an apparent increase in the presence of the inhibitor as can be seen in Figure 4.12. This means that by increasing the substrate concentration, enzyme inhibition can be overcome. However, the V_{max} remains unaltered.

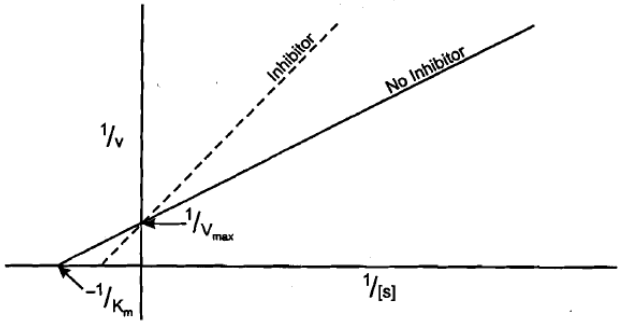

Figure 4.12: Lineweaver-Burk plot for an enzyme-substrate reaction in competitive inhibition

Next, let us learn about the noncompetitive inhibition.

2) *Noncompetitive inhibition*

In this type of inhibition, the inhibitor binds at a site on the enzyme other than catalytic site. As there is no competition between the substrate and the inhibitor, the inhibition cannot be reversed in this case by increasing the substrate concentration. It appears that as if inhibitor is removing the enzyme, thus causing a decrease in Vmax as can be seen in Figure 4.13. No change of Km, however, occurs. l/[sl

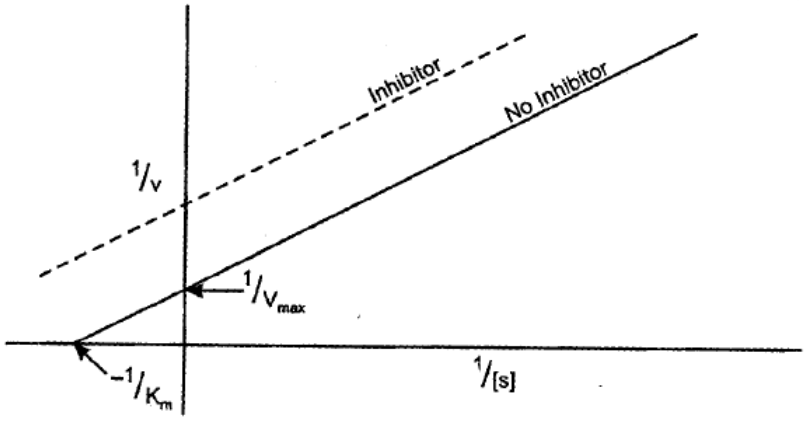

Figure 4.13: Lineweaver-Burk plot for an enzyme-substrate reaction in noncompetitive inhibition

A noncompetitive inhibitor can combine with either the free enzyme or the enzyme-substrate complex, interfering both. The most common type of noncompetitive inhibition is affected by the substances that cambine with some functional group of the enzyme (outside the catalytic site) that is essential for maintaining the conformation of the enzyme molecule required for its activity. For example, enzymes possessing the essential —SH group are sometimes inhibited by metals like mercury or copper. Finally, let is get to know about the third type of enzyme inhibition i.e. uncompetitive inhibition.

3) *Uncompetitive inhibition*

In this type of inhibition, the inhibitor only binds with the enzyme-substrate complex making it inactive. As a result, the product formation becomes difficult. In uncompetitive inhibition, both Km and V max changes as can be seen in Figure 4.14. The former increases while the latter decreases. This kind of inhibition is rare in one substrate reactions but common in two substrate reactions.

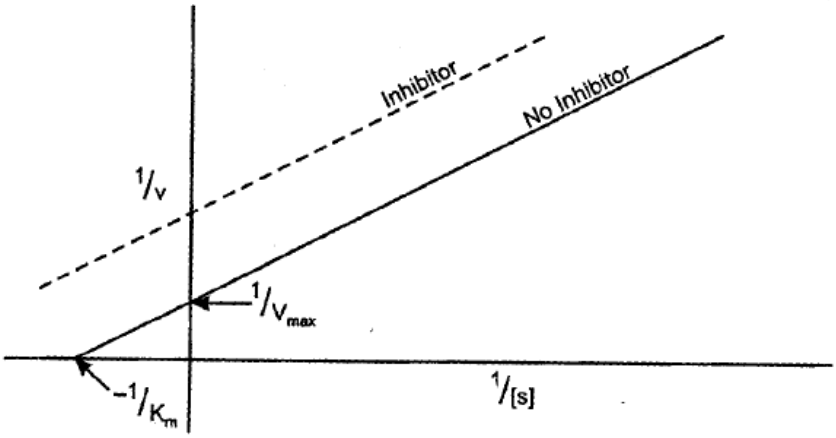

Figure 4.14: Lineweaver-Burk plot for an enzyme-substrate reaction in uncompetitive inhibition

So far we studied about the enzyme mechanism and the factors which influence and inhibit its activity. Our study of enzymes shaIl be incomplete, without the understanding of the role of enzymes and coenzymes in metabolism. The next section focuses on this aspect. But before moving on to the next section, let us recapitulate what we learnt till now.

STUDENTS ACTIVITY - 3

1) Name the environmental factors on which enzyme activity depends. Explain any two of these.

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2) What do you me by 'enzyme inhibition'? What is its significance

NOTES

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4) How does the Km of an enzyme catalyzed reaction change in the presence of a competitive inhibitor?

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4.10 ROLE OF ENZYMES AND COENZYMES IN METABOLISM

You have already learnt that many enzymes require a non-protein part for their optimal activity, which may be a coenzyme or a metal ion. This has also been stated earlier that many water soluble vitamins are acting as coenzymes in their native or derived forms. So, it is important to learn how the coenzymes are assisting different enzymes in catalyzing biochemical reactions. In this sub-section, you will learn about:

the chemical nature of different coenzymes, and

the general role of co-enzymes in assisting different enzymes during metabolism.

As coenzymes participate in a variety of functions, they can be classified broadly into two groups:

i) Hydrogen transferring coenzymes, and

ii) Group transferring coenzymes

1) *Hydrogen Transferring Coenzymes*

This group consists of three important coenzymes all of which assist different enzymes in oxidation-reduction reactions. These are:

a) Nicotinamide nucleotides

b) Flavin nucleotides

c) Lipoic acid

A brief review of these coenzymes follows.

(a) *Nicotinamide nucleotides*

These coenzymes are derived from the vitamin, niacin. They are of two types, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Collectively, they are called as pyridine nucleotides.

In NAD^+, the pyridine ring is attached to a ribose molecule through glycosidic bond and the phosphate provides a link between adenosine and nicotinamide riboside. In $NADP^+$, an a4ditional phosphate group is present in carbon atom 2 of the ribose molecule of adenosine component.

Both NAD^+ and $NADP^+$ act hydrogen acceptors during oxidation-reduction reactions in the body. Dehydrogenation is the primary mechanism of biological oxidation in which two hydrogen atoms are removed from the substrate in presence of an acceptor. The hydrogen atoms ionize to yield two and two electrons.

The nicotinamide ring of NAD^+ or $NADP^+$ accepts a proton and two electrons which are equivalent to W. The other remains as such. All reactions catalyzed by them are reversible. Table 4.3 lists some of the enzymes, all dehydrogenases, which are dependent on these coenzymes

Table 4.3: Enzymes requiring nicotinamide nucleotide coenzymes

Enzyme	Coenzyme	Substrate	Product
Lactate dehydrogenase	NAD^+	Lactate	Pyruvate
Alcohol dehydrogenase	NAD^+	Primary and secondary alcohols	Corresponding aldehydes and ketones
Glutamate dehydrogenase	NAD^+	Glutamate	α-ketoglutarate and ammonia
Glyceraldehyde-3-phosphate dehydrogenase	NAD^+	Glyceraldehyde-3-phosphate	1,3-diphosphoglyceric acid
Glucose-6-phosphate dehydrogenase	$NADP^+$	Glucose-6-phosphate	6-phosphogluconoloctone
Malate dehydrogenase	NAD^+	Malate	Oxaloacetate
Isocitrate dehydrogenase	$NADP^+$	Isocitrate	α-ketoglutarate

(b) *Flavin nucleotides*

Flavin nucleotides are derived from the vitamin B2 (riboflavin) and are actively involved in hydrogen transfer reactions. You may recall reading in the last unit about the two coenzymes that are produced from riboflavin, i.e. flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Look at Figure 4.16 for the structure

of FAD. FMN is the active component of riboflavin and is formed by the addition of a phosphate group and FAD is formed by the combination of FMN with one molecule of adenosine triphosphate (ATP). Both, FMN and FAD, are involved in many oxidation-reduction reactions. Enzymes that require the presence of FMN for their catalytic activity include glycolic acid oxidase, L-amino oxidase etc. FAD containing enzymes are succinate dehydrogenase, D-amino oxidase etc.

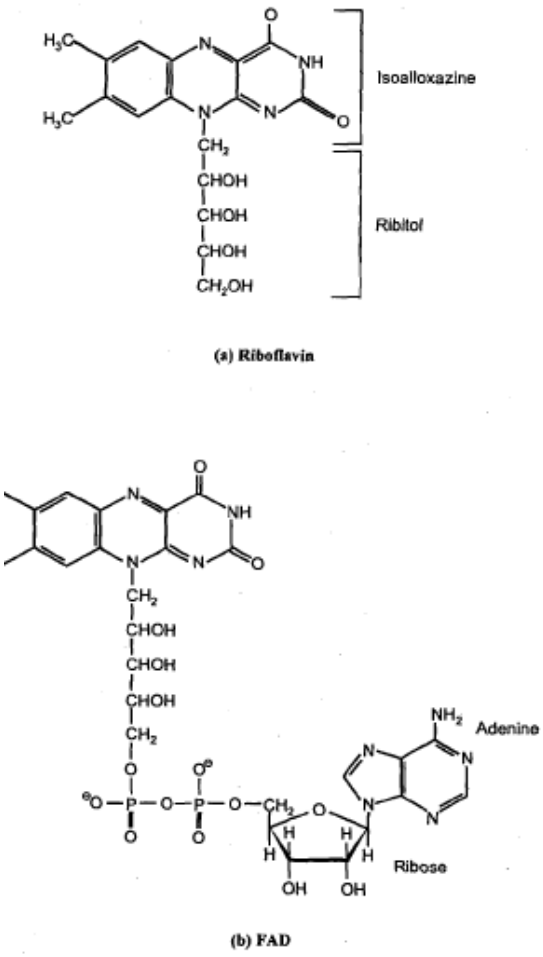


Figure 4.16: Structures of Riboflavin and its coenzyme FA

(c) *Lipoic acid*

Lipoic acid acts in the transfer of hydrogen during oxidative decarboxylation reactions. It occurs both in oxidized and reduced forms. Refer Figure 4.17 (a) for its structure. Figure 4.17 (b) shows the structure of lipoamide, where lipoic acid is bound in an amide linkage to the e-amino group of a lysine residue (blue) of dehydrolipoamide acyl-transferases. The complex reactions of the carbohydrate metabolism catalyzed by pyruvate dehydrogenase system and a-ketoglutarate dehydrogenase system require the participation of lipoic acid. These reactions will be discussed later in detail in Unit 6 covering carbohydrate metabolism.

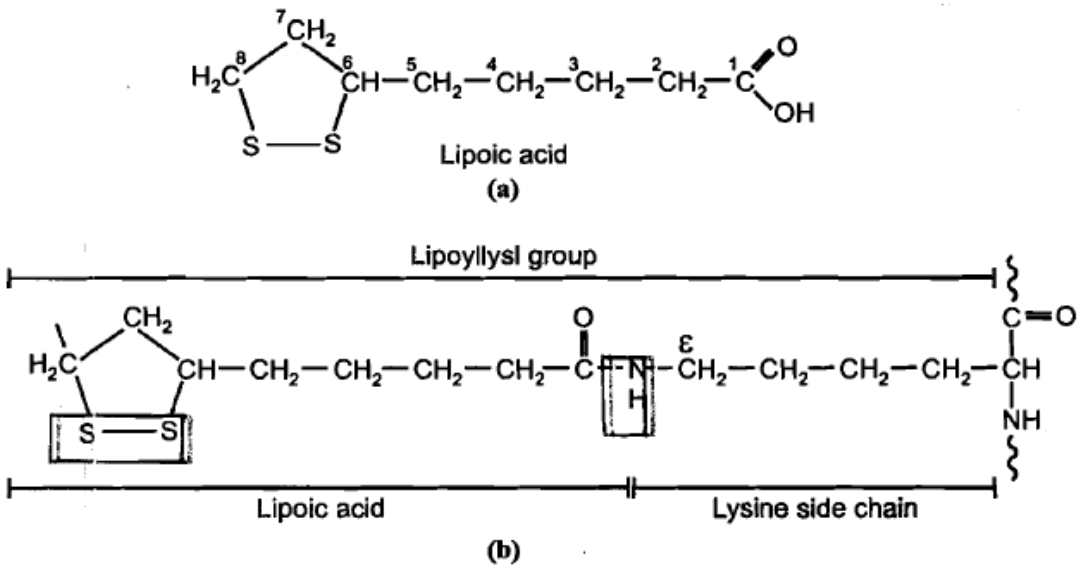


Figure 4.17: Structures of (a) Lipoic acid (b) Lipoamide

II) *Group Transferring Coenzymes*

This group consists of the following coenzymes involved in different metabolic reactions, where the transfer of any functional group is taking place:

Biotin

Thiamin diphosphate (TDP)

Pyridoxal phosphate

Coenzyme A (CoA)

Tetrahydrofolic acid (THF)

Cobamide coenzymes

Adenosine triphosphate (ATP)

You may recall reading about these enzymes in the last unit on vitamins, particularly under the section on water soluble vitamins. There we talked about them as derivatives of water soluble vitamins. We shall look at these enzymes once again to help you understand them better.

1) *Biotin*

This water soluble B group vitamin participates in the transfer of carboxylic groups. Two important enzymes carrying out carboxylation reactions are pyruvate carboxylase and acetyl CoA carboxylase. Pyruvate carboxylase contains four biotin molecules attached to the enzyme protein. The biotin dependent enzymes require ATP (adenosine triphosphate) which is converted to ADP (adenosine diphosphate) during the reaction.

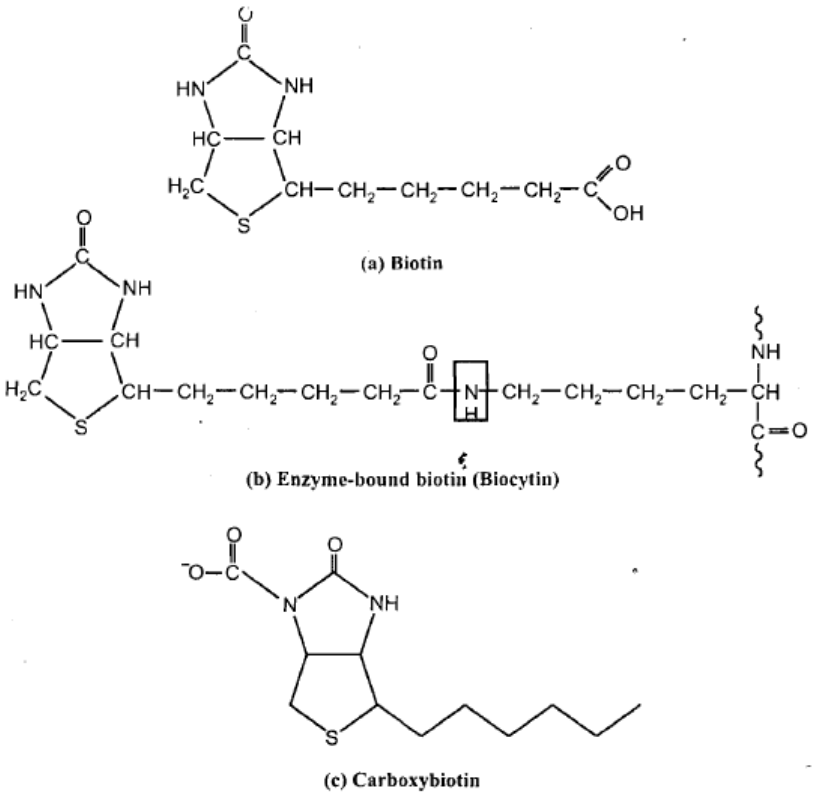


Figure 4.18: Structure of Biotin and enzyme-bound biotin

2) *Thiamine diphosphate*

Thiamine diphosphate (TDP) is the coenzyme responsible for the transfer of aldehyde and glyoxal groups. Figure 4.19 presents the structure of TDP.

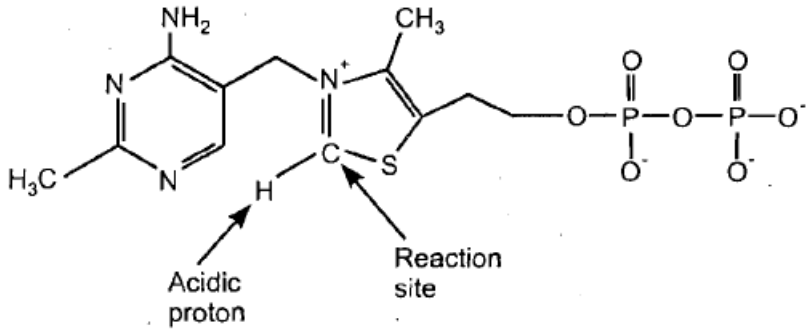


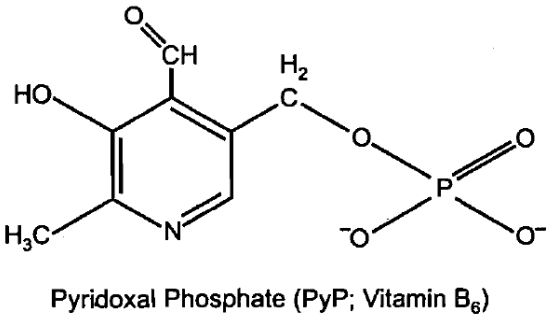
Figure 4.19: Structure of thiamine diphosphate

The most important metabolic reaction catalyzed by T DP is the oxidative decarboxylation of a-keto acids such as pyruvic acid. The involvement of T DP in the decarboxylation of pyruvic acid was so well known that the coenzyme was also popularly called as co-carboxylase. It is also actively taking part in the conversion of pyruvic acid to acetyl-CoA and a-ketoglutarate to succinyl-CoA that are catalyzed by pyruvate dehydrogenase and a-ketoglutarate dehydrogenase complex, respectively during carbohydrate metabolism. You shall learn about this

in more details in Unit 6. Another important Inetabolic reaction catalyzed by T DP is the conversion of xylulose-5-phosphate to sedoheptulose-7-phosphate catalyzed by the enzyme transketolase in pentose phosphate pathway of carbohydrate metabolism. Look up Unit 6, section 6.8 for knowing more about the pentose phosphate pathway.

3) *Pyridoxal phoshphate*

Pyridoxal phosphate is derived from pyridoxine (vitamin B6) and is involved in amino acid metabolism. The other two compounds, pyridoxal and pyridoxamine, about which you learnt in the last unit, having the properties of vitamin B6 also occur as phosphate derivatives. Enzymes that are dependent on B6 phosphate coenzymes catalyze a variety of reactions such as transamination (transfer of amino group from an amino acid to a keto acid), decarboxylation (removal of carboxyl group) and racemization (transformation of one isomer to another). The structure of pyridoxal phosphate is shown in Figure 4.20. o



Pyridoxal Phosphate (PyP; Vitamin B_6)

Figure 4.20: Structure of pyridoxal phosphate (Pyp; Vitamin B6)

Enzyme glutamate oxaloacetate transaminase catalyzes the reaction between glutamate and oxaloacetate with the formation of a-ketoglutarate and aspartate due to transfer of one amino group from glutamate to oxaloacetate. Pyridoxal phosphate acts as the carrier of the amino group. Whenever it accepts the amino group, it transforms to pyridoxamine phosphate, and after the release of the amino group, it again becomes pyridoxal phosphate. Aspartate is decarboxylated by aspartate decarboxylase taking the help of pyridoxal phosphate.

L-alanine transforms to D-alanine by alanine racemase which also requires pyridoxal phosphate as the co-enzyme.

4) *Coenzyme A*

Coenzyme A is derived from the vitamin pantothenic acid. This is abbreviated as CoA. This can be divided into two components, adenosine 3,5-diphosphate and pantotheine, which is formed by the combination of pantothenic acid and mercaptoethylamine. Refer Figure 4.21 to understand its structures well. It gives rise to acyl-CoA derivatives that are mainly formed in ATP dependent synthetase

reactions. These are highly reactive and participate in various types of reactions. For example, oxaloacetate is converted to citrate in presence of citrate synthetase by accepting acyl group of acyl-CoA. During carbohydrate metabolism, the pyruvate is converted by the pyruvate dehydrogenase complex to acetyl-CoA by the active participation of coenzyme A. Also, in the oxidation of fatty acids, .

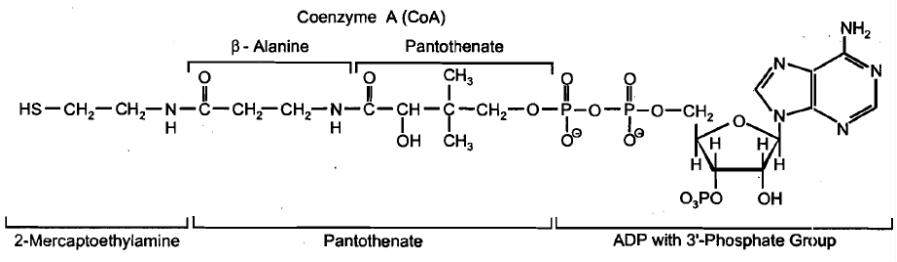


Figure 4.21: Structure of coenzyme A

5) *Tetrahydrofolate*

Coenzyme tetrahydrofolate, as you may already know, is derived from the vitamin folic acid. Have a look at the Figure 4.22 for structures of folic acid and tetrahydrofolate — the coenzyme. It is responsible for the transfer of one carbon fragrnents at the oxidation level of formate, formaldehyde and methanol. The two most important metabolic reactions in which tetrahydrofolate participates are the biosynthesis of purine and methionine.

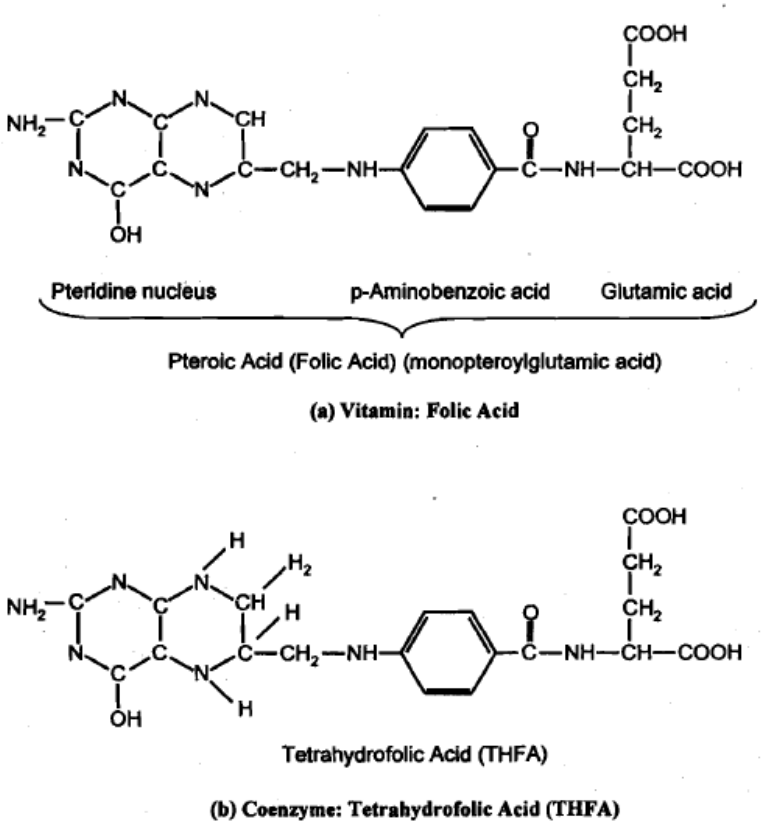


Figure 4.22: Structures of folic Acid and tetrahydrofolic acid

6) *Cobamide coenzymes*

Cobamide coenzymes are the derivatives of vitamin Bn. The structure of cobamide-coenzyme is very complex and is shown in Figure 4.23. They participate in many biochemical reactions. L-methylmalonyl CoA is converted to succinyl CoA by the action of the enzyme methylmalonyl CoA isomerase. In this reaction, the —CO—SCoA group is transferred by the cobamide-coenzyme. An important reaction catalyzed by this coenzyme as an integral part of the ribonucleotide reductase is the reduction of ribonucleotides to deoxyribonucleotides.

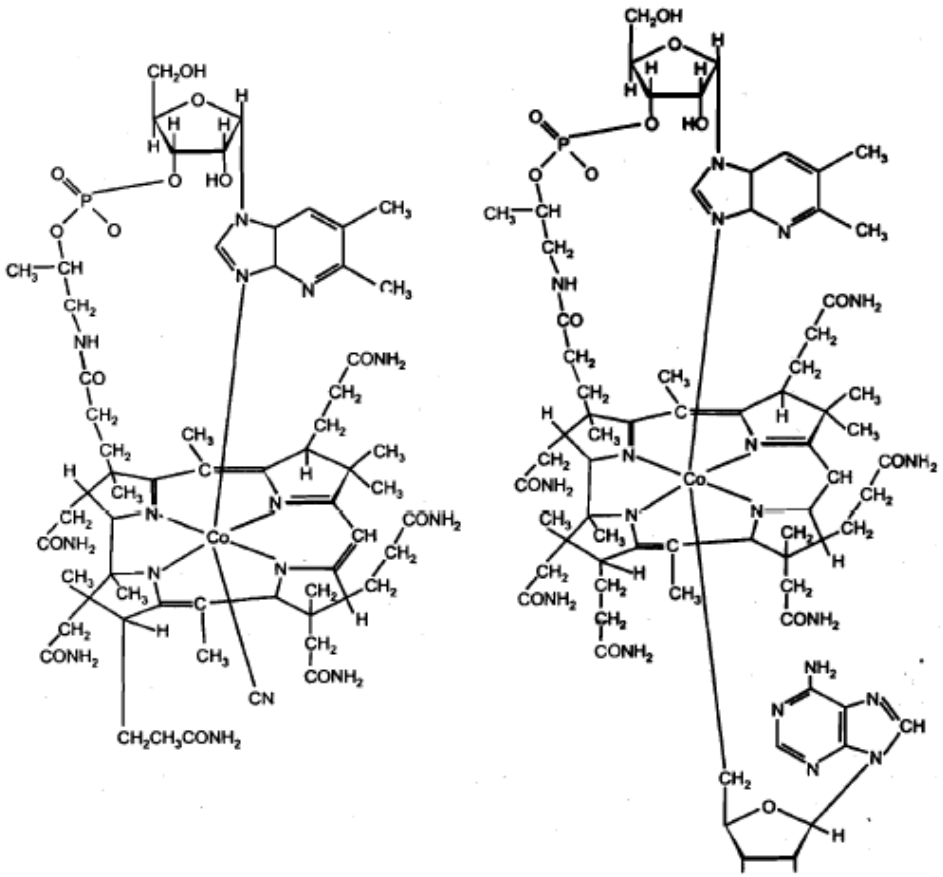


Figure 4.23: Structure of vitamin Bn •nd its coen4tne

7) *Adenosine triphosphate*

Though adenosine triphosphate (ATP) often functions as a second substrate, it can also serve as a coenzyme by modulating the activity of specific enzymes. The compound consists of adenine connected to a ribosyl 5'-triphosphate. See Figure 4.24 for its structure. As a co-substrate, ATP is utilized by various kinases for the transfer of the terminal phosphate group to various acceptors.

For example, glucose is converted to glucose 6-phosphate in presence of the enzyme hexokinase by accepting phosphate from ATP.

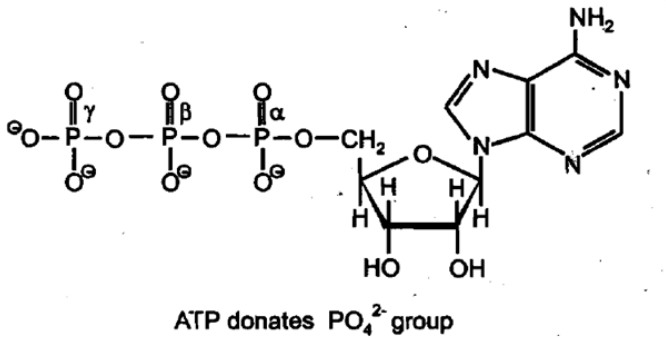


Figure 4.24: Structure of ATP

So far we have studied about the different enzymes and coenzyme involved in metabolism. Interestingly, it is known that enzymes catalyzing essentially the same reaction may differ in various ways. These are called isozymes. The next section focuses on isozymes.

4.11 ISOZYMES

Sometimes an enzyme present in the same organism is found to have different molecular forms but catalyzing the same reaction. These are called isozymes. The 1964 Committee recommended that "multiple enzyme forms" in a single species should be known as isozymes. Among many enzymes known to have isozymes, the most studied is lactate dehydrogenase (LDH), which is an important enzyme of carbohydrate metabolism. This enzyme exists in five possible forms in most vertebrates. In fact two basically different Owes of LDH occur predominantly in the heart and muscle. The former consists of four identical monomers (H). The latter (muscle LDH) also forms due to the combination of four identical monomers (M) but having different amino acid composition, in comparison to the former. Different combinations of H and M monomers yield three additional hybrid enzymes possessing four monomers each. The possible combinations of H and M monomers therefore, produce five isozymes ofLDH. These are

$$H_4.\ H_3M,\ H_2M_2,\ HM_3 \text{ and } M_4$$

Isozymes differ from each other not only in the amino acid composition, they also have different electrophoretic property, thermolability, immunological properties and kinetic properties such as substrate affinities i.e. different $_m$ values.

Estimation of isozymes of some enzymes can be used for the clinical diagnosis of different diseases. Let us learn about this aspect next.

4.12 NZYMES IN CLINICAL DIAGNOSIS

The rationale for measuring plasma or serum enzyme levels is based on the premise that these levels reflect changes that have occurred in a specific tissue or

organ. Enzymes present in the blood are of two types — one type such as thrombin (associated with blood coagulation) has a functional role and is present in high concentration, the other type has no functional role in the blood and is present in very small amount.

The latter types of enzymes mainly originate from different tissues or organs. An insult in the form of any disease may cause changes in cell membrane permeability or increased cell death, resulting in the release of intracellular enzymes into the blood:

As a result, the concentration of these enzymes increases in the blood. In the diagnosis of specific organ involvement in a disease process, it would be ideal if enzymes unique to each organ could be identified. But, this seldom occurs as the metabolism of different organs is not unique.

Alcohol dehydrogenase and acid phosphatase are two such enzymes, the levels of which in the blood can be used as specific tools for the diseases of liver and prostrate, respectively. However, the ratio of enzymes varies from organ to organ. This fact, combined with a study of the kinetics of appearance and disappearance of particular enzymes from the blood, enables one to the involvement of a specific organ in a disease. Both glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) are present in the liver but the concentration of GPT is more than GOT. In liver dysfunction, both these enzymes are leaked out from liver and as a result, their level in the blood increases but the rise is more in case ofGPT than GOT making it a reliable marker for liver diseases.

Following a heart attack, many enzymes such as GOT, creatine phosphokinase, LDH etc. are released from the myocardium but their time of release differs, enabling one to establish when the attack occurred and whether the treatment is effective or not.

Enzymes that are often estimated in the plasma or serum for confirmatory or suggestive diagnosis of different diseases are listed in Table 4.4 along with their normal values. This has to be kept in mind that these values will vary according to the procedure used for their estimation and generally all laboratories standardize their own normal values.

This is to note that though increase of enzyme activities in the cerebrospinal fluid sometimes occurs e.g. increase in lactate dehydrogenase activity in meningitis, it is not reflected in the blood.

Isozymes are often estimated to speciß' a diseased organ/tissue. Two different isozymes of alkaline phosphatase are present in liver and bones. So, a rise of serum alkaline phosphatase may occur either due to liver damage or due to problems related to bones. In this situation, estimation of specific isozymes will give a clear picture for the rise in the enzyme level in serum and this enables one to pin point the affected organ/tissue.

Table 4.4: Common enzymes of diagnostic importance

Enzyme	Normal value in the serum	Concentration increases in conditions
Acid phosphatase	1-5 units/dl	Metastatic carcinoma in the prostrate.
Alkaline phosphatase	5-13 units/dl	Rickets, hyperparathyroidism, obstructive jaundice, kidney disease, metastatic carcinoma, osteoblastic sarcoma. Isozymes of alkaline phosphatase can distinguish liver lesions from bone lesions in cases of metastatic carcinoma.
Amylase	0.8-32 IU/L	High intestinal obstruction, acute pancreatitis.
Acetyl cholinesterase	3-5 IU/ml	Nephrotic syndrome.
Creatine phosphokinase	5.5-75 units/L (Male) 6-50 units/L (Female)	Muscular dystrophy, myocardial infarction.
Glutamate-oxaloacetate transaminase (SGOT)	5-40 units/dl	Myocardial infarction, slightly elevated in liver diseases.
Glutamate-pyruvate transaminase (SGPT)	5-35 units/dl	Acute liver diseases, slightly elevated in cardiac necrosis.
Lipase	0.2-1.5 units/dl	Acute pancreatitis, pancreatic carcinoma.
Glucose-6-phosphate dehydrogenase (in RBC)	3.5-7.5 units/ml	Haemolytic anaemia often associated with administration of anti-malarial or sulphonamide drugs and after eating fava bean.
Lactate dehydrogenase	90-200 IU/L	Myocardial infarction, acute hepatitis, renal tubular necrosis.

4.13 LET US SUM UP

In this unit we covered the structural and functional aspects of enzymes and coenzymes — two of the most important group of biomolecules. While enzymes are almost always protein in nature, coenzymes are mainly derived from water-soluble vitamins. You now have a clear idea about the different classes of enzymes and the type of reactions they catalyze. This knowledge will greatly help you to understand the different metabolic reactions that will be described in the subsequent sections.

One of the main differences between an inorganic catalyst and enzyme is the high degree of specificity of the latter which has been discussed in the light of models for enzyme-substrate interaction. Enzyme being a protein is particularly sensitive to different environmental factors and its activity changes accordingly. These along with other important factors governing the activity of an enzyme have been dealt with in this unit.

Enzyme kinetics was further discussed in this Unit and so also the application of Michaelis-Menten equation. You now understand how the affinity of an enzyme for a particular substrate can be judged on the basis of its Km value. Impact of different inhibitors on the Km and V of an enzyme is also taken into consideration.

Further, in this unit we got the idea how different coenzymes are taking part in different enzyme-catalyzed reactions with suitable examples. This will also greatly help us in learning different metabolic reactions in the subsequent unie of this course. Importance of serum enzymes and isozymes in the diagnosis of different diseases is well known. This has been discussed comprehensively. Thus this unit has covered all important aspects of enzymoloy but emphasis has been given only on the topics relevant to our need as nutritional biochemists.

4.14 GLOSSARY

Active site : the site on the surface of an enzyme to which substrate or substrates bind.

Cleft : partially divided.

Conformation : specific arrangement of the molecule.

Denaturation : change from original structures.

Electrophoretic : ability to move in an electric field.

Haemolytic : liberation of haemoglobin from erythrocyte causing anaemia.

International : the amount of enzyme that catalyses the transformation of nzyme 1 μmol of substrate into product in 1 minute.

Isozymes : an enzyme having different molecular forms but catalyzing the same reaction.

Katai : the amount of enzyme that transforms I mol of substrate into product in one second.

Metabolism : synthesis or breakdown of bio-molecules.

Metastatic : cancer that can be transferred from one part of the body to carcinoma other unrelated parts.

Muscular : defects in the muscle due to faulty nutrition. ystrophy

Myocardial : necrosis of myocardium due to interruption of blood infarction supply.

Myocardium : the middle layer of the heart wall.

Necrosis : cell death.

Nephrotic syndrome : kidney disease due to degeneration of renal tubule.

Osteoblastic sarcoma : a kind of tumor.

Pancreatitis : inflammation of the pancreas.

Prosthetic group : a non-protein part of the enzyme which remains tightly bound to the protein part.

Rickets : a condition in children due to vitamin D deficiency.

Thermolability : temperature sensitivity.

Trivial : less important.

Turnover number : the number of molecules of substrate transformed per catalytic site of the enzyme per minute,

4.15 CHECK YOUR PROGRESS

1) How can affinity of an enzyme for a substrate be judged

2) Give the relationship between substrate concentration and reaction velocity.

3) What do you understand by enzyme specificity? List the four different types of enzyme specificities

4) How are enzymes different from inorganic catalysts?

5) What are coenzymes? How are the coenzymes grouped?

6) Explain the following terms giving suitable examples.
   a) Competitive inhibition
   b) Uncompetitive inhibition
   c) Non-competitive inhibition

NOTES

UNIT

5

DIGESTION, ABSORPTION AND TRANSPORT OF CARBOHYDRATES, PROTEINS AND LIPIDS

STRUCTURE

5.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

recognize the organs of the digestive system and their functions,

understand the process of digestion in stomach and intestine,

discuss the role of various enzymes in facilitating the digestion process,

explain the hormonal and neural control of secretions of salivary glands, gastric glands, pancreas, liver and intestine, and

differentiate between the types of absorption and transport of final end products across the intestinal brush border into the portal and lymphatic system.

5.2 INTRODUCTION

Nutrition emphasizes the role of foodstuffs in meeting the enery and specific requirements of the body for continuation of cellular activity. Although food assumes a wide variety of forms, it is categorized into major six chemical forms

i.e. carbohydrates, proteins, lipids, vitamins, minerals and water and these are collectively termed as 'nutrients'. The first three units in this block focused on the chemistry of these nutrients and their properties. In this unit, we shall deal with their digestion, absorption and transportation.

Next, once ingested, what happens to these nutrients in our body? These nutrients, as you may already know, are utilized to perform the various functions i.e. provide energy, body building, protection against diseases etc. in our body. Of the major six nutrients, only carbohydrates, proteins and lipids can give energ,'. The ingested food material is broken down into smaller constituents which are then passed into the gastrointestinal tract and then into the bloodstream. The process through which the major nutrients namely carbohydrates, proteins and lipids are converted to simple substances and then passed into the gastrointestinal tract and then into the bloodstream is called digestion and absorption. In this unit we shall focus on the processes of digestion, absorption and transport of nutrients in our body, with emphasis on the role of various enzymes which facilitate the process.

5.3 DIGESTION, ABSORPTION AND TRANSPORT - BASIC CONCEPT

What is digestion? The ingested food material is broken down into smaller constituents which are assimilable by the blood. The process through which the major nutrients namely carbohydrates, proteins and lipids are converted to simple sugars, amino acids, fatty acids and glycerol respectively, while passing from mouth to small intestine, constitutes digestion. The process of digestion is facilitated with the help of secretions from salivary glands, stomach, pancreas and liver. Both hormonal and neural controls regulate these secretions. Microvilli of the small intestine are the major site of absorption. So then what is absorption? Absorption involves the transfer of materials through the mucosa of the alimentary tract into blood and lymph vessels. The transfer comprises of different transport mechanisms. In the next few sections we shall learn about each of these processes i.e. digestion, absorption and transport of carbohydrates, proteins and lipids. We shall begin with digestion.

5.4 DIGESTION

Digestive enzymes break down food particles into smaller units. You will see that the final breakdown products of protein digestion are single amino acids or small chains of two or three amino acids. The final products of carbohydrate digestion are monosaccharides. The final digestive products of triacylglycerol digestion are free fatty acids and glycerol and monoacylglycerols. Vitamins, minerals, water and some larger fat-like compounds such as cholesterol are not broken down before they are absorbed.

Where does the digestion of food occur in our body?

Obviously, it occurs in the digestive system. The human digestive system is a coiled, muscular tube (6-9 meters long when fully extended) extending from the mouth to the anus. Several specialized compartments occur along this length-mouth, pharynx, orsophagus, stomach, small intestine, large intestine and anus. Basically, the digestive system is made up of two groups of organs:

Alimentary tract, which includes mouth, pharynx, oesophagus, stomach, small intestine, large intestine, appendix, rectum, anal canal, and

Accessory organs, which include tongue, teeth, salivary glands, liver, gall bladder and pancreas.

The major function of the digestive system is to ingest the food materials, digest it to absorbable end products, absorb these products and eliminate the unusable material. That sounds simple. But actually the process involved is not all that simple. Starting from the mouth, the process of digestion through the different compartments of the digestive system is discussed next.

5.4.1 Digestion in the Mouth

The mouth receives food. The tongue serves in swallowing, manipulating the food for chewing and in perceiving taste. The teeth mechanically subdivide the food for easier digestion. Then, there are the salivary glands. There are three pairs of salivary glands namely parotid, submaxillary and sublingual which secrete the colourless viscous fluid called 'saliva'. Saliva helps in swallowing the food by lubricating the food and the neutral pH of saliva prevents the decalcification of teeth and keeps the mouth clean. In the mouth, is also present the salivary amaylase, originally called ptyalin. 'E*he presence of salivary amylase starts the starch digestion by breaking it into dextrins. Dextrins are polysaccharides containing fewer simple sugar units than starches. The salivary secretion is controlled by nervous reflexes. From the mouth, the partly digested food enters the stomach. What happens there? Let's find out.

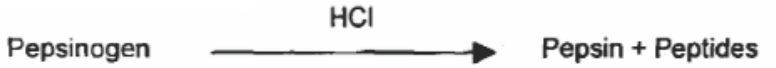
5.4.2 Digestion in the Stomach

To understand the digestion mechanism in the stomach, it is important to know about the anatomy of the stomach.The stomach is a 'J' shaped organ and secretes gastric juice. The major components of gastric juice are hydrochloric acid and the enzyme pepsin, which is secreted in an inactive form called pepsinogen. Small amount of lipase is also produced but remains inactive because of the acidic nature of gastric juice. Rennin is present only in infants and helps in the digestion of milk protein 'casein'. Hydrochloric acid (pH I .2 — 1.5) activates the inactive pepsinogen to the active form pepsin' as indicated in the Figure 5.1. Hydrochloric acid denatures the food proteins for easy digestion. The germicidal effect of hydrochloric acid also prevents the growth of microorganisms in the stomach.

Digestion of proteins is initiated in the stomach by pepsin. Pepsin acts upon food proteins and converts them to proteoses and peptones which are large polypeptide derivatives as can be seen in Figure 5.1. Pepsin is an endopeptidase

since it hydrolyses peptide bonds within the main polypeptide structure. Pepsin specifically acts upon the peptide bonds formed by amino acids with an aromatic ring (e.g. tyrosine) or dicarboxylic amino acid i.e. amino acids with 2 COOH groups (e.g. glutamate).

In infants, the presence of rennin helps in digestion of casein to paracasein which is then acted upon by pepsin.



The pepsin formed as an active proteolytic enzyme activates the remainder of the pepsinogen molecules by proteolytically converting them to pepsin.

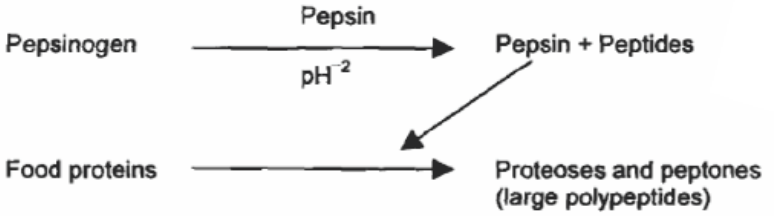


Figure 5.1: Role of pepsin

A word about the control of gastric secretion. Gastric secretion is initiated by nervous reflexes and the continued secretion is controlled by the hormone 'gastrin' produced by the stomach Histamine, the decarboxylated amino acid 'histidine' also stimulates gastric juice production.

Next, from the stomach the partly digested food passes into the intestine via the pancreas.

5.4.3 Role of Pancreas in Digestion

Pancreatic juices secreted from the pancreas aid in digestion of the food. About 600-800 ml of fltiid i.e. the pancreatic juice is secreted per day by pancreas. Pancreatic juice is alkaline in nature (pH about 8.0) and contains both organic and inorganic substances.

The enzyme components are trypsin, chymotrypsin, elastase and carboxypeptidase, all of which are secreted as inactive precursors -d zymogens. The other enzymes are amylase, lipase, cholesteryl ester hydrolase, phospholipase, ribonucleases, deoxyribonucleases and phosphodiesterases. Sodium, chloride and bicarbonate are the major inorganic constituents.

Before we proceed further, let us understand what we mean by zymogens. Zymogen is actually the enzyme molecule synthesized, but in an inactive form. Once the food comes into the stomach/intestine, the inactive zymogen gets converted into the active enzyme. The example given herewith explains the concept clearly. Pepsinogen, we learnt earlier in the sub-section 5.3.2 is the inactive form, which actually is a zymogen. HCI in the stomach activates the inactive pepsinogen to the active enzyme pepsin.

Pepsinogen ⟶ Pepsin
(Z~mogen) (Active Enzymes)

NOTES

A. *Trypsin*

Trypsin is secreted in the inactive form Trypsinogen which is converted into the active form trypsin by the enzyme enterokinase secreieu by the duodenal mucosa. Trypsin acts upon the native proteins, proteoses and peptones and converts them to polypeptides and peptides as indicated in Figure 5.2. It attacks the peptide linkages containing arginine or lysine residues.

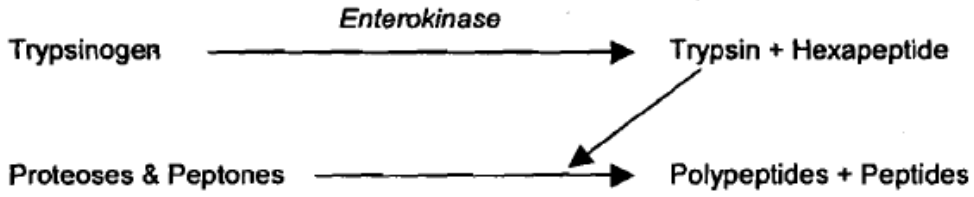


Figure 5.2: Role of trypsin

B. *Chymotrypsin*

Chymotrypsin is secreted in an inactive form 'chymotrypsinogen', which is activated by trypsin as shown in Figure 5.3. Chymotrypsin is specific for peptide bonds containing uncharged amino acid residues such as aromatic amino acids.

C. *Elastase*

The inactive proelastase is activated by trypsin to the active form elastase as can be seen in Figure 5.3. Elastase attacks peptide bonds next to the small amino acid residues such as glycine, alanine and serine and has a broader specificity than the other two enzymes.

All the three enzymes viz. trypsin, chymotrypsin and elastase are endopeptidases (a subclass of peptide hydrolases that hydrolyse the more centrally situated peptide bonds). You have already seen that pepsin is also an endopeptidase.

D. *Carboxypeptidase*

The inactive zymogen procarboxypeptidase is activated by trypsin as shown in Figure 5.3. The further action on the polypeptides is carried out by carboxypeptidase which attacks the carboxy terminal peptide bond, liberating single amino acids. So you realize that carboxypeptidase hydrolyses peptide bonds from the end of the peptide chain. Therefore, it is called as an exopeptidase (unlike trypsin, chymotrypsin and elastase). Further, since this enzyme acts on the peptide bonds of the peptide chain containing free COOH (carboxylic) group, it is specifically called carboxypeptidase.

The action of each of the specific enzyme discussed above is summarized in Figure 5.3.

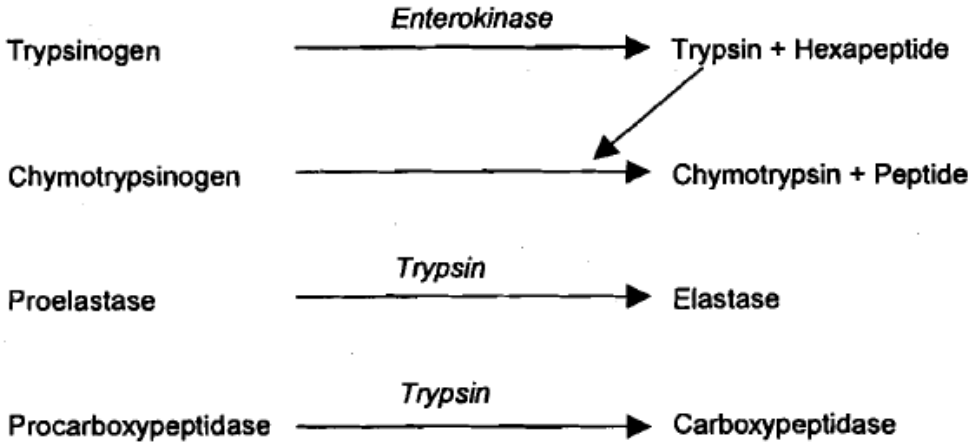


Figure 5.3: Activation of zymogens by trypsin

E. *Amylase*

The presence of pancreatic amylase brings about the breakdown of starch and glycogen and its action is similar to salivary amylase. The hydrolysis of starch and glycogen produces maltose, maltotriose and a mixture of branched oligosaccharides (a- limit dextrin), non branched oligosaccharides and some glucose.

F. *Lipase*

Pancreatic lipase specifically hydrolyses the primary ester linkages i.e. at the position 1 and 3 of triacylglycerols. The presence of bile salt helps in emulsification of fat, thereby, increasing fat digestion. 2-monoglyceride (2-monoglycerol) and fatty acids are the major end products of fat digestion and one fourth of the dietary triacylglycerol is completely broken down to glycerol and fatty acids as shown in Figure 5.4.

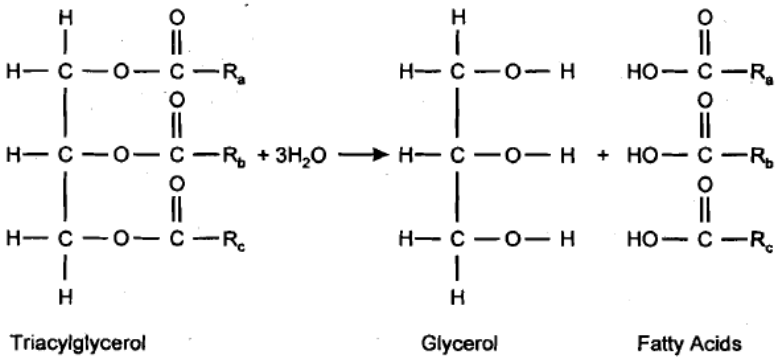


Figure 5.4: Breakdown of Triacylglycerol to glycerol and fatty acids

Cholestryl ester hydrolase acts upon cholesteryl esters to liberate free cholesterol and fattv acids. Phospholipases break down phospholipids.

G. *Ribonucleases, Deoxyribonucleases, Phosphodiesterases*

These enzymes are responsible for the degradation of dietary nucleic acids.

Ribonucleases and deoxyribonucleases secreted by the pancreatic juice hydrolyze RNA and DNA to oligonucleotides. These are further hydrolyzed by pancreatic phosphodiesterases producing a mixture of 3' and 5' mononucleotides. Nucleotidases remove the phosphate groups hydrolytically releasing nucleosides. These are absorbed by the mucosal cells or may be further degraded to free bases before uptake.

We have so far studied about the different enzymes produced by the pancreas. How are these pancreatic secretions controlled? Let's find out

The pancreas releases its digestive juice when stimulated by secretin, a hormone produced in the duodenum in response to chyme (the complex contents of the stomach). Secretin causes the secretion of an almost protein free electrolyte solution that has a high concentration of bicarbonate. Pancreozymin or cholecystokinin-pancreozymin is a hormone released from the mucosa of the duodenum, which causes the release of an enzyme — rich juice from the pancreas.

5.4.4 Role of Bile in Digestion

Bile is produced by liver and stored in gall bladder. During digestion, the gall bladder contracts and supplies bile, rapidly to the duodenum through the common bile duct.

As compared to the bile produced by liver (hepatic bile), the bile stored in gall bladder is more concentrated (since water is absorbed during the storage period in 'the gall bladder) and high in bile salts, cholesterol ari pigments. Bile acids-are cholic acid and deoxycholic acid and salt of these acids with glycine and taurine are known as bile salts. Bile salts have a considerable ability to lower surface tension. By this property, they prevent the coming together of the small fat droplets in the intestine and thus allow more rapid digestion of the fat. This is called the emulsification action of bile. Bile salts also combine with fatty acids and render them more easily absorbed. This is called hydrotropic action of bile. The presence of bile in the intestine is to accomplish digestion and absorption of fat and also of fat-soluble vitamins.

Finally, the partly digested food enters the intestine. Let us study about the process of digestion in the intestine.

5.4.5 Digestion in the Intestine

About 2 to 3 liters of alkaline fluid is secreted every day by the intestine and completes the digestive process. Sodium bicarbonate and sodium chloride are the main inorganic components. The organic components include enzymes such as aminopeptidases, dipepetidases of various specificity, specific disaccharidases, phospholipases etc. Let us get to know about these enzymes and about the control of these (intestinal) secretions.

Enzymes of intestinal juice

Aminopepetidase is an exopeptidase and cleaves peptide bonds next to N-terminal amino acids of polypeptides and oligopeptides (unlike carboxypeptidase which acts on peptide bond at the C (carboxy) terminal). Dipeptidase completes the digestion of dipeptides to free amino acids.

Next, let us see how the intestinal secretions are controlled.

Control of intestinal secretion

There are specific disaccharidases and oligosaccharidases i.e. a glucosidase (maltase) which removes single glucose residues from α (1—>4) linked oligosaccharides and disaccharides, starting from the non-reducing ends. Isomaltase (α-dextrinase) hydrolyses1→6 bonds in a-limit dextrins. ß-galactosidase (lactase) removes galactose from lactose (i.e. hydrolyses lactose to galactose and glucose) and sucrase hydrolyses sucrose to glucose and fructose.

The intestinal juice contains phospholipase that attacks phospholipids to produce glycerol, fatty acids, phosphoric acid and bases such as choline.

The intestinal secretion is stimulated by enterokinase produced by jejunum, of the small intestine, which in turn is released by the entry of acid chyme from the stomach to duodenum.

In this section we studied about the process of digestion in the mouth → stomach pancreas → intestine. We have seen that the process of digestion is achieved through the secretions (enzymes) secreted by the organs of the digestive system. Next, we shall look at the digestion of food material. But, first let us try to answer the questions given in check your progress 1. These will test your understanding on the topic covered so far.

STUDENTS ACTIVITY - 1

1) What do you understand by the term 'digestion'? What is the major site of absorption?

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2) List the various organs involved in digestion process.

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3) List the enzymes of intestinal juice.

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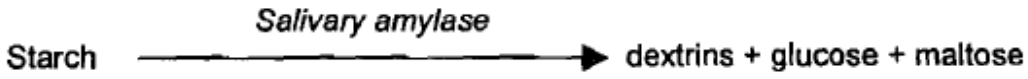
5.5 DIGESTION OF FOOD MATERIALS

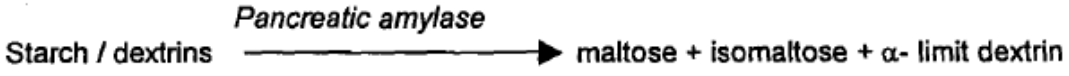
Digestion, you know, involves the mixing of food, its movement through the digestive tract and chemical breakdown of the large molecules of food into smaller molecules. Digestion, you also learnt, begins in the mouth, when we chew and swallow, and is completed in the small intestine. The chemical process varies somewhat for different kinds of food/nutrients i.e. for carbohydrates, proteins, fats etc. In this section, we shall learn about the chemical process involved during the digestion of these nutrients in the body. We shall start with the digestion of carbohydrates.

5.5.1 Digestion of Carbohydrates

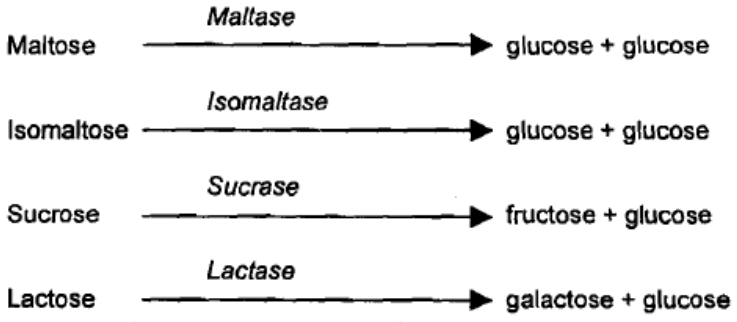
In the last section we saw that the digestion of food starts in the mouth itself by the action of enzyme salivary amylase and the carbohydrates present in the food, particularly the starch, are broken down by the salivary amylase to starch dextrins, glucose and maltose. The presence of hydrochloric acid in the stomach stops further action of salivary amylase when the food enters the stomach.



When the chyme enters the duodenum, the alkaline pH of the pancreatic juice helps in the digestion of starch / dextrin and glycogen by pancreatic amylase. The end products are maltose, isomaltose etc.



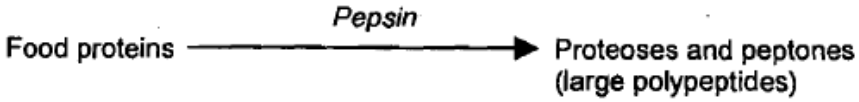
The enzymes from brush border membrane of small intestine complete the digestion of various disaccharides of the diet and the product of pancreatic amylase action such as maltose, isomaltose, sucrose, lactose. The final products of carbohydrate digestion are monosaccharides i.e. glucose, fructose and galactose as shown herewith.



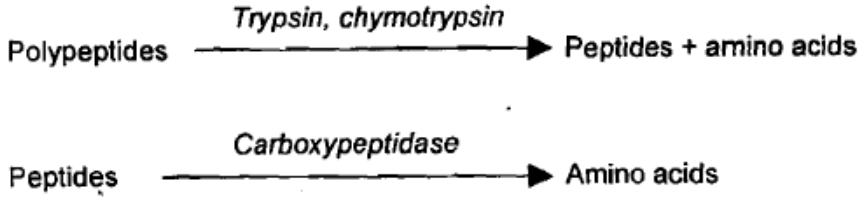
Remember, cellulose is not digested in the human GI tract due to the absence of the enzyme cellulase.

5.5.2 Digestion of Proteins

The proteolytic enzymes secreted by gastric juice, pancreatic juice and intestinal juice cause the hydrolysis of proteins in the gastrointestinal tract. With the exception of the intestinal peptidases, all proteolytic enzymes are activated by the conversion of inactive 'lgrge precursors called zymogens to functional enzymes. Recall reading about this in the last section. The enzyme pepsin acts on food proteins and converts them into proteoses and peptones.



The polypeptides formed in the stomach are digested in the intestine by trypsin,chymotrypsin, elastase and carboxypeptidases secreted in the pancreatic juice.



The products of these enzymes are free amino acids, dipeptides and small peptides. The residual peptides are hydrolysed in the intestinal mucosal cells by aminopeptidases and dipeptidases. Figure 5.5 graphically presents the process of protein digestion. The final breakdown products of protein digestion are single amino acids or small chains of two or three amino acids.

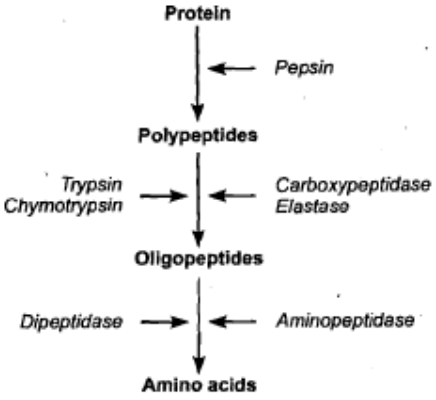


Figure 5.5: Process of protein digestion

The proteolytic enzymes have some remarkable specificity for clearing protein chain at certain amino acid residues. The specificities are summarized in Table 5. 1.

Table 5.1: Specificity of some proteolytic enzyme

Enzyme	Occurrence	pH Optimum	Major site of action
Trypsin	Intestine	7.5 to 8.5	Arginyl, lysyl bonds
Chymotrypsin	Intestine	7.5 to 8.5	Aromatic amino acid bonds (Phe, Trp, Tyr)
Pepsin	Stomach	1.5 to 2.5	Wide rang_e of spe ificit
Carboxypeptidase	Intestine	7.5 to 8.5	C-tenninal amino acid
Aminopeptidase	Intestinal mucosa		N-tenninal amino acid

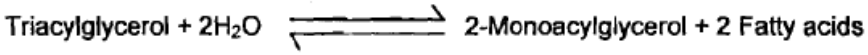
5.5.3 Digestion of Lipids

Even though lipase is present in the stomach, it remains inactive due to the acidic environment of the stomach contents. Therefore, the ingested fat is primarily digested in the small intestine.

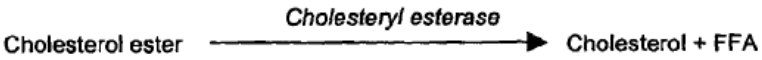
Fat present in the food is mixed with bile and pancreatic secretion while reaching the duodenum. The bile salts emulsi$' the lipids before the action of pancreatic lipase.

Pancreatic lipase acts on the dietary triacylglycerols after it has been incorporated into the mixed micelle in the intestinal lumen. The lipase acts at interface between water and the triacylglycerol molecules, and its catalytic action is facilitated by the presence of colipase (a small protein cofactor needed by pancreatic lipase for efficient dietary lipid hydrolysis), which is also produced by the pancreas.

Pancreatic lipase is specific for the fatty acid residues at positions I and 3 of the glyceryl moiety thus releasing 2-monoacylglycerol and two fatty acids from a triacylglycerol molecule. The final digestive products of triacylglycerol digestion are free fatty acids, glycerol and monoacylglycerols.



After hydrolysis, the products diffuse from the micelle to the intestinal mucosal cell membrane. The phosphoglycerides present in the diet are digested by pancreatic phospholipase and cholesteryl esters are hydrolyzed to cholesterol and fatty acids through the action of cholesteryl esterase.



The action of various phospholipases is presented in Figure 5.5. Phospholipase A1 hydrolyzes fatty acids attached to carbon I of the phospholipids, whilephospholipase removes the fatty acid attached to carbon 2. It is found in pancreatic juice and snake venom. In each case, the product is called lysophospholipid. Phospholipase B can hydrolyse both the fatty acids (at carbons 1 and 2).

Phospholipase C attacks the ester bond in position 3 and removes phosphate + base, forming 1,2-diacylglycerol. This enzyme is present in toxins secreted by

bacteria. Phospholipase D, found normally in plants, hydrolyses the nitrogenous base from phospholipids, forming phosphatidic acid.

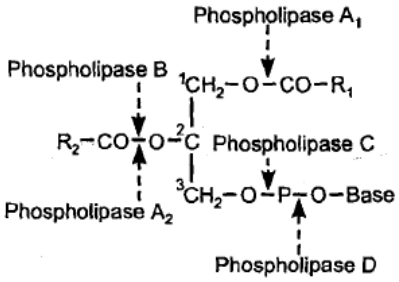


Figure 5.6: Action of various phospholipases

5.5.4 Digestion of Nucleic Acids

Several nucleases present in the pancreatic juice like RNAase and DNAase digest various nucleic acids. These enzymes are endonucleases which hydrolyze internal phosphodiester bonds which are located more centrally in the nucleic acid. They form shorter length chains with 3'-hydroxyl and 5'-phosphotyl or 5'-hydroxyl and 3'-phosphoryl terminus. Some act on both the strands, whereas, others can hydrolyze only single strand of nucleic acids. Phosphatases present in the intestinal juice remove phosphate from hexophosphates, glycerophosphates and nucleotides derived from the food. Further, the nucleotidases and nucleosidases present in the intestinal juice hydrolyze it to the level of respective bases as shown in Figure 5.7.

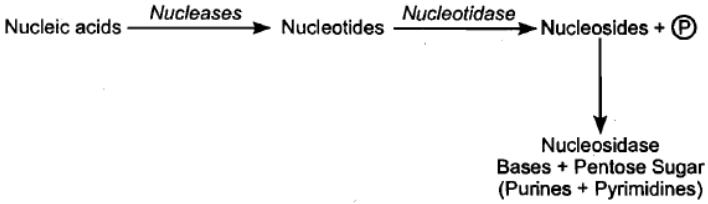


Figure 5.7: Digestion of nucleic acids

With this, we end our study on digestion. We move to the absorption process next.

But before that, let us recapitulate what we have learnt till now.

STUDENTS ACTIVITY -2

1) Name the various enzymes involved in the digestion of carbohydrates. Enumerate the reactions catalyzed by these.

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2) What are the final breakdown products of proteins? Explain giving suitable reactions involved.

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3) Explain the role of lipase in lipid digestion.

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4) What are 'endonucleases'? What is their site of action and end products?

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5.6 ABSORPTION AND TRANSPORT

We already know that the movement of substances into or across tissues, in particular, the passage of nutrients and other substances into the walls of the gastrointestinal tract and then into the bloodstream is referred to as absorption.

The small intestine is the main digestive and absorptive organ. Most absorption occurs in the duodenum and jejunum (second third of the small intestine).

Transport across the intestine may be active or passive. Active transport requires energy, whereas passive transport does not. Also, active transport may involve movement of a substance against a concentration gradient (that is, from a region of lower to higher concentfation), while substances that are passively transported always move with the concentration gradient. Facilitated diffusion is a type of passive transport which, unlike simple diffusion, uses a carrier. It is therefore more rapid than simple diffusion. Active transport mechanisms have been identified for intestinal absorption of many substances including glucose, galactose, amino acids, calcium, iron, folic acid, ascorbic acid, thiamin and bile acids. Fructose, riboflavin and vitamin B_{12} (in combination with intrinsic factor) are among the substances absorbed by facilitated diffusion.

5.6.1 Absorption of Carbohydrates

Absorption of carbohydrates is via both passive and active transport. Let us learn how.

1) *Passive transport*

The end products of carbohydrate digestion are monosaccharides. They are absorbed from the jejunum into the portal blood system, through which they are transported first to the liver and then to the rest of the body. Pentoses and fructose pass across the intestinal barrier by simple passive diffusion and also by facilitated diffusion involving carrier proteins.

1) *Active transport*

D-glucose and D-galactose are absorbed by an active transport mechanism.

The active transport of glucose is illustrated in Figure 5.8. aansport of glucose is facilitated by a carrier protein, which has separate binding sites for glucose and sodium. Both are transported through the plasma membrane and after releasing sodium and glucose into the cytosol (liquid medium of the cytoplasm), the carrier protein return to take up fresh load. The sodium is transported down its concentration gradient and at the same time causes the carrier to transport glucose against its concentration gradient. The free enerO' required for this active transport is obtained from the hydrolysis of ATP linked to sodium pump which expels Na^+ from the cell.

The active transport of glucose is inhibited by Ouabain (a cardiac glycoside), an inhibitor of the sodium pump and by phlorihizin, a known inhibitor of glucose re-absorption in the kidney tubules.

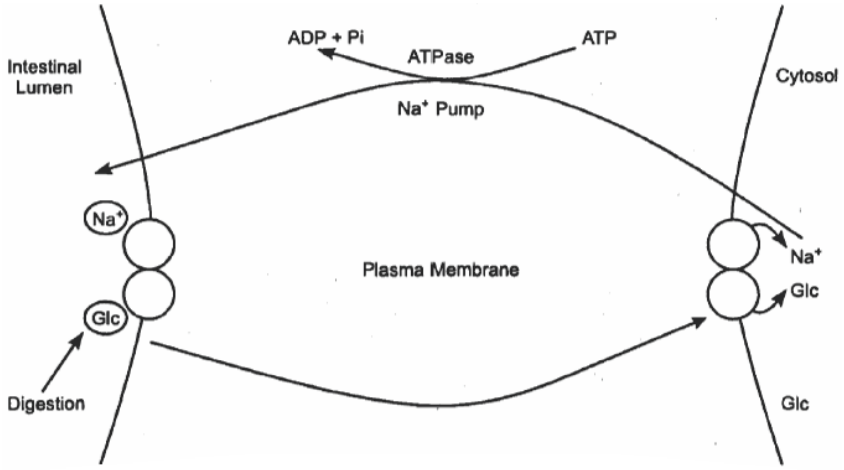

Figure 5.8: Active transport of glucose

There is also a sodium independent carrier of glucose.

5.6.2 Absorption of Proteins

in sub-section 5.3.2, we have seen how the proteolytic enzymes break the dietary proteins into amino acids or di- and tripeptides. In fact, it is in this form that the dietary proteins are absorbed. Dietary proteins are, with very few exceptions, not absorbed. Digestion of proteins and absorption of amino acids goes on throughout the small intestine. We shall look at the mechanism of absorption of amino acids and peptides next.

Absorption of amino acids and peptides

Generally, the dietary proteins are almost completely digested to their constituent amino acids and these are rapidly absorbed from the intestine into the portal blood. Some of the dipeptides are hydrolyzed by peptidases located in the absorptive cells so that only amino acids are released into the portal blood. D-amino acids are absorbed by simple diffusion but the L-amino acids (occurring in foods) require a

carrier system in the absorption.

The mechanism by uhich amino acids are absorbed is conceptually identical to that of monosaccharides. The luminal plasma membrane of the absorptive cell bears at least four sodium-dependent amino acid transporters (one each for acidic, basic, neutral and aromatic amino acids), as highlighted in Table 5.2. Each system transports amino acids that are structurally similar. These transporters bind amino acids only after binding sodium. The fully loaded transporter then undergoes a conformational change that dumps sodium and the amino acid into the cytoplasm, followed by its reorientation back to the original form. Thus, absorption of amino acids is also absolutely dependent on the electrochemical gradient of sodium across the epithelium. The energy dependent carrier system also involves vitamin B6 (pyridoxal phosphate) during the transport of amino acids.

Apart from amino acids, a substantial amount of small peptides are also absorbed by stereospecific (determined by structure of amino acid) transport systems. These small peptides are absorbed without dependence on sodium, probably by a single transport molecule. The uptake mechanisms for peptides are separate from those for amino acids.

Table 5.2: Amino acid transport systems

Amino acid specificity	Examples of amino acid transported
1 Small neutral amino acids	Alanine, serine and threonine
2 Large neutral and aromatic amino acids	Isoleucine, leucine, valine, tyrosine, tryptophan, phenylalanine
3 Basic amino acids	Arginine, lysine, ornithine
4 Acidic amino acids	Glutamic acid, aspartic acid

Absorption of proteins in infants

In infants, permeability of the intestine appears to be greater than in later life, and some large protein molecules such as antibodies in the mother's milk and protein allergens pass by diffusion through the wall. Pinocytosis appears to account for the absorption of these large molecules in children and adults. Pinocytosis, as you may recall reading in the Applied Physiology Course, is a process by which certain cells can engulf and incorporate droplets of fluid. Look up Unit 2, sub-section 2.3.1 in the Applied Physiology Course for understanding this process.

Next, let us.look the absorption of lipids.

5.6.3 Absorption of Lipids

Lipids are absorbed by a mechanism distinctly different from what we have seen for monosaccharides and amino acids. In fact absorption of dietary triacylglycerols and medium chain triacylglycerols in the food also varies.

Let us get to know this process in greater detail.

Absorption of long-chain triacylglyerols

The triacylglycerols present in the ordinary food that we eat contains long chain fatty acids (mostly 16 and 18 carbon-atom saturated and unsaturated fatty acids). Pancreatic lipase specifically acts upon the fatty acid residues at positions I and 3 of the glyceryl moiety thereby producing 2-monoacylglycerols and the released fatty acids can pass through cell membrane, and they are absorbed by diffusion into the mucosal cells of the jejunum and ileum. There is resynthesis of triacylglycerol moiety inside the mucosal cells and this reformed triacylglycerol is coated with protein, cholesterol or phospholipid to form tiny globules called chylomicrons as shown in Figure 5.9. Instead of being absorbed directly into capillary blood, these chylomicrons are transported first into the lymphatic vessel that penetrates into each villus. Chylomicron-rich lymph then drains into the system lymphatic system, which rapidly flows into blood. Blood-borne chylomicrons are rapidly disassembled and their constitutent lipids utilized throughöut the body.

Absorption of medium-chain triacylglycerols

Medium chain triacylglycerols with fatty acids contain less than 10 to 12 carbons are absorbed intact either onto the mucosal cell villi or into the cells and are hydrolyzed to free fatty acids and glycerol by the lipase present inside the cell. The released fatty acids pass directly into the portal vein. Plasma albumin acts as a carrier for these fatty acids and delivers them to the liver as fatty acids by portal circulation.

The difference in digestion and absorption of dietary triacylglycerols and medium chain triacylglycerols is illustrated in Figure 5.9.

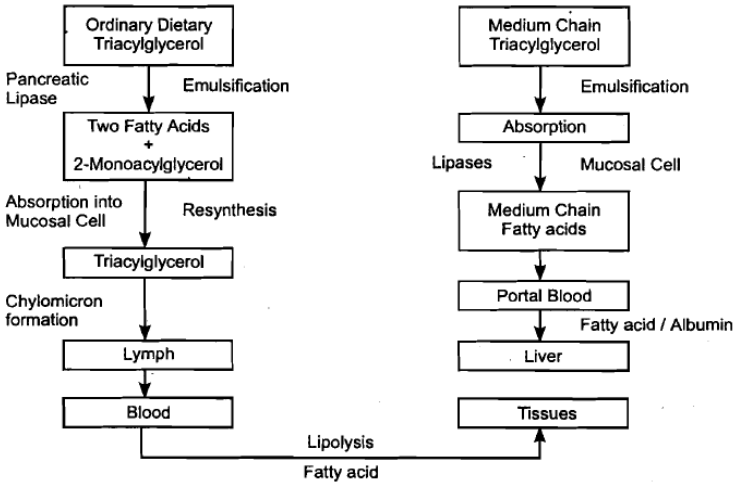

Figure 5.9: Difference in digestion and absorption of dietary triacylglycerols and medium chain triacylglycerols

Another lipid of importance that is absorbed in the small intestine is cholesterol.

A specific transport protein has been identified that ferries cholesterol from the intestinal lumen into the enterocyte. From there, cholesterol is incorporated into chylomicrons and shuttled into blood by the mechanisms described above.

NOTES

So we have looked at how the fats are absorbed in the body. Now the fats (lipids) absorbed from the diet must be transported in the blood. Blood plasma is a watery environment. We also know that lipids are insoluble in water. So how are they transported in blood? Well, fats are insoluble in water and so lipid compounds such as cholesterol, fatty acids, oil soluble vitamins and triglycerides need to be associated with proteins, forming water soluble lipoproteins, in order to be transported around the body.

Most lipids are transported in the blood as triacylglycerols within lipoprotein (particles containing a core of hydrophobic lipids surrounded by a shell of proteins, phospholipids and cholesterol). Four major groups of lipoproteins help in transport of lipids. These include:

1. Chylomicrons-function is to carry dietary triacylglycerols
2. Very low density lipoprotein (VLDL)-function is to carry endogenously made triacylglycerols
3. Low density lipoprotein (LDL)-function is to carry cholesterol and cholesterol esters, and
4. High density lipoprotein (HDL)-functions in reverse cholesterol transport and exchange of apoproteins.

We shall learn more about lipoproteins later in Unit 7 under lipoprotein metabolism. With this, we come to an end of our study of digestion, absorption and transport of carbohydrates, lipid and proteins.

STUDENTS ACTIVITY -3

1) List the various transport mechanisms involved in the absorption of various nutrients in the intestine, giving suitable examples.

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2) How are carbohydrates absorbed in our body?

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3) Explain the active transport of glucose.

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5.7 LET US SUM UP

In this unit we studied about the digestion, absorption and transport of carbohydrates, fats and proteins in our body. The salient features discussed include:

1. The digestive system takes in food, digests it to the end products, which are absorbable into the system. The non-absorbable products are eliminated.

2. The ingested food is broken down into end products through mechanical, chemical and enzymatic digestion.

3. Digestion involves the participation of mouth, esophagus, stomach, intestine, liver, pancreas and gall bladder.

4. Digestion also involves the participation of a number of enzymes viz., amylase, pepsin, trypsin, chymotrypsin, endopeptidases, aminopeptidases, carboxypeptidases, nucleases and lipases, which break down the dietary carbohydrates, proteins and lipids into simple units.

5. Control of pancreatic, liver and intestinal secretion is by hormones.

6. Most of the above enzymes are secreted in the form of pro-enzymes and get converted into active form just before their action.

7. The end products are absorbed into the system through various transport mechanisms such as active, passive mechanism etc.

5.8 GLOSSARY

Absorption : taking in the molecules from the GI tract.

Active transport : using carriers, energ (ATP) and enzymes of cell to cause a substance to cross a membrane.

Bile : a clear yellow or orange fluid produced by the liver.

Brush border : a specialization of the free surface of a cell, consisting of minute cylindrical processes (microvilli) that greatly increase the surface area.

Chyme : the thick semi-fluid mixture of partially digested foods and digestive juices found in the stomach and small intestine.

Dextrins : polysaccharides containing fewer simple sugar units than starches.

Digestion : breaking of foods using chemical or mechanical- means to convert foods into chemical compounds, which could be absorbed.

Facilitated diffusion : a type of passive trarisport that uses a carrier.

Micelle : a submicroscopic aggregation of molecules, as a droplet in a colloidal system. Micelles are essentially small aggregates of mixed lipids and bile salts suspended within the ingesta

Pinocytosis : the ingestion of dissolved materials by endocytosis. The cytoplasmic membrane invaginates and pinches off placing small droplets of fluid in a pinocytic vesicle. The liquid contents of the vesicle are then slowly transferred to the cytosol.

Serum lipoproteins : spherical or ellipsoidal particles containing proteins, cholesterol esters and triacylglycerols, encased within a monolayer of phospholipids and cholesterol.

Zymogen : an inactive form of an enzyme; becomes active prior to its action.

5.9 CHECK YOUR PROGRESS

1). Describe the digestive process occuring in the stomach. .

2) How are lipids transported in blood? List the four major groups of lipoproteins involved in lipid transport.

3) Describe the composition of pancreatic juice.

4) Explain the process of absorption of amino acids and peptides..

5) Explain the emulsification action of bil

UNIT

6

CARBOHYDRATE METABOLISM

STRUCTURE

6.1 LEARNING OBJECTIVE

After going through this unit, you will be anle to:

describe the conversion of various monosaccharides into glucose,

identify the various metabolic pathways available for glucose in the cell,

work out the energy (ATP) production when glucose is oxidized in various metabolic pathways, glycolysis, citric acid cycle etc.,

illustrate the reactions involved when the availability of glucose is more than needed through glycogenesis and when the availability of glucose is less than needed through glycogenolysis,

find out the interconversion of various monosaccharides with 3 carbon to 7 carbon along with their biochemical significance,

discuss the synthesis of glucose from non carbohydrate sources (gluconeogenesis),

elaborate on the role of various hormones in the carbohydrate metabolism,

describe the regu lation of blood glucose levels, and

explain electron transport chain and oxidative phosphorylation.

NOTES

6.2 INTRODUCTION

Metabolism comprises of various cellular reactions. These reactions are important for maintaining the structural integrity of the cell. Structural integrity imparts the functional characteristics to the cell. Metabolism provides the energy for the cellular activities. In the earlier unit we learnt that the chemical nature ofthe energy in a cell is ATP (Adenosine triphosphate).

Glucose is the principal carbohydrate involved in ATP production. Glucose produces ATP by undergoing various structural changes catalyzed by several enzymes. The sequences of enzymatic reactions collectively constitute metabolic pathways wherein the product of one enzyme reaction becomes the substrate to the next reaction in the sequence. These successive products of the reaction are termed as metabolites or metabolic intermediates. In this unit, various metabolic pathways that glucose can take in a cell are described i.e. glycolysis, gluconeogenesis.

6.3 CARBOHYDRATE METABOLISM: AN OVERVIEW

We already studied that carbohydrates are broken down into monosaccharides which are absorbed into the blood stream. In the liver and muscles, most of the glucose is changed into glycogen by the process of glycogenesis (anabolism). Glycogen is stored in the liver and muscles until needed at some later time when glucose levels are low. If blood glucose levels are low, then epinephrine and glucagon hormones are secreted to stimulate the conversion of glycogen to glucose. This process is called glycogenolysis (catabolism).

If glucose is needed immediately upon entering the cells to supply energy, it begins the metabolic process called glycolysis (catabolism). The end products of glycolysis are pyruvic acid and ATP. Since glycolysis releases relatively little ATP, further reactions continue to convert pyruvic acid to acetyl CoA and then citric acid in the citric acid cycle. The majority of the ATP is made from oxidations in the citric acid cycle in connection with the electron transport chain.

During strenuous muscular activity, pyruvic acid is converted into lactic acid rather than acetyl CoA. During the resting period, the lactic acid is converted back to pyruvic acid. The pyruvic acid in turn is converted back to glucose by the process called gluconeogenesis (anabolism). If the glucose is not needed at that moment, it is converted into glycogen by glycogenesis. These processes are summarized in the Figure 6.1. A detailed discussion on each of these processes is included in the following sections.

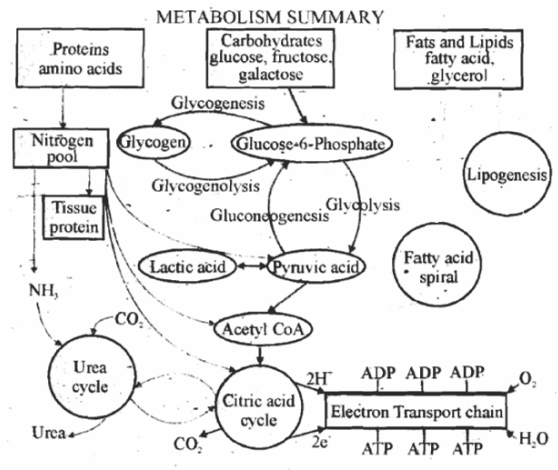

Figure 6.1: Carbohydrate metabolism summary

6.4 GLYCOLYSIS

The glycolytic pathway is also called the Embden-Meyerhof Pathway (EM Pathway) and is employed by all tissues for the utilization of glucose to generate energy (in the form of ATP) and intermediates for other metabolic pathways. Pyruvate, as you have read above, is the end product of glycolysis in cells with mitochondria and an adequate supply of oxygen.

A series of ten reactions are called aerobic glycolysis because oxygen is needed to reoxidise the NADH formed during the oxidation of glyceraldehyde-3-phoSVhate. In anaerobic glycolysis, the glucose is converted to pyruvate, which is reduced by NADH to form lactate and there is no net formation of AT P. Aerobic glycolysis yields net 8 molecules of ATP per molecule of glucose, whereas anaerobic glycolysis results in net 2 molecules of ATP generation from one molecule of glucose. Anaerobic glycolysis allows the continued production of ATP in tissues that lack mitochondria (for e.g. red blood cells) or in cells deprived of sufficient oxygen.

6.4.1 Glycolytic Pathway

Glycolysis, a series of ten reactions that occur in the cytoplasm, is a process in which one glucose molecule is converted into two molecules of pyruvate. The glycolytic pathway comprises of two stages:

In the first phase, energy is utilized in the synthesis of phosphorylated form of glucose.

In the second phase, energy is generated in the form of ATP. 8 molecules of ATP are produced per molecule of glucose metabolized when pyruvate is the end product and only 2 molecules of ATP are produced per molecule of glucose metabolized when lactate is the end product.

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The sequence of reactions involved in the entire glycolysis pathway is given in Figure 6.2.

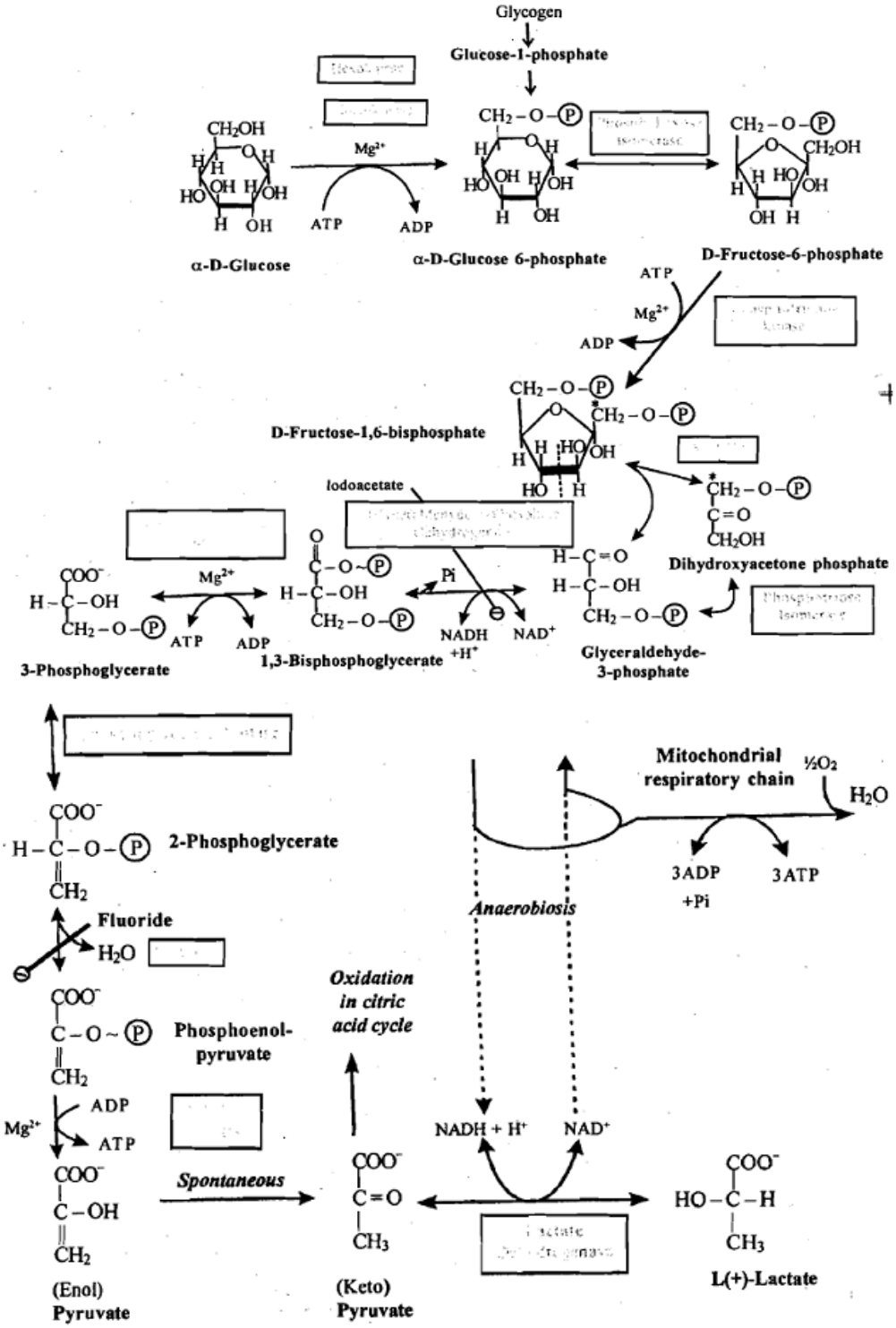

Figure 6.2 : Reactions of Glycolysis

First phase

As mentioned above, the first step in glycolysis is the synthesis of phosphorylated forms of glucose. This and the other steps are enumerated herewith:

1) Phosphorylation of glucose: Glucose is converted to glucose-6-phosphate since phosphorylated intermediates do not readily penetrate cell membrane and this commits glucose to further metabolism in the cell. Hexokinase catalyses this irreversible reaction in most tissues and in liver; glucokinase is the predominant enzyme for the phosphorylation of glucose. Hexokinase is an allosteric enzyme that is strongly inhibited by the product glucose-6-phosphate.

   Hexokinase exists in many isozyme forms and can act upon any aldo- or keto- hexose but has a low (20 gM) (i.e. high affinity) for glucose. Glucokinase has high Km (12 mM) for glucose, not inhibited by the product glucose-6-phosphate and is an inducible enzyme induced by carbohydrate rich diets and insulin.

2) Isomerization of glucose-6-phosphate: This step is catalyzed by phosphoglucoisomerase to form fructose-6-phosphate. This is- a reversible reaction as can be seen in Figure 6.2.

3) Phosphorylation of fructose-6-phosphate: This is an irreversible reaction, catalyzed by phosphofructokinase, (PFK-I) a rate-limiting enzyme of glycolysis in most tissues and is the most important regulatory enzymes of glycolysis. PFK-I is activated by high concentrations of AMP and fructose—2,6-bisphosphate. Inhibitors are citrate and ATP.

4) Cleavage of fructose-I,6-bisphosphate: Aldolase cleaves fructose—I, 6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate in the reversible reaction shown in Figure 6.2.

5) Isomerization of dihydroxyacetone phosphate: Triosephosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Dihydroxyacetone phosphate must be isomerized to glyceraldehyde-3-phosphate for further metabolism in the glycolytic sequence. This isomerization results in the production of two molecules of glyceraldehyde-3-phosphate from the cleavage of fructose-I ,6-bisphosphate.

The sequence of reactions within the first phase was enumerated above. Now we move on to the second phase.

Second Phase

This phase is the energy-producing stage from triose phosphate to pyruvate. The reaction starts with the oxidation of glyceraldehyde-3-phosphate. Let us look at the reactions in this phase.

6) Oxidation of glyceraldehyde-3-phosphate: The conversion of glceraldehyde-3-phosphate to 1,3-bisphosphoglycerate is catalysed by glyceraldehyde-3-phosphate dehydrogenase and the required cofactors are NAD^+ and Pi. The NADH formed is reoxidized either via the respiratory chain or by the NADH linked conversion of pyruvate to lactate. The high-energy phosphate group at carbon of 1 , 3-bisphosphoglycerate conserves much of the free energy produced by the oxidation of glyceraldehyde-3-phosphate. This is an example of substrate level phosphorylation in which the production of a high-energy phosphate is coupled directly to the oxidation of a substrate instead of resulting •from oxidative phosphorylation via the election transport chain.

7) Formation of ATP from 1,3-bisphosphoglycerate and ADP: The high-energy phosphate group of 1,3-bisphosphoglycerate is used to synthesise ATP from ADP catalysed by phosphoglycerate kinase and is a reversible reaction. The reaction product is 3-phosphoglycerate. Two molecules of ATP are produced since two molecules of 1,3-bisphosphoglycerate are formed from one molecule of glucose. 3-phosphoglycerate may also be formed as follows: 1,3-bisphosphoglycerate is converted to 2,3-bisphosphoglyceyate (BPG) by the action of bisphosphoglycerate mutase which is present at high concentration in erythrocytes. 2,3-bisphosphoglycerate is hydrolyzed to 3-phosphoglycerate by a phosphatase. This constitutes a bypass reaction occurring in erythrocytes and no ATP is formed. However, it serves to provide 2,3-BPG which binds to haemoglobin, decreasing its affiniw for oxygen and promoting unloading ofoxygen in the tissue.

8) Shift of the phosphate group from carbon 3 to carbon 2: This reversible reaction is catalyzed by phosphoglycerate mutase.

9) Dehydration of 2-phosphoglycerate: Enolase causes dehydration of 2-phosphoglycerate to phosphoenol pyruvate.

10) Formation of pyruvate: Pyruvate kinase converts phosphoenolpyruvate to pyruvate in this irreversible reaction with the release of high-energy phosphate to form ATP as can be seen in Figure 6.2. This is the 2nd example of substrate level phosphorylation. This is one of the regulatory sites of glycolysis. Pyruvate kinase is an allosteric enzyme activated by fructose-I,6-bisphosphaté and inactivated by glucagon via cyclic AMP. Other inhibitors are ATP, alanine, fatty acid and acetyl CoA.

6.4.2 Fate of Pyruvate

In the last step of the glycoly%is cycle, we saw that pyruvate kinase converts phosphoenolpyruvate to pyruvate which is the end-product of glycolysis. In this sub- section we shall look at the fate of pyruvate.

Pyruvate has three different fates. Under aerobic conditions, pyruvate enters

mitochondria and is converted to acetyl CoA as illustrated in Figure 6.1

. The acetyl CoA enters the citric acid cycle. Reducing equivalents produce of the citric acid cycle enter the electron transport chain where oxidation is completed and ATP is synthesized. A second fate of pyruvate is conversion to lactate. This takes place in anaerobic microorganisms and in our own bodies when glycolysis occurs faster than the oxygen dependent citric acid cycle and electron transport chain can operate. Some microorganisms convert pyruvate to ethanol, the third fate of pyruvate.

In each of these three processes, NAD+ is regenerated so that glycolysis can continue. We shall learn in greater details about the first two fates of pyruvate now, starting with formation of lactate.

1) Formation of lactate and its consumption: If anaerobic conditions prevail, the reoxidation of NADH through the respiratory chain is prevented. Then the regeneration of NAD^+ from NADH is carried out by the action of lactate dehydrogenase on- pyruvate resulting in lactate and NAD^+ as shown in Figure 6.2 thereby allowing the glycolytic cycle to continue even in the absence of oxygen.

Thus tissues that function under hypoxic conditions tend to produce lactate. The advantage of using private for reoxidation of N ADH lies in the fact that pyruvate is the end product of glycolysis and would readily be available in the cells. In exercising skeletal muscle, there is accumulation of lactate, which is released into the blood and taken up by the liver where it is converted to glucose by the process called gluconeogenesis. In liver and heart; the ratio of NADH/NAD+ is lower than in exercising muscle. These tissues oxidize lactate obtained from the blood to pyruvate.

In liver, pyruvate is either converted to glucose by gluconeogenesis or oxidized in the citric acid cycle as illustrated in the Figure 6.1 and heart muscle oxidizes lactate to C02 and H20 via the citric acid cycle. Glycolysis in erythrocytes, even under aerobic conditions, always terminates in lactate since erythrocytes laek mitochondria.

Next, let us look at the second fate of pyruvate i.e. its conversion to acetyl CoA.

2) Oxidative decarboxylation of pyruvate: This is an important step in tissues with high oxidative capacity such as cardiac muscle, whereby pyruvate is converted to acetyl CoA, a major fuel of the citric acid cycle and the building block for fatty acid synthesis about which we will learn in the next unit. Besides oxygen, this pathway requires the participation of mitochondrion with a functional electron transport chain.

The third fate of pyruvate is specific to microorganisms. The carboxylation of pyruvate by pyruvate decarboxylase to form ethanol occurs in yeast and certain

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micro- organisms but not in humans. Hence, we shall not go into any further details on this aspect now.

6.4.3 Energy Production in Glycolysis

We learnt earlier that in the first phase of glycolysis, from one molecule of glucose, 2 molecules of glyceraldeyde-3-phosphate are formed. After that in the reaction of glycolysis, each product yields two molecules as highlighted herewith:

ATP generated reactions	*ATP Formed*
1) Glyceraldehyde-3-phosphate ⟶ 1,3-Bisphosphoglycerate (*respiratory chain oxidation of 2NADH. Oxidation of one NADH by the electron transport chain leads to formation of 3ATP)*	6
2) 1,3-Bisphosphoglycerate ⟶ 3-Phosphoglycerate (phosphorylation)	2
3) Phosphoenolpyruvate ⟶ Enol pyruvate (phosphorylation)	2
ATP utilized reactions	
1) Glucose ⟶ Glucose-6-phosphate	1
2) Fructose-6-phosphate ⟶ Fructose-1,6-bisphosphate	1
Therefore Net ATP generated	**8**

But in anaerobic conditions, the total number of ATP will be only two up to lactate. The regeneration of NAD+ from NADH formed in the reaction by glyceraldehyde- 3-phosphate dehydrogenase step is not via the respiratory chain but utilized by lactate dehydrogenase to fonn lactate. Thus only two ATP molecüles are synthesized in anaerobic glycolysis.

6.4.4 Regulation of Glycolysis

There are three markedly exergonic reactions in the glycolytic pathway, which are considered physiologically irreversible. These reactions are catalyzed by the enzymes hexokinase (glucokinase), phosphofructokinase-I and pyruvate kinase and these reactions are the major sites of regulation of glycolysis. The activities ofall these enzymes are increased by glucose.

Phosphofructokinase-I is activated by AMP and inhibited by ATP and citrate. When ATP is utilized in energy requiring process, the concentration of AMP is highly increased. Thus a large increase in AMP acts as & metabolic amplifier of a small change in ATP. This mechanism allows the activity of phosphofructokinase-I to be highly sensitive to even small changes in energy status of the cell and to control the quantity of carbohydrate undergoing glycolysis prior to its entry into citric acid cycle. The increase in AMP also explains why glycolysis is increased during hypoxia when ATP decreases.

The regulation of glycolysis by allosteric activation or inhibition or the

phosphorylation / dephosphorylation of rate limiting enzyme is short term i.e., they exert their action and affect glucose consumption over periods of minutes or hours, whereas, the effect of hormones on the amount of enzyme protein synthesized is more pronounced. The hormonal effects can result in 10 to 20-fold increase in enzyme activity that typically occurs over hours to days.

Consumption ofa meal rich in carbohydrate or administration of insulin initiate an increase in the amount of glucokinase, phosphofructokinase-I and pyruvate kinase in liver. These changes reflect an increase in gene transcription resulting in an increased enzyme synthesis. High activity of these 3 enzymes favours the conversion of glucose to pyruvate, a characteristic of the well-fed state. When plasma insulin is low and glucagon is high, the gene transcription and synthesis of these key enzymes are decreased as seen in starvation or diabetes. .

Other inhibitors

Iodoacetate is the inhibitor of glyceraldehyde-3-phosphate dehydrogenase.

Arsenite inhibits synthesis of ATP, in the conversion of 1,3-bisphosphoglycerate to 3- phosphoglycerate, by causing uncoupling of oxidation and phosphotylation in the mitochondrial electron transport chain.

Fluoride inhibits enolase enzyme involved in the conversion of 2-phosphogl~kerateto phosphoenol pyruvate.

With the study of t,he reactions and the mechanisms involved in regulating the utilization of glucose to release energy, we come to an end of our study on glycolysis. Next, we shall study in details the fate of pyruvate in terms of its oxidation to acetyl CoA. But first let us recapitulate our understanding so far

STUDENTS ACTIVITY - 1

1) Name the three irreversible reactions in the glycolytic pathway.

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2) Describe the nature of the enzymes involved in the phosphorylation of glucose.

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6.5 OXIDATION OF PYRUVATE TO ACETYL CoA

In Figure 6.1, you would have seen, and subsequently studied that the oxidation of pyruvate to acetyl CoA is the irreversible route from glycolysis to the citric acid cycle. What is citric acid cycle? It is a cycle which stores energy, released by the

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oxidation of carbohydrates, fats and proteins, in the form ofATP. We will learn about this cycle later in section 6.5.

Before pyruvate can enter the citric acid cycle, it must be transported into the mitochondria via a special pyruvate transporter, which involves a symport mechanism whereby one proton is cotransported. In this case, both pyruvate and H* are transported from the cytosol into the mitochondria. Oxidative decarboxylation of pyruvate to acetyl CoA as shown in Figure 6.3 takes place inside the mitochondrion and this is catalyzed by a multi-enzyme complex designated as pyruvate dehydrogenase complex, which is located in the mitochondrial matrix. The irreversibility of this reaction precludes the formation of pyruvate from acetyl CoA, and explains why glucose cannot be formed from acetyl CoA in gluconeogenesis.

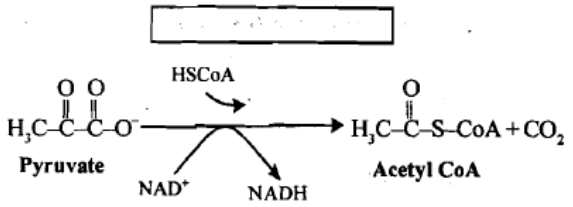

Figure 6.3: Oxidation of pyruvate to acetyl CoA

The pyruvate dehydrogenase (PDH) complex is a multi-molecular aggregate of thrée enzymes namely, pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase. Each catalyzes a part of the overall reaction. Their physical association links the reactions in proper sequence without the release of intermediate. Table 6.1 presents the components of PDH, with their reactions and prosthetic group.

Table 6.1-:-Components of pyruvate dehydrogenase (PDH)

Enzyme	Reaction	Prosthetic group
Pyruvate Dehydrogenase (E1)	Oxidative decarboxylation of pyruvate	Thiamine diphosphate TDP
Dihydrolipoyl	Transfer of acetyl group	Lipoamide
Transacetylase (E2)	to CoA	Lipoic acid covalently attached to a amino group of specific lysine residue of the enzyme.
Dihydrolipoyl Dehydrogenase (E3)	Regeneration of wxidized form of limpoamide	FAD

As can be seen from Table f. l, the complex requires different coenzymes (prosthetic groups), FAD, TDP, lipoic acid etc. The reactions involved in the oxidation of pyruvate are enumerated next.

6.5.1 Reactions Involved in the Oxidation of Pyruvate to Acetyl CoA

Four distinct enzymatic activities are associated with the overall reaction as illustrated in Figure 6.4. EaZh enzymatic activity requires different substrates and cofaétors that comprise the enzyme complex. The steps involved include:

Step 1. Oxidative decarboxylåtion of pyruvate

a) This reaction is catalysed by the El subunit of PDH.

b) The cofactor thiamine diphosphate (TDP) is required.

c) In this step, CO_2 is formed and the a hydroxyethyl group derived from pyruvate becomes covalently bound to TDP.

Step 2. Tansfer of the 2 c, unit from El to E2

a) E2 requires the coenzyme lipoic acid, which is covalently attached to the lysine residue of the enzyme protein and hence is called lipoamide.

b) In this step, the hydroxyethyl group is simultaneously oxidized to an acetyl group and transferred to the oxidized form of lipoamide.

c) TDP is regenerated in this step.

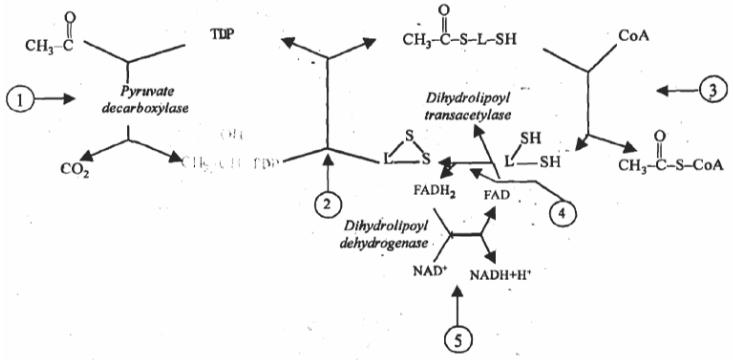

Figure 6.4: Action of Pyruvate Dehydrogenase complex

Slep 3: Formation of Acetyl CoA

a) This reaction is catalyzed by the E2 subunit of PDH. -

b) The reaction involves transfer ofthe acetyl grpup from lipogmide to coenzyme-A to form acetyl CoA.

c) Lipoamide is in the reduced state after this transfer.

Step 4: Regeneration of oxidized lipoa-mide

a) This reaction is catalyzed by E3 subunit ofPDH.

b) NAI)+ and tightly bound FAD are cofactors for E3.

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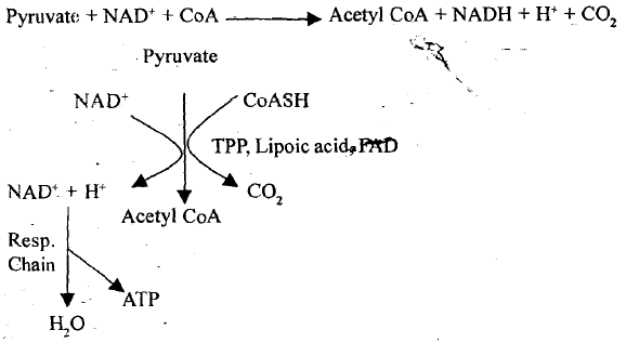
c) FAD reoxidizes lipoamide and is reduced to FADH2.

Step 5: Regeneration of oxidized FAD

$FADH_2$ is deoxidized to FAD* by NAD^+. NADH and H^+ are produced. NADH and H^+ are oxidized in the adjacent electron transport chain forming NAD^+ and 3 molecules of ATP. Thus the overall equation for oxidation of pyruvate is:



Next, we shall learn about the regulation of pyruvate dehydrogenase.

6.5.2 Regulation of Pyruvate Dehydrogenase

The activity of the PDH cömplex is highly regulated by a variety of factors. These include:

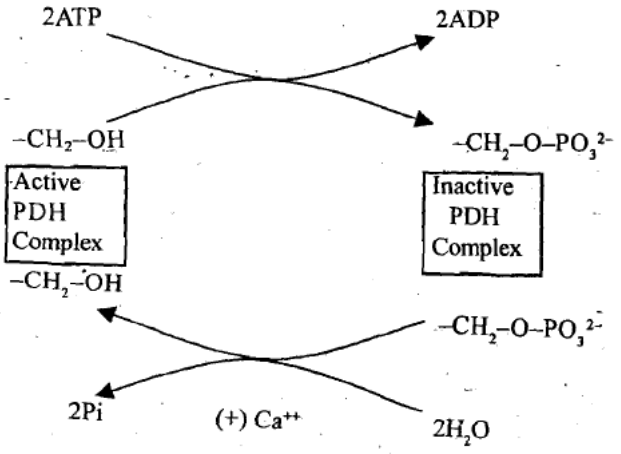
a) Product inhibition: Both acetyl CoA and NADH inhibit py-uvate dehydrogenase.

b) Availability Of substrate: Adequate concentration of the acceptor molecule CoA and NAD^+ must bé present for the complex to function.

c) Covalent modification PDH exists in 2 forms

i) Inactive, phosphorylated

ii) Active, dephosphorylated



The active form of PDH is phosphorylated by a protein kinase with the help of ATP and Mg" to the inactive form of PDH. Acetyl CoA and NADH are activators for this action and COA, NAD and pyruvate are inhibitors. The inactive form of

PDH is dephosphorylated by phosphoprotein phosphatase to the active form in the presence of increased Ca^{++} ion concentration.

The importance of the PDH complex can be further appreciated with the knowledge that certain diseases are associated with the deficiency of PDH complex. These defects are discussed next.

6.5.3 Genetic Defect in Pyruvate Dehydrogenase

A defed in any of the protein subunits of PDH can result in decrease or complete loss of activity. Severe cases are usually fatal. Symptoms of deficiency include

a) Lactic acidosis, and

b) Neurologic disorders.

Chronic alcoholics suffer fiom the deficiency of,thiamin, which results in the accumulation of pyruvic acid. Excess pyruvate is also reduced to lactate leading to potentially fatal pyruvic and lactic acidosis

When the defect is in El, administration of large doses of thiamin may be effective and for the defect in E2, administration of large doses of lipoic acid may be effective. A ' ketogenic diet, high in fat and low in carbohydrate, helps to lower the level of pyruvate and lactate, which is formed fiom the excess Dmvate

STUDENTS ACTIVITY - 2

1) Which enzyme acts on pyruvate in mitochondria and converts it to acetyl CoA? Name its components and cofactors associated with it.

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2) Describe the regulation of pyruvate dehydrogenase through covalent modification

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6.6 CITRIC ACID CYCLE

The citric acid cycle (also called the krebs cycle.or the tricarboxylic acid (TCA) cycle) is a series of enzymatically catalyzed reactions that form a common pathway for the final oxidation of all metabolic fuels (carbohydrates, free fatty acids, ketone bodies and amino acids) which are catabolized to the substrate (acetyl coA) of the citric acid cycle as you may have noticed in Figure 6.1 earlier. Its central function is the oxidation of acetyl CoA (i.e. acetyl group) to CO_2 and H_2O. This oxidation is the major site of oxygen consumption and ATP production in most animals including humans. The enzymes of citric acid cycle are located in the mitochondrial matrix and are therefore in close proximity to enzymes of the respiratory chain thereby facilitating the transfer ofelectrons from the reduced coenzymes formed during citric acid cycle to oxygen. Thus it is an aerobic process.

In the citric acid cycle, the oxaloacetate is first condensed with acetyl CoA, and then regenerated as the cycle is completed. But these reactions are not part of a closed circle system and are more similar to a traffic circle with compounds entering and leaving as required. Only a small quantity ofoxaloacetate is needed for the oxidation of a large quantity of acetyl CoA, as it is regenerated. Hence, oxaloacetate may be considered to play a catalytic role.

We begin our study of the citric acid cycle, by first learning about its functions

6.6.1 Functions of Citric Acid Cycle

The citric acid cycle is an amphibolic pathway i.e. it is involved in both anabolic and catabolic processes. Let us see how?

Anabolic reactions: The intermediates of citric acid cycle are used as precursors in the biosynthesis of many compounds like synthesis of glucose from carbon skeletons of amino acids, and providing building blocks for heme synthesis.

Catabolic reactions: The cycle provides a means for the degradation of two carbon acetyl residues which are derived from carbohydrates, fatty acid and amino acids.

Further, the citric acid cycle generates ATP by oxidative phosphorylation when electrons generated in the cycle are transferred to the electron transport chain.

Hence, citric acid cycle is the one which stores energy. Let us get to know the reactions involved in this important cycle next.

6.6.2 Reactions of tbe Citric Acid Cycle

The citric acid cycle is illustrated in Figure 6.5. Do not get intimidated by the reactions involved in this cycle. While reading the reactions highlighted here in the text, look up the corresponding reactions in the cycle. This will help you understand the sequence of reactions. So here we begin, step by step.

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a) Synthesis of citrate from acetyl CoA and oxaloacetate: Citrate synthasc catalyses this aldol condensation reaction with the release ofCoA. There are certain inhibitors to this reaction, which include:

Inhibitors: Citrate synthase is inhibited by ATP, NADH, succinyl CoA and acyl CoA derivative of fatty acids (fatty acyl COA). The rate of the reaction is also determined by the availability of the substrate.

What is the role of citrate in this cycle? Let us get to know, next.

Role of Citrate

Citrate in addition to being an intermediate ofcitric acid cycle provides a source of acetyl CoA for the cytosolic synthesis of fatty acids.

e Citrate inhibits phosphofructokinase-I, the rate limiting enzyme of glycolysis, and

Citrate activates acetyl CoA carboxylase (the rate limiting enzyme of fatty acid synthesis)

b) Isomerization of citrate: In this step, as shown in Figure 6.5, citrate is isomerized to isocitrate by aconitase which has iron-sculpture centre as its prosthetic group.

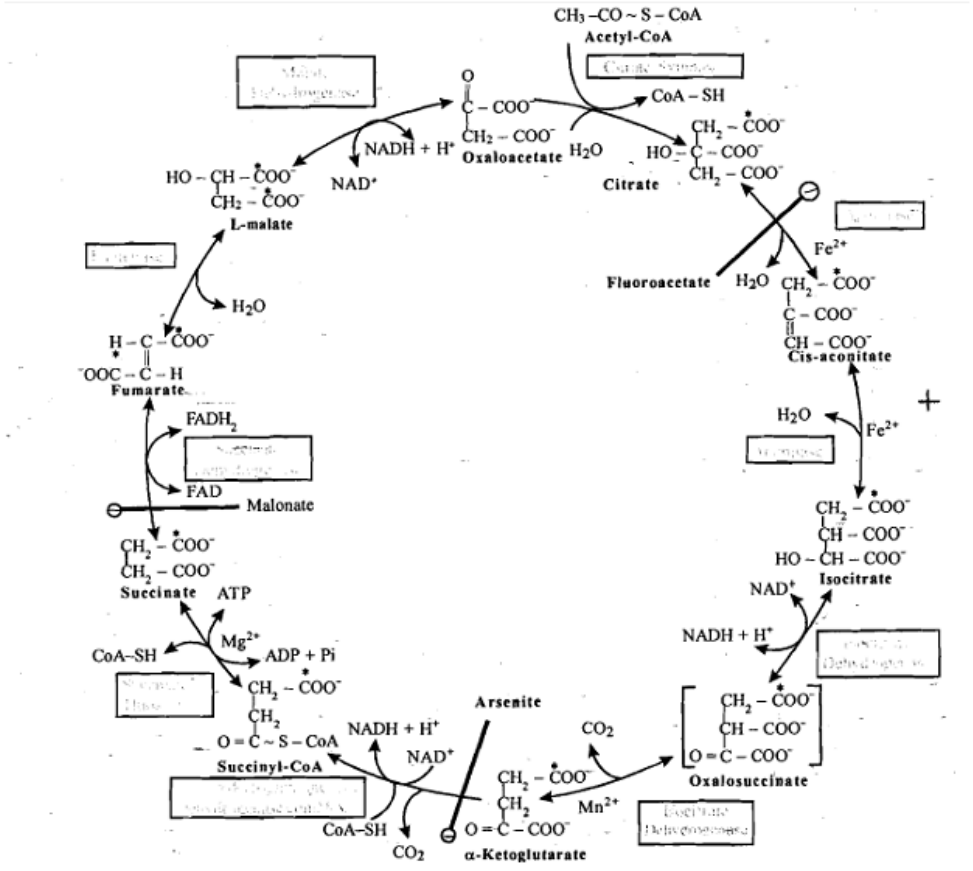


Figure 6S : Reaction of citric acid cycle

c) Oxidation and decarboxylation of isocitrate to α-ketoglutarate: Isocitrate dehydrogenase catalyseS this reaction, yielding the first three NADH molecules produced by the cycle, and the first release ofCOr Note, the aim of the citric acid cycle is to oxidize acetyl units to 2 molecules ofCO_2. In fact, this is one of the rate limiting steps of the citric acid cycle. The enzyme isocitrate dehydrogenase is activated by ADP and inhibited by ATP and NADH.

d) Oxidative decarboxylation of a-ketoglutarate to succinyl CoA: This conversion of a-ketoglutarate to succinyl CoA is catalyzed by the a-ketoglutaráte dehydrogenase complex and the mechanism of action js similar to that of PDH. This reaction releases the 2nd C02 and produces the 2nd NADH of the cycle. The equilibrium of the reaction is farin the direction of succinyl CoA, high energy thioester similar to acetyl CoA.

The coenzymes required in this reaction are similar to those involved in PDHcompléx discussed earlier, which include: thiamine diphosphate, lipoic acid, FAD, NAD+ and CoA.

The enzyme a-ketoglutarate dehydrogenase complex is inhibited by AT P, GTP, NADH and succinyl CoA (all indicators of high energy status in the cell) but not regülated by phosphorylation / dephosphorylation reaction as described for pyruvate dehydrogenase complex.

e) Cleavage of succinyl CoA to succinate: Succinate thiokinase (succinyl CoA synthetase) cleaves the high energy thioester linkage in succinyl CoA to release succinate and CoA along with the substrate level phosphorylation ofGDPto GTP (GTP and ATP are inter-convertible by nucleoside diphosphate kinase reaction). Succinyl CQA is also used in the biosynthesis of heme. This is the only reaction in citric acid cycle in which ATP is generated by substrate-level phosphorylation.

f) Oxidation of succinate to fumarate: This reaction is catalyzed by succinate dehydrogenase and FAD* is needed as a cofactor. Malonate, a structural analogue ofsuccinate, competitively inhibits succinate dehydrogenase. It is also competitively inhibited by oxaloacetate.

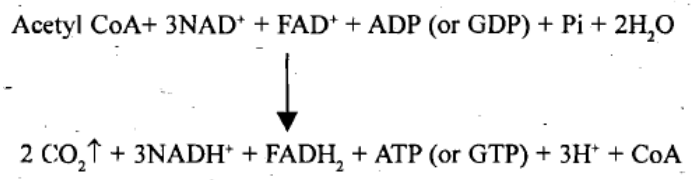
g) Hydration of fumarate to L-malate: Fumarase catalyses-this reversible reaction.

h) Oxidation of malate to oxaloacetate: Malate is oxidized to oxaloacetate by malate dehydrogenase and NAD+ is required as coenzyme. This is the third step of NADH production in the citric acid cycle by the electron transport chain along with the generation-of ATP molecules.

So starting with oxaloacetate and acetyl CoA, we move round the circle to once again produce oxaloacetate. ne relationship between the reactants and the product of the chemical reactions of the citric acid cycle and the summary is highlighted

next.

Stoichiometry of the citric acid cycle



Summary of Reactions

a) Two carbon atoms enter the cycle as acetyl CoA and leave in the form of CO

b) Four pairs of electrons are released from the substrate; three pairs leave in the form of NADH and one pair leave as $FADH_2$.

c) One high energy phosphate bond is generated in the form of adenosine (or guanosine) triphosphate (ATP or GTP) by substrate level phosphorylation.

d) Although intermediates of the citric cycle may be inter-converted, the cycle does not consume or produce solely from acetyl CoA any intermediate of the cycle.

What is the net energy output of citric acid cycle? The equation of ATP production is presented next.

ATP Production

Oxidation of one NADH by the electron transport chain leads to formation of 3ATP, whereas oxidation of-FADH2 yields 2 ATP.

Isocitrate ⟶ α-ketoglutarate (NADH ⟶ NAD^+)	3
α-ketoglutarate ⟶ Succinyl CoA (NADH ⟶ NAD^+)	3
Succinyl CoA ⟶ Succinate (ADP ⟶ ATP or GDP ⟶ GTP)	1
Succinate ⟶ Fumarate ($FADH_2$ ⟶ FAD)	2
Malate ⟶ Oxaloacetate (NADH ⟶ NAD)	3

Thus, 12 molecules of ATP are produced from oxidation of one molecule of acetyl CoA (using both substrate level and oxidative phosphorylation).

How are the reactions involved in the citric acid cycle regulated? The next sub-section focuses on this aspect.

6.6.3 Regulation of the Citric Acid Cycle

The citric acid cycle is regulated by certain enzymes and by the availability of ADP. These factors are discussed next.

a) Regulatory enzymes: Citrate synthase, isocitrate dehydrogenase and a -ketoglutarate dehydrogenase complex are the key enzymes which regulate the citric acid cycle. Table 6.2 summarizes the enzymes their inhibitors and activators.

Table 6.2 : Key enzymes which regulate the citric acid cycle

lnhibitor	Activator	Enzyme
ATP, NADH, Succinyl CoA, Acyl CoA derivative	ADP	Citrate synthase
ATP, NADH	ADP	Isocitrate dehydrogenase
ATP, GTP, NADH, Succinyl CoA	ADP	a-ketoglutarate dehydrogenase complex

Besides the enzymes, the availability of ADP also regulates the citric acid cycle. How? Read and find out.

b) Regulation by availability of ADP: When the ADP levels increase due to hydrolysis of ATP in various biosynthetic reactions, the rate of reaction to generate ATP is accelerated and this is mainly by oxidative phosphorylation. There are 4 reactions in which the reducing equivalents are transported to respiratory chain coupled with the generation of ATP in this cycle and thus increase in ADP causes oxidation of acetyl CoA by the citric acid cycle. On the contrary a low level of ADP inhibits the formation of ATP by oxidative Phosphorylation. The rate of oxidative phosphorylation is proportional to [ADP] [Pil / [ATP] which is known as respiratory control ofenergy production. When low ADP levels prevail, the oxidation of NADH and FADH2 also cease and get accumulated because the processes of oxidation and phosphorylation are highly coupled and occur simultaneously. The accumulation of reduced form of coenzymes inhibits the oxidation of acetyl CoA by the citric acid cycle due to lack of oxidized coenzyme forms.

Having studied about the reactions and mechanisms involved in regulating the citric acid cycle, it is clear that 12 ATP molecules are produced from oxidation of one molecule of acetyl CoA.

Now what is the total picture which emerges in terms of the total high energy phosphates formed from one mole of glucose? We have studied about the energy production through glycolysis, next, in oxidation of pyruvate and now in the citric acid cycle. Can you now estimate the total energy production? Go ahead and do the exercise. Tally your answer with the estimation given here in the next sub-section.

6.6.4 Generation of High Energy Phosphates (From Oxidation of Glucose)

The overall high energy phosphate formed starting with one molecule of glucose is estimated herewith. The reactions responsible for the generation of ATP during

oxidation of glucose are given in the Table 6.3.

Table 6.3: Reactions responsible for the generation of ATP during oxidation of glucose

Pathway	Reaction catalysed by	Method of ~ P production	No. of ~P formed per mole of glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Phosphoglycerate kinase	Oxidation at substrate level	2
	Pyruvate kinase	Phosphorylation at substrate level	2 10
	Consumption of ATP by reaction	Catalyzed by hexokinase and phosphofructokinase-I **Net**	-2 **8**
	Pyruvate dehydrogenase	Respiratory chain oxidation of 2NADH	6
Citric acid cycle	Isocitrate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	α-ketogluatarate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Succinate thiokinase	Phosphorylation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of $2NADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of $2NADH_2$	6
	Total (net)per mole of glucose under aerobic condition		**38**
	Total (net) per mole of glucose under anaerobic condition		**2**

When I mole of glucose is combusted in a calorimeter to CO_2 and H_2O, approximately 2870 KJ of energy is liberated as heat. When oxidation occurs in the tissues, some of this energy is not lost immediately as heat but is captured as high energy phosphate. A total of 38 molecules of ATP are generated per molecule of glucose oxidized to CO_2 and H_2O. Assuming each high energy bond to be equivalent to 51.6 KJ (in tissues), the total energy captured in ATP per mole of glucose oxidized is 1961 KJ (38 x 51.6), or approximately 68% of the energy of combustion. Most of the ATP is formed as a consequence of oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain. The remainder is generated by phosphorylation at the substrate level.

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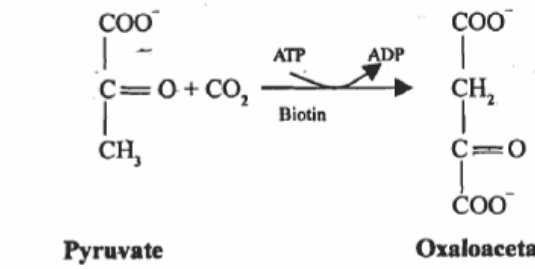
Before we end our study of citric acid cycle, we need to learn about anaplerotic reactions. What are these reactions and what is their significance in citric acid cycle? The last sub-section in this section focuses on this aspect.

6.6.5 Anaplerotic Reactions

Anaplerotic reactions are reactions that replenish the intermediates of citric acid cycle. The special enzymatic mechanisms by which the pool of citric acid cycle intermediates can be replenished are called anaplerotic (filling-up) reactions. Anaplerotic reactions can increase the concentration of citric acid cycle intermediates, allowing an increased rate of oxidation of two-carbon units. As more intermediates are available, more moles of acetyl CoA can be processed. The intermediates may also be used for other biosynthetic reactions and need to be replaced. These anaplerotic reactions include:

1) Pyruvate carboxylase which forms oxaloacetate through the following reaction.



Pyruvate **Oxaloacetate**

2) Phosphoenol pyruvate (PEP) + CO_2 + GDP $\xrightarrow{\text{PEP carboxy kinase}}$ Oxaloacetate + GTP

3) Pyruvate + HCO_3^- + NADPH $\xrightarrow{\text{malic enzyme}}$ Malate + $NADP^+$

4) Glutamate dehydrogenase, which also provides α-ketoglutarate.

Glutamate + NAD^+ + H_2O $\longleftrightarrow$ α-ketoglutarate + NH_3 + NADH + H^+

5) Succinyl CoA formation from isoleucine, valine, methionine and threonine

For a reaction to be classified as anaplerotic reaction, net synthesis of citric acid cycle intermediates must occur. Accordingly the reaction catalysed by glutamate-oxaloacetate transaminase as presented herewith is not anaplerotic since formation of oxaloacetate is counterbalanced by utilization of a-ketoglutarate.

α-ketoglutarate + aspartate ⟶ glutamate + oxaloacetate

With anaplerotic reactions, we come to an end of our study on citric acid cycle.

Check Your Progress Exercise 3

1.) What is'the function of the citric acid cycle?

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2) Name the high-energy complex generated during the conversion of succinyl CoA to succinate in the citric acid cycle.

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6.7 GLUCONEOGENESIS

Gluconeogenesis (i.e synthesis of new glucose) is the synthesis of carbohydrate from non-carbohydrate, source. The major substrates for gluconeogenesis are the glucogenic amino acids, lactate, glycerol and (important in ruminant) propionate. We shall get to know about them later in sub-section 6.6.2. Liver and kidney are the major tissues involved in gluconeogenesis due to the availability of the necessary enzymes.

But, first, what is the significance of gluconeogenesis? Read the next sub-section and find out.

6.7.1 Functions of Glifconeogenesis

The significance of gluconeogenesis jnclude:

1) During starvation or during periods of limited carbohydrate intake, when the levels of liver glycogen are low, gluconeogenesis is important in maintaining adequate blood sugar concentration since a continual supply of glucose is necessary as a source of energy for the nervous system and the erythrocytes.

2) Even when most of the energy requirement ofthe organism is met by the supply of fat, there is always a certain basal requirement for glucose which is provided by gluconeogenesis.

3) During extended exercise, when high catecholamine levels have mobilized carbohydrate and lipid reserves, the gluconeogenic pathway allows the use of lactate from glycolysis and of glycerol from fat break down.

4) During metabolic acidosis, gluconeogenesis in the kidney allows the excretion of an increased number of protons.

5) Gluconeogenesis also allows the use of dietary protein in carbohydrate pathway after disposal of the amino acid nitrogen as urea.

6) Gluconeogenesis is important to human beings everyday, making it possible for us to make it through the night and from meal to meal without nibbling on a source of carbohydrate continuously.

So, you would have realized that the production of glucose from other substrates is necessary for use as fuel, Hence, it is important for us to learn about these substrates and their reactions in gluconeogenesis. The next sub-section presents a discussion on these substrates.

6.7.2 Gluconeogenesis — Substrates

Earlier in this section, you may recall reading that the major substrates for gluconeogenesis are the glucogenic amino acids, lactate, glycerol etc. Let us get to know about these substrates and their role in gluconeogenesis. We start with lactate as a substrate.

A) *Lactate*

Lactate is transported to the liver in the Cori cycle (lactic acid cycle) and is converted to pyruvate as shown in Figure 6.6. Hepatic gluconeogenesis then converts lactate back to glucose. Glucose is then free to circulate back to peripheral tissue to re-enter anaerobic glycolysis. This is the Cori cycle. It functions to:

maintain glucose substrate for vital tissues, and

prevent excessive acidosis due to an excess o lactate.

The process invoJved in the Cori cycle is enumerated herewith along with the graphic illustration in Figure 6.6. Read the steps given here and at the same time follow the sequence in Figure 6.6

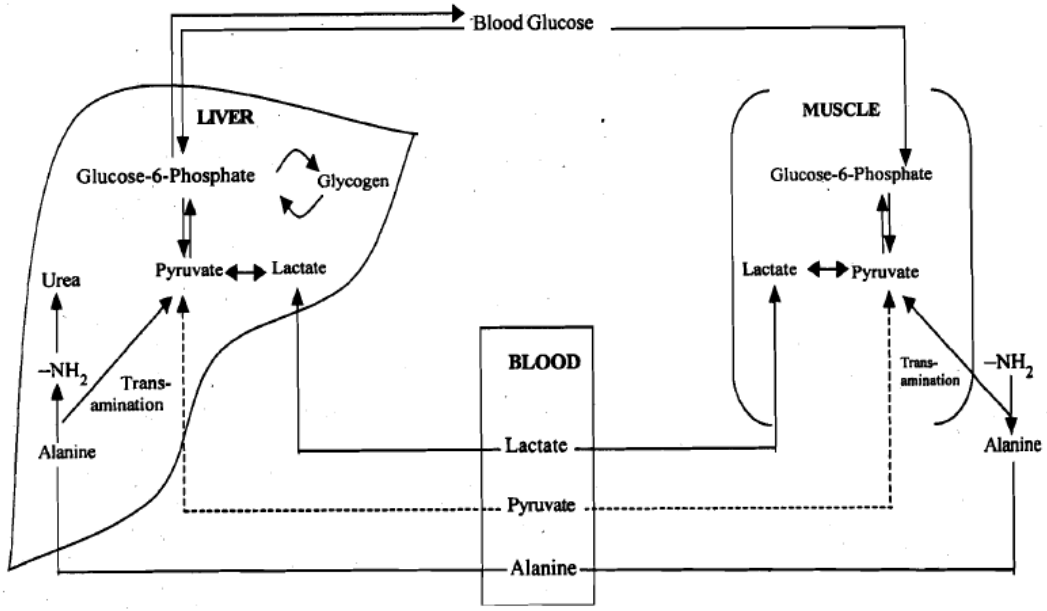

Figure 6.6 : The Cori cycle and Alanine cycle

1) *The Cori cycle*

a) Pyruvate formed from glucose is converted to lactate by lactate

dehydrogenase in the muscle cell.

b) Lactate is released into the blood and taken up by the liver.

c) Lactate is converted to pyruvate by the isoenzyme of lactate dehydrogenase using NAD+ as cofactor in the liver.

d) Pyruvate is converted to glucose by gluconeogenic mechanism in the liver and released into the blood where it can be used as energy source for muscle and other tissue.

In Figure 6.6, you would have noticed that pyruvate formed in the muscle can be converted to alanine as well. Hence alanine too can function as a substrate for gluconeogenesis, as discussed next.

2) *The Alanine cycle*

Look at Figure 6.6. Follow the alanine link in the alanine cycle. The process goes as under:

1) Pyruvate fomed from glycolysis in the muscle is converted to alanine by transamination reaction.

2) Alanine is released by the muscle into the blood and is taken up by the liver.

3) In the liver, alanine is converted back to pyruvate by the reverse of the transamination reaction that ockurred in the'muscle

4) Pyruvate is converted to glucose via gluconeogenic pathway.

5) The NH3 liberated in convert' to urea in the liver.

Next, let us study about the substrate — glycerol.

B) *Glycerol*

The process includes:

1) Glycerol is formed infreadiposetissue by lipolysis of triacylglycerol when metabolic fuel is scarce.

2) Glycerol is released intothe blood and taken upbythe liver, where it is firstconverted to glycerol-3-phosphate by glycerokinase and AT

3) Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase in presence of NAD+. Dihydroxyacetone phosphate is then converted to glucose.

C) *Amino acids*

Figure 6.7 illustrates the citric acid cycle intermediates from the amino acids. Here, as you can see:

1) the glucogenic amino acids are converted tothe intermediates ofcitric acid

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cycle either by transamination or deamination, and

2) these intermediates are converted to oxaloacetate and finally converted to glucose by the enzymes of gluconeogenesis.

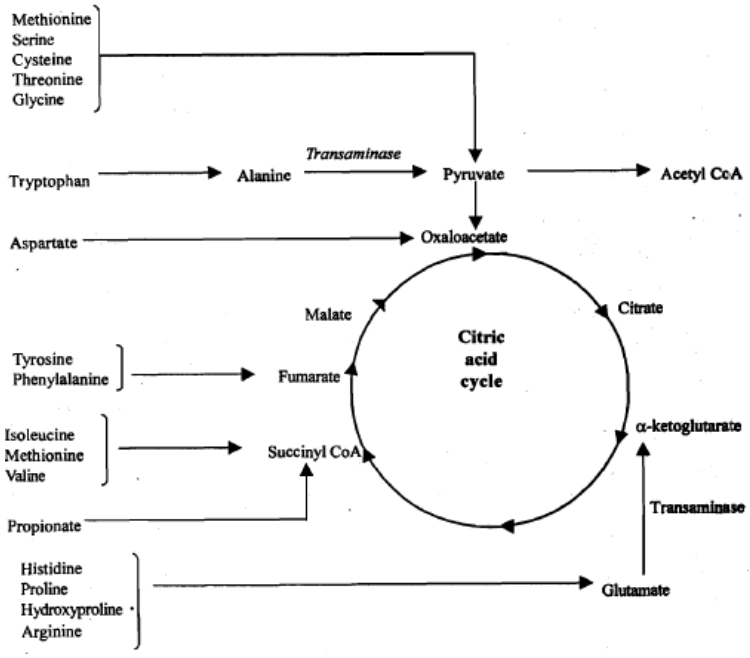

Figure 6.7 : The citric acid cycle intermediates from the amino acids

Another important substrate, particularly in ruminant is propionate. Let us leam about it.

D) *Propionic acid*

Propionic acid is formed as a residual unit (propionyl CoA) in "-oxidation of odd-carbon fatty acids. The conversion ofpropionate to succinyl CoA involves a long process as given herewith and in Figure 6.8.

1) Propionate is first activated by thiokinase with ATP and CoA to form propionyl CoA.

2) Propionyl CoA undergoes CO_2 fixation reaction to form D-methyl malonyl CoA catalyzed by propionyl CoA carboxylase and biotin is required as a coenzyme.

3) D-methyl malonyl CoA is converted to L-methyl malonyl CoA by methyl malonyl CoA racemase.

4) L-methyl malonyl CoA is isomerised to succinyl CoA by methyl malonyl CoA isomerase which requires vitamin B_{12} as a coenzyme.

5) Succinyl CoA enters citric acid cycle and is converted to oxaloacetate and then further to glucose via gluconeogenic pathway.

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Look up Figure 6.7 , which illustrates the substrates for gluconeogenesis. The discussion above on substrate was indeed quite extensive. The idea behind giving the mechanism for each substrate was to help you understand how the substance forms a substrate for gluconeogenesis. Hope you enjoyed reading it. Next, we shall move on to the gluconeogenesis — the pathway involved in the process.

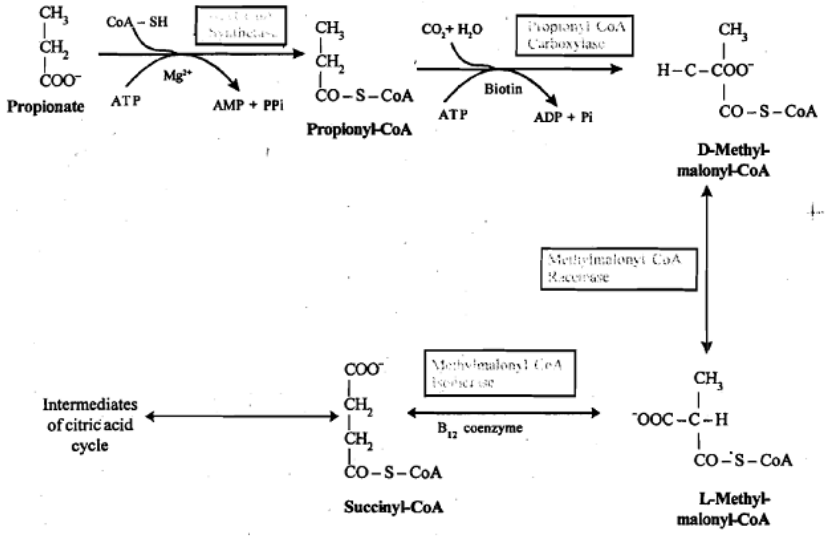

Figure 6.8 : Metabolism of Propionate

6.7.3 Gluconeogenic Pathway

The metabolic pathways in connection with gluconeogenesis are the modification of the EM pathway and citric acid cycle. The synthesis of glucose from substrates is essentially a reversal of glycolysis. However, Krebs pointed out that energy barriers obsüuct a simple reversal of glycolysis and must be bypassed for gluconeogenesis to be completed.

These reactions are:

i) Between pyruvate and phosphoenolpyruvate (PEP)

ii) Between fructose I ,6 bisphosphate and fructose 6-phosphate

iii) Between glucose-6-phosphate and glucose, and

iv) Between glucose-I -phosphate and glycogen.

You may recall reading about these reactions in the glycolysis pathway.

To help you understand the process, a summary of the gluconeogenesis pathway with gluconeogenesis enzyme names in red and names of reversible glycolysis enzymes in blue is presented in Figure 6.9.

The above mentioned reactions are circumvented by the special reactions

highlighted in Figure 6.9 (Under A, B and C) and also discussed herewith.

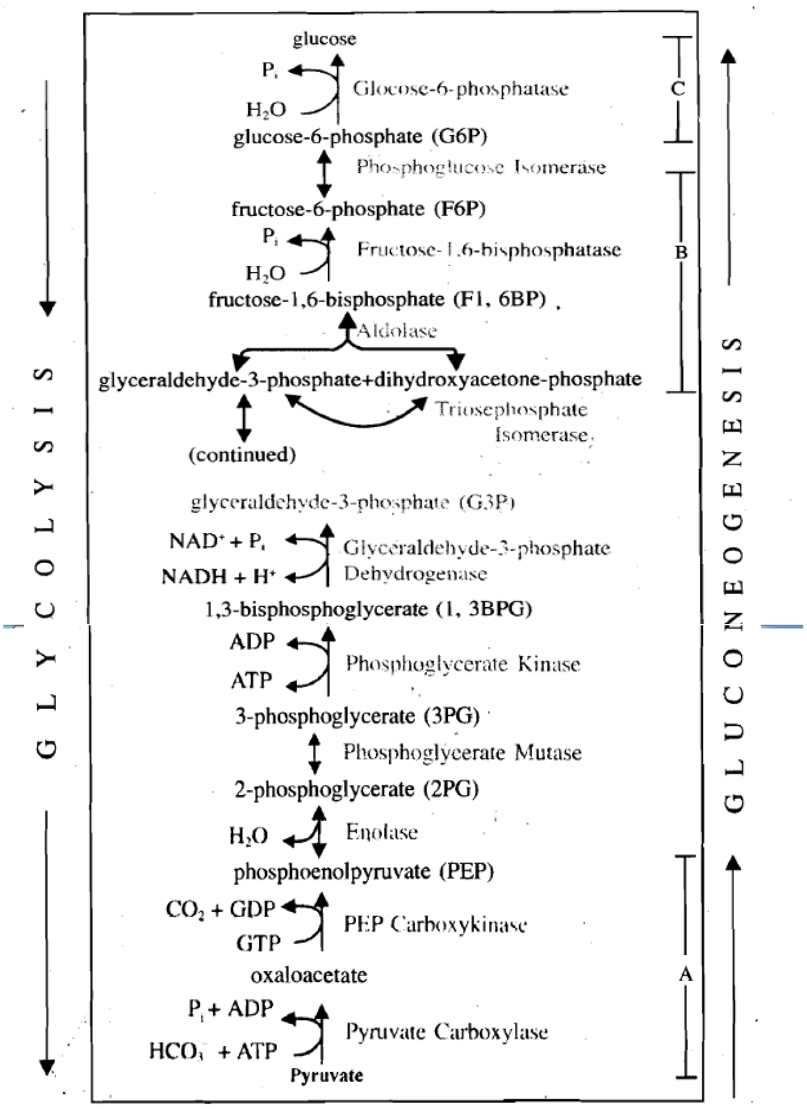

Figure 6.9 : Summary of the pathway

A) *Pyruvate and Phosphoenolpyruvate (Look at Figure 6.9, A)*

1) Pyruvate carboxylation: In this reaction, pyruvate, C02 and ATP are converted to oxaloacetate, ADP and Pi catalysed by the enzyme pyruvate carboxylase and the cofactors required are biotin and ions. This reaction occurs in the mitochondrial mafrix.

2) Conversion of oxaloacetate to phosphoenolpyruvate: In this reaction, oxaloacetate and guanosine triphosphate (GTP) are converted to PEP, CO_2 and guanosine diphosphate (GDP). This reaction is catalyzed byphosphoenolpyruvate carboxykinase (PEPCK) which requires Mn^{++}

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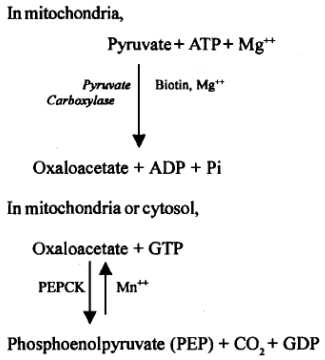
for its activation. In humans, this enzyme is equally distributed between mitochondria and the cytosol. In mitochondria,



With the help of the above 2 enzymes and LDH, lactate can also be converted to PEP.

3) Oxaloacetate to Malate: Oxaloacetate cannot permeate mitochondrial membrane well and it must be transported across the membrane in the form of malate. This reaction is catalyzed by malate dehydrogenase.

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Malate} + \text{NAD}^+$$

a) In the mitochondria, a mitochondrial malate dehydrogenase catalyses the above reaction, and

b) In the cytosol, a cytosolic malate dehydrogenase catalyses the reverse reaction which regenerates oxaloacetate so it can be converted to PEP. Look at Figure 6.10.

$$\text{Malate} + \text{NAD}^+ \longrightarrow \text{Oxaloacetate} + \text{NADH} + \text{H}^+$$

c) In this process, malate also serves to transfer reducing equivalents from the mitochondria to the cytosol. The NADH formed is used in gluconeogenesis.

B) Fructose-I,6-bisphosphate and Fructose-6-phosphate: (Look at Figure 6.9, B)

The conversion of fructose- 1,6- bisphosphate to fructose-6-phosphate is catalysed by fructose-I, 6-bisphosphatase which is the major regulatory enzyme in gluconeogenesis. This enzyme is present in liver, kidney and striated muscle but absent from adipose tissue, heart muscle and smooth muscle. Fructose-1,6 bisphosphatase is an allosteric enzyme, activated by citrate and inhibited by AMP and fructose-I ,6-bisphosphate.

These allosteric effects are exactly the opposite of those observed with phosphofructokinase, the regulatory enzyme in glycolysis. This is an example of reciprocal control ofopposing metabolic pathways.

C) *lucose-6-phosphate to Glucose: (Look at Figure 6.9, C)*

Glucose-6-phosphate is converted to glucose by glucose-6-phosphatase which is present in intestine, liver and kidney but absent from muscle and adipose tissue.

D) *Glucose-I-phosphate to Glycogen*

The conversion of glucose- I -phosphate to glycogen is through UDPG and glycogen synthase. We shall learn about this later in section under glycogen synthesis.

The conversion of 2 moles of pyruvate and 1 mole of glucose uses 4 moles of ATP and 2 moles of GTP, the total equivalent of6 moles of high energy phosphate since GTP, like ATP, possesses high energy phosphate bonds.

Overall, each i .e. glycolysis and gluconeogenesis may be summarized as follows:

Glycolysis:

glucose + 2NAD* + 2ADP + 2 Pi ⟶ 2 pyruvate + 2NADH + 2ATP

Gluconeogenesis:

2 pyruvate+2 glucose+2NAD^+ + 4ADP + 2GDP ⟶ glucose+2NAD^+ + 4ADP + 2GDP+6Pi

Having studied the gluconeogenic pathway, we also need to learn about the factors which regulate the pathway. A brief discussion follows in sub-section 6.6.4.

6.7.4 Regulation of Gluconeogenesis

Gluconeogenesis and glycolysis are reciprocally regulated. Figure 6.10 illustrates the regulation of gluconeogenesis and glycolysis. All factors increasing gluconeogenesis, simultaneously decrease glycolysis. Similarly decrease in gluconeogenesis is accompanied by increase in glycolysis.

The key enzymes of gluconeogenesis, as we studied earlier, include:

a) Pyruvate carboxylase

b) Phosphoenol pyruvate carboxykinase

c) Fructose- I ,6-bisphosphatase, and

d) Glucose-6-phosphatase.

Important aspects related to regulation include:

1) The hormones glucagon and glucocorticoids which are secreted during starvation stimulate glucose-6-phosphatase to enhance gluconeogenesis.

2) During starvation, the increased level of glucagon also stimulates the enzyme phosphoenolpyruvate carboxykinase and thus increases gluconeogenesis,

3) During starvation, increased fatty acid oxidation provides more acetyl CoA which allosterically activates the enzyme pyruvate carboxylase, thereby forming oxaloacetate and enhancing gluconeogenesis.

4) The released glucagon also stimulates gluconeogeneyis by decreasing the concentration of fructose-2,6-bisphosphate which in turn cannot activate phosphofructokinase-I but activates the enzyme fructose-I ,6-bisphosphatase.

5) High carbohydrate diets increase the insulin / glucagon ratio end thus minimizes the gluconeogenic mechanism by-reducing the activity of key enzymes.

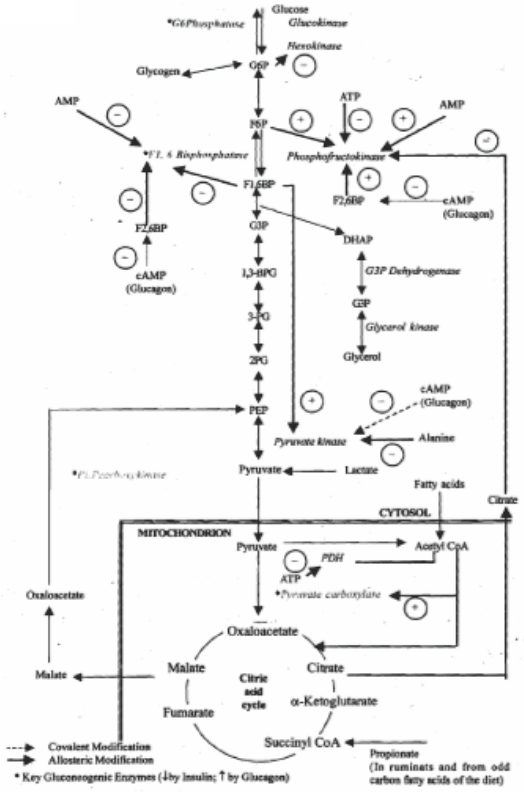


Figure 6.10 : Regulåtion of and Glycolysis

6) ATP and citrate, as can be seen in Figure 6.10, are the activators of fructose-1,6 bisphosphatase and hence gluconeogenesis is increased. But high level of AMP in liver cells inhibits fructose-I,6-bisphosphatase activity and thus reduces gluconeogenesis.

7) Increased ADP allosterically inhibits pyruvate carboxylase and thus reduces gluconeogenesis.

8) The hormones glucagon, epinephrine and glucocorticoids stimulate the

synthesis of pyruvate carboxylase and thus enhances gluconeogenesis. But the hormone insulin depresses the enzyme pyruvate carboxylase and thus reduces gluconeogenesis.

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Earlier in the section on glycolysis we studied about the regulation of glycolysis at the PFK-I reaction. The fructose-I,6-bisphosphate (Fl,6BPase) reaction is a major point of control of gluconeogenesis. The regulatory role of fructose-2,6-bisphosphate is discussed herewith and highlighted in Figure 6.11.

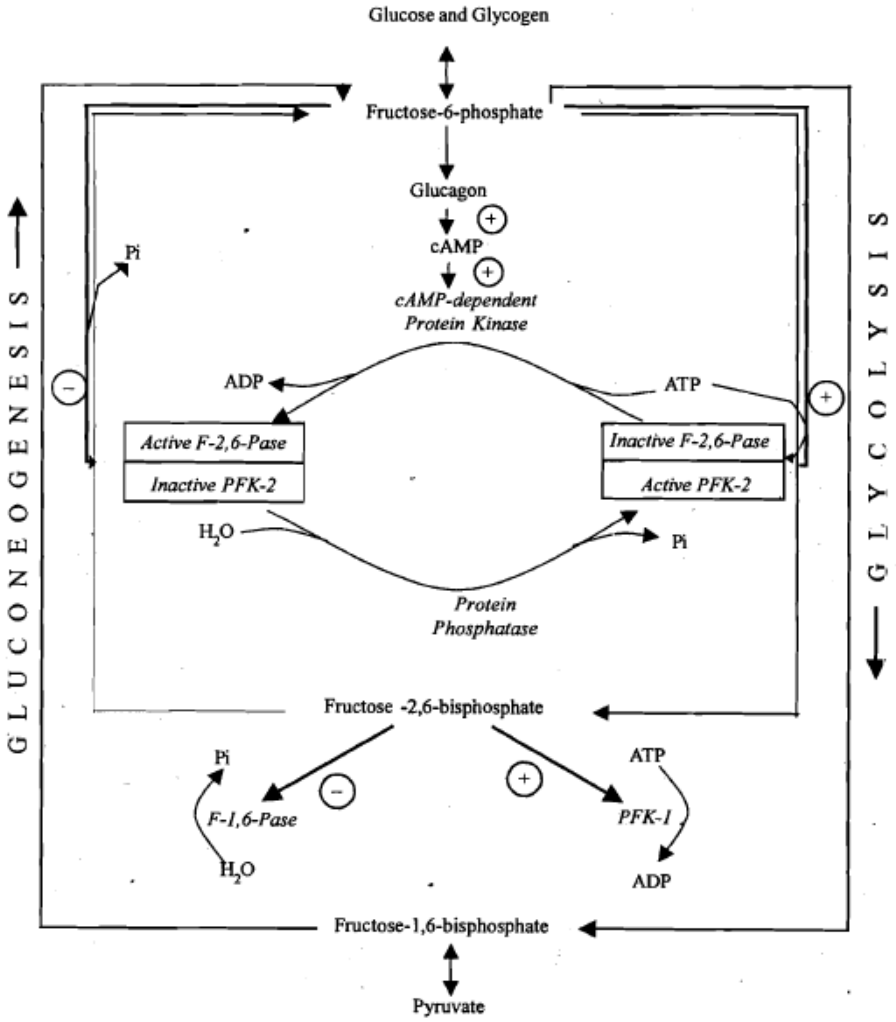

Figure 6.11 : Role of Fructose-2,6-Bisphosphate

Regulatory Role of Fructose-2,6-Bisphosphate

1) Fructose-2,6-bisphosphate is the most potent allosteric effector of phosphofructokinase-I and inhibitor offructose-l ,6-bisphosphatase in liver.

2) It relieves inhibition of phosphofructokinase-I by ATP and increases affinit for fructose-6-phosphate.

3) It inhibits fructose-I,6-bisphosphatase by increasing the km for fructose-1,6-

bisphosphate.

4) Fructose-2,6-bisphosphate is formed from fructose-6-phosphate by the phosphofructokinase-2 (PFK-2) (a bifunctional enzyme). Thus the same enzyme also causes its breakdown by possessing fructose-2,6-bisphosphatase activity. It is allosterically controlled by fructose-6-phosphate.

5) When glucose is less, glucagon stimulates the production of cAMP which inactivates phosphofructokinase-2 and activates fructose-2,6-bisphosphatase by phosphorylation.

6) When there is abundance of glucose, the concentration offructose-2,6-bisphosphate stimulates glycolysis by activating phosphofructokinase-I and inhibiting fructose 1 ,6-bisphosphatase.

7) In glucose shortage, gluconeogenesis is stimulated by a decrease in the concentration offructose-2,6-bisphosphate, which deactivates phosphofructokinase-I and deinhibits fructose- 1 ,6-bisphosphatase. This mechanism also shows that glucagon stimulation of glycogenolysis in liver results in glucose release rather than glycolysis.

8) Recently, it has been indicated that glucose- 1 ,6-bisphosphate plays a similar role in some extrahepatic tissue.

6.8 METABOLISM OF GLYCOGEN

Glycogen is a highly branched, very large polymer ofglucose molecules, linked along its main line by a-I glycosidic linkages, branches arise by a- glycosidic bonds at about every 10th residue.

The storage form of glucose is glycogen and the major storage sites are liver and muscle. Although the concentration of glycogen is higher in the liver, the much greater mass of skeletal muscle stores a greater total amount of glycogen. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within muscle itselfand muscle glycogen is significantly depleted after prolonged exercise. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of the blood glucose, particularly between meals and after 12-18 hours of fasting, the liver glycogen is completely depleted.

After a meal, when there is a rise in blood glucose level, the synthesis of glycogen in liver and muscle is initiated. Thisprocess is called glycogenesis. This not only prevents excessive rise in blood glucose level, but also helps to store glycogen for future use. In the liver, glycogen is metabolized to glucose and then released into the circulation in a fasting person. In the muscle, although glycogen cannot be converted into glucose it can still be used for obtaining energy during muscle contraction. This breakdown of glycogen in the liver (glycogen—nlucose) and muscle (glycogen—+glucose- 1 -phosphate) is called glycogenolysis.

NOTES

Glycogen synthesis (glycogenesis) and glycogen usage (glycogenolysis) occur in separate pathways, which are discussed next.

6.8.1 Glycogenesis

The synthesis of glycogen in liver and muscle, we learnt earlier, is called glycogenesis.

What does this process involve? Figure 6.12 illustrates the synthesis of glycogen.

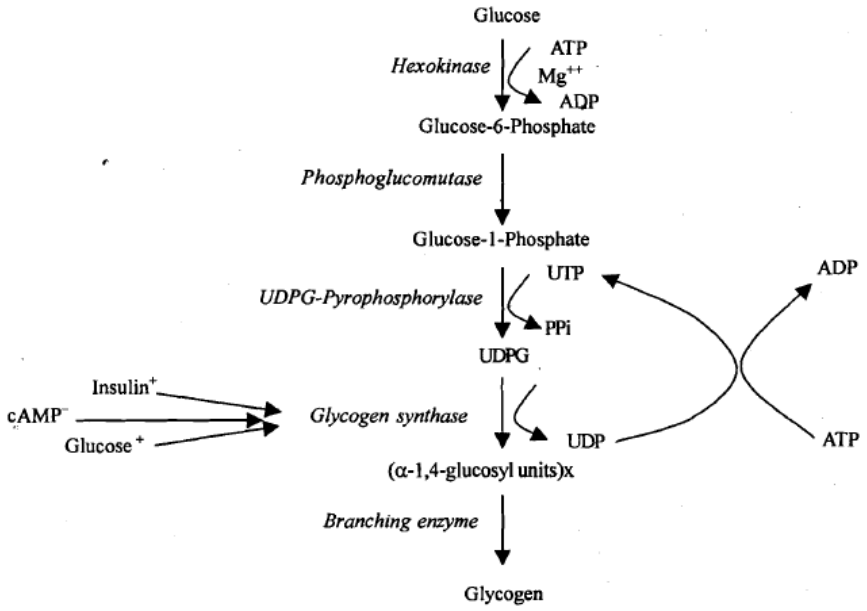

Figure 6.12 : Glycogenesis

The process is enumerated herewith:

1) Glucose is phosphorylated to glucose-6-phosphate by hexokinase (glucokinase in liver) in the presence of ATP and Mg^{++} ions. The action of glucokinase in liver is to remove glucose from the blood following a meal.

2) Glucose-6-phosphate is acted upon by phosphoglucomutase to form glucose-1- phosphate. This is a reversible reaction.

3) Glucose-I-phosphate reacts with uridine triphosphate (UTP) to form the active nucleotide uridine diphosphate glucose (UDPG) catalyzed by the enzyme UDPG pyrophosphorylase. The released pyrophosphate (PPi) is rapidly broken down by pyrophosphorylase to 2 inorganic phosphate (Pi) molecules, thereby rendering the reaction essentially irreversible.

4) The synthesis of new glycogen requires the presence of a glycogen primer (i.e. a preformed molecule) and a- glucosyl residues from UDP glucose. The residues ar9 successively transferred to the C-4 terminus (non-reducing end) of an existing glycogen chain in a-1,4 glycosidic linkage. This process is repeated till about 10 to 12 molecules have been added. This reaction,

which is the rate limiting step in glycogen synthesis, is catalyzed by glycogen synthase (glycogen synthetase).

5) After the chain has been lengthened to a minimum of 11 glucose residues, the branching enzyme (amylo [11.6] transglucosidase transfers a part ofthe I +4 chain (minimum length of6 glucose residue)to a neighbouring chain to form 1-+6 linkage, thus, establishing a branch point in the molecule. The branches grow by further addition of 1—+4 glucosyl units and further branching.

Perhaps as a revision exercise, you could write the pathway of glycogenesis giving the chemical structure of all the intermediates.

Next, let us look at the factors which regulate glycogenesis.

6.8.2 Regulation of Glycogenesis

Glycogen synthase, the key enzyme in glycogenesis, is activated by insulin and glucose and inhibited by cAMP as shown in Figure 6.13.

Glycogen synthase exists in two forms: the phosphorylated form designated as 'D' form js the inactive one and the dephoshorylated form designated as 'I' form is the active one. The 'D' form is an allosteric enzyme, activated by high concentration of 5'—AMP and the 'I' form does not require 5'—AMP for its action. Hence the names D and I forms — D for dependent on 5'—AMP activity and I for independent of 5'—AMP concentration. The interconversion of D and I form is catalyzed by cAMP dependent protein kinase. The level of cAMP is in turn regulated by adenylate cyclase enzyme, which is activated by glucagon and epinephrine as shown in Figure 6.13.

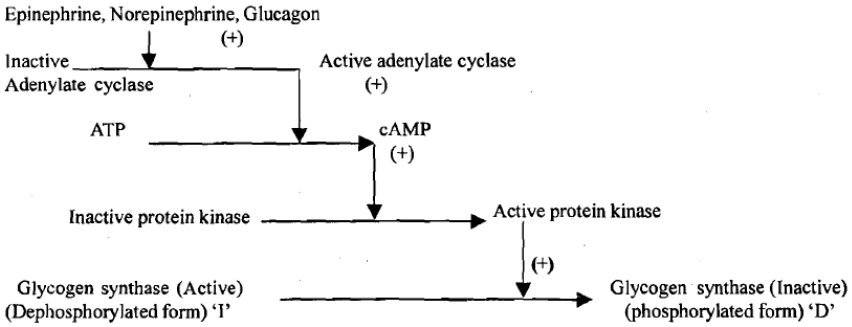

Figure 6.13 : Control of Glycogen Synthase

Having studied about glycogen synthesis and regulation, next it is the turn of glycogenolysis i.e. the breakdown of glycogen.

6.8.3 Glycogenolysis

Unlike glycogenesis, glycogenolysis is the breakdown of glycogen. Glycogen is broken down in the liver and muscle catalysed by the enzyme glycogen phosphorylase. Inorganic phosphate (Pi) is used for the lysis and hence is called

phosphorolysis. Phosphorylase specifically acts upon a I +4 linkage of glycogen to produce glucose- 1 - phosphate. The removal of a 1 ,4 glucosyl residues continues until about 4 glucose residues remain on either side of a-1,6 branch, then the debranching enzyme (amylo a-1,6 glucosidase) causes the hydrolytic splitting ofa 1 ,6 linkages.

Here free glucose is formed (since no phosphate is used for lysis). However, since a-1,6 linkages are very few compared to a 1 -+4 linkages, the major end product of glycogenolysis is glucose with small amounts of glucose-I -phosphate. By the combined action of both the enzymes, glycogen is catabolized.

The reversible reaction of phosphoglucomutase causes the conversion of glucose- 1 -phosphate to glucose-6-phosphate.

In liver and kidney (but not in muscle), there is a specific enzyme glucose-6 phosphatase, which acts upon glucose- 6-phosphate to release free glucose from the cell to the extracellular compartment as illustrated in Figure 6.14.

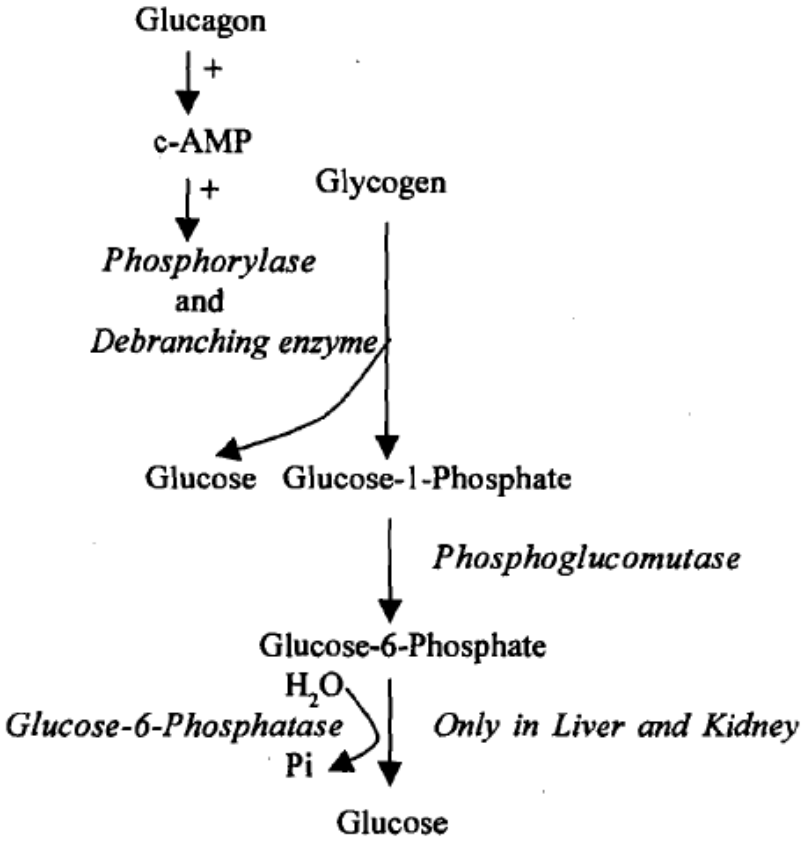

Figure 6.14 : Glycogenolysis

Glycogen phosphorylase is a dimeric (2 polypeptide) enzyme that utilizes pyridoxal phosphate as a prosthetic group. Different isozymes of glycogen phosphorylase are present in different tissues. Phosphorylase from liver is activated by glucagon stimulated cAMP levels whereas muscle phosphorylase is activated only by

epinephrine via cAMP. In lysosomes, another enzyme a-1,4 glucosidase is involved in debranching.

Having understood the glycogenolysis process, let us now leam about its regulation.

6.8.4 Regulation of Glycogenolysis

Figure 6.15 illustrates the cascade regulation of glycogen phosphorylase activity, which we learnt above, is the major enzyme involved with breakdown of glycogen, and hence also plays a main role in its regulation.

Glycogen phosphorylase exists in two distinct states — phosphorylase a, the active state and phosphorylase b, the inactive state. The regulation mechanism involves:

a) The hormones catecholamines (epinephrine, norepinephrine) and glucagon cause the increase in cAMP levels in cells. This cAMP activates protein kinase, which stimulates the key enzyme phosphorylase for glycogenolysis. Briefly, phosphorylase b is phosphorylated, and rendered highly active, by phosphorylase kinase.

b) Immediately afterthe onset ofmuscle contraction, glycogenolysis is highly increased in muscle by the rapid activation of phosphorylase due to the activation of phosphorylase kinase by Ca^{++} ions.

c) Calmodulin (a Ca^{+}t dependent regulatory protein) causes further activation of phosphorylase kinase for glycogenolysis.

So having looked at the mechanism involved, you may have realized that the regulation ofglycogen phosphorylase is complex. Its regulation ensures that glucose remains stored as glycogen until it is mobilized from liver for maintaining blood glucose homeostasis, or to supply energy to the muscle cell. This enzyme is phosphorylated in response to hormone signals in a cascade. The enzyme that directly catalyses the phosphorylation of glycogen phosphorylase is phosphorylase kinase, which itself can be activated either by phosphorylation or allosterically by calcium.

The cascade is initiated by binding of hormone (glucagon or epinephrine) to its specific receptor. Hormone binding activates adenylyl cyclase. Adenylyl cyclase produces cyclic AMP that activates protein kinase. To accelerate glycogenolysis, protein kinase (active) catalyses the phosphorylation of phosphorylase kinase converting it from its inactive b form to its active a form.

Subsequently, phosphorylase kinase-a (active form) catalyses the phosphorylation of the tense (inactive) glycogen phosphorylase-b to generate the relaxed (active) glycogen phosphorylase-a form. Thus glycogen degradation is triggered.

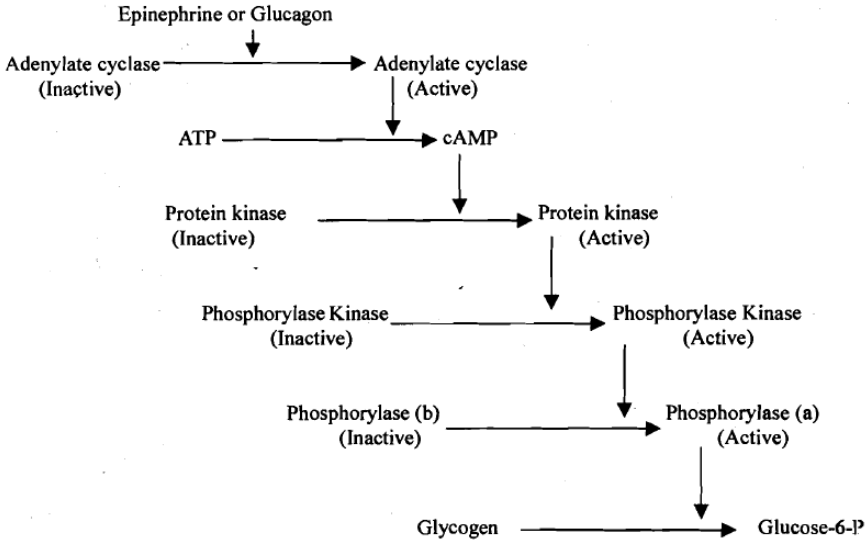

Figure 6.15 : Cascade regulation of glycogen phosphorylare activity

In the sub-sections above, we have studied about glycogenesis and gluconeogenesis. Next we shall learn about regulation of glycogen metabolism.

6.8.5 Regulation of Glycogen Metabolism

It is important for you to understand that glycogenesis and gluconeogenesis are regulated reciprocally. There is a hormonal regulation system functioning at the muscle and liver level, which regulates the glycogen metabolism.

We have studied about the phosphorylation cascade involving the hormones epinephrine and glucagon above. Epinephrine promotes glycogenolysis and inhibits glycogenesis. It stimulates the formation of cAMP by activating adenylate cyclase in the muscle. Insulin, another hormone, increases glycogenesis and decreases glycogenolysis in the muscle. It heightens the entry of glucose unit in the muscle cells. It reduces cAMP levels by speeding up the destruction of cAMP by phosphodiesterase.

Glucagon activates adenylate cyclase in the liver cell membrane and thus turns on glycogenolysis and reduces glycogenesis. Insulin increases glycogenesis in the liver by increasing the activity of glycogen synthase. The glucagon: insulin ratio appears to be more important than the absolute level of either hormone since glycogen metabolism is strongly influenced by the predominant hormone.

In addition to the hormonal regulation of glycogen metabolism, the role of covalent modification in regulation is also important. With separate systems for the synthesis and degradation of glycogen and with glucose-I-phosphate acting as a common intermediate, the possibility ofa futile cycle of glycogen must be considered.

What is a futile cycle? If two reactions occur uncontrolled, the situation would represent a waste of ATP without any metabolic work being done. Futile

cycles or substrate cycles occurs at several points in the patbWay that interconnect glycogen and pyruvate. Other such pairs include glucokinase and glucose-6-phosphatase, pyruvate kinase and pyruvate carboxylase plus phosphoenolpyruvate carboxykinase. However, this does not occur extensively, due to the various control mechanisms, which ensure that one reaction is inhibited as the other is stimulated. At the same time allowing some futile cycling is physiologically advantageous. It helps in 'fine tuning' of metabolic control. The heat generated also helps in maintaining body temperature.

The futile cycle is avoided because covalent modification, by phosphorylation, has opposite effects on the enzymes concerned with the synthesis and degradation of glycogen. Look at the following reactions :

i) An adequate level of cAMP stimulates formation of the inactive 'D' form of glycogen synthase and the active form of phosphorylase. Thus, glycogenesis is limited and glycogenolysis is increased.

ii) With a low level of cAMP, the active I form of glycogen synthase predominates and the active form of phosphorylase kinase is low because the common phosphorylating enzyme, cAMP dependent protein kinase, is inactive. There, glycogenesis is increased and glycogenolysis is decreased.

Having reviewed the processes of glycogenesis and glycogenolysis, we have more or less completed our study of glycogen metabolism. However, before we end our study on this topic, we must also study about the disorders linked with glycogen metabolism. This aspect is discussed next.

6.8.6 Glycogen Storage Diseases

Glycogen storage diseases are caused by genetic defects that result in deficiencies in certain enzymes of glycogen metabolism. These deficiencies lead to excessive accumulation of glycogen and 1 or the inability to use that glycogen as a fuel source. The structure of glycogen may also be abnormal.

6.9 HEXOSE MONOPHOSPHATE PATHWAY

The hexose monophosphate pathway (HMP also called the pentose phosphate pathway, or phoshogluconate pathway) consists oftWo irreversible oxidative reactions, followed by a series of reversible sugar phosphate interconversions. This is an alternate oxidative pathway for the metabolism of glucose in the liver, lactating mammary gland and adipose tissue in addition to Embden—Meyerhof pathway for glycolysis.

In this pathway, 3 molecules of glucose-6-phosphate yield 3 molecules of C02 and 3 molecules of five carbon residues (pentose sugar). The latter are converted ultimately to 2 molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3-

phosphate. NADP serves as a hydrogen acceptor in this pathway.

Unlike glycolysis or the citric acid cycle in which the direction of the reactions is well defined, the interconversion reactions of the HMP pathway can function in several different directions.

The rate and direction of the reactions at any given time are determined by the supply of and demand for intermediates in the cycle. The HMP pathway like glycolysis occuFS in the cytosol of the cell. However, C02 which is not produced in glycolysis, is a characteristic product in HMP pathway.

Further, in this pathway no ATP is generated, which you know, is the major product of glycolysis. Again oxidation uses NADP+ unlike NAD+ in glycolysis. It would be a useful exercise for you to list the similarities and differences in glycolysis and HMP pathway, later after having gone through the section of HMP pathway here. So let's get moving and get to know the metabolic reactions in the HMP pathway.

6.9.1 Metabolic Reactions in the HMP Pathway

The hexose monophosphate pathway is responsible for the generation of a substantial fraction of the cytoplasm NADPH required for biosynthetic reactions, and for the generation ofribose-5-phosphate for nucleotide synthesis. Hence, there are the following two phases of HMP pathway:

1) In the oxidation pathway, glucose-6-phosphate is converted to ribulose-5-phosphate by dehydrogenation and decarboxylation reactions. 2) In the no oxidative phase, ribulose-5-phosphate is converted back to glucose-6- phosphate by a series of reactions involving transketolase and transaldolase.

1) *The oxidative phase generates NADPH*

The oxidative branch of the pathway generates NADPH and pentose-5-phosphate, through the following reactions:

i) Glucose-6-phosphate is dehydrogenated to 6-phosphogluconate via 6-phosphoglucano-lactone by glucose-6-phosphate dehydrogenase in presence of $NADP^+$ and the cofactors Mg^{++}, Mn^{++} or Ca^{++}.

Glucose-6-phosphate dehydrogenase deficiency is an inherited disease characterized by haemolytic anaemia if the patient is treated with an oxidant drug (such as primaquine and sulphonamide) or ingests fava beans.

ii) 6-phosphogluconate is oxidized by 6-phosphogluconate dehydrogenase in the presence of coenzyme $NADP^+$ and cofactors Mg^{++}, Mn^{++} or Ca^{++} to 3-keto 6 phosphogluconate which is decarboxylated to form ribulose-5 phosphate

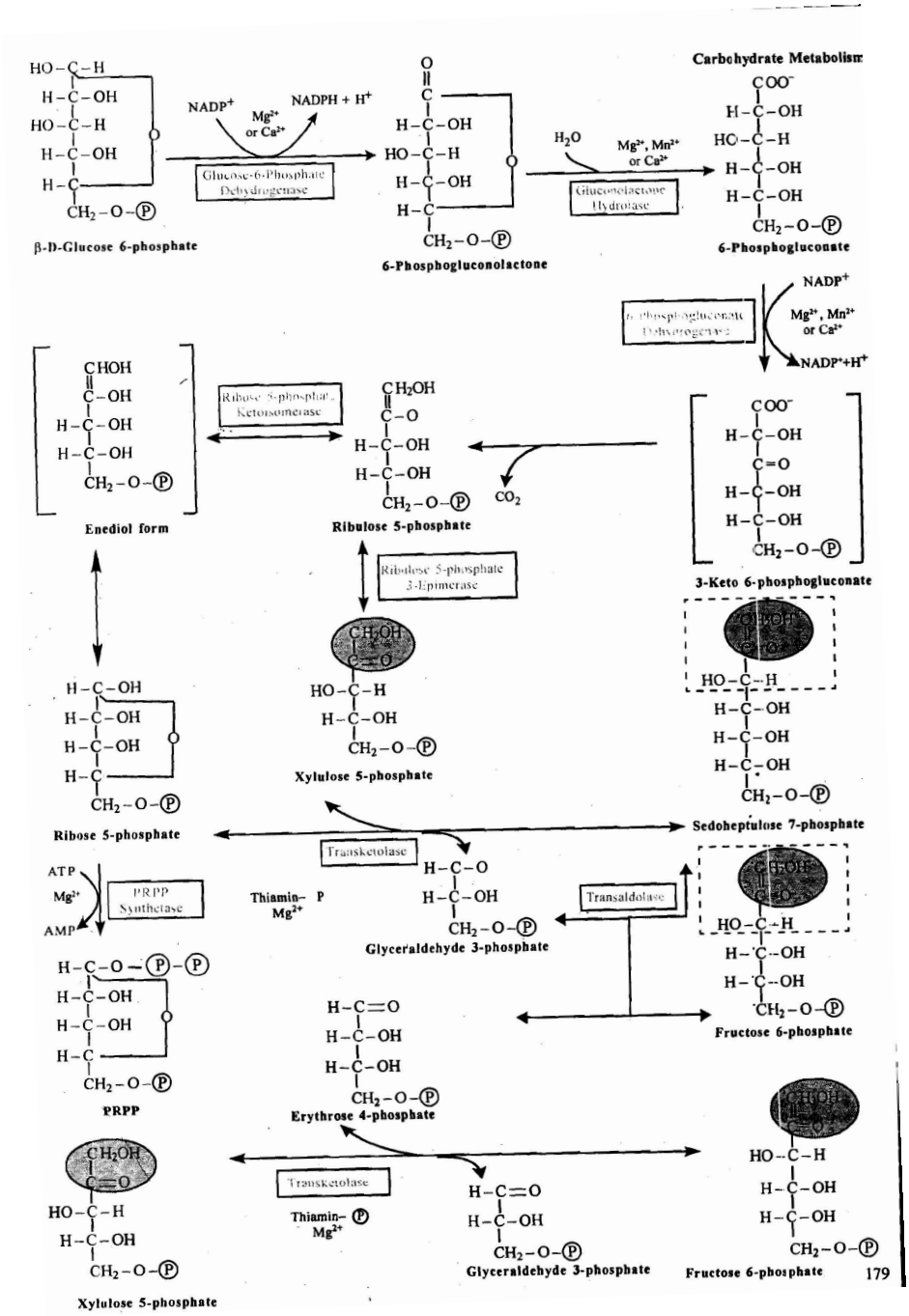

Figure 6.16 : Pentose Phosphate Pathway (HMP Shunt)

2) *The non-oxidative phase generates ribose precursors*

The non-oxidative phase of the pathway, including the following reactions, converts pentose-5-phosphate to other sugars.

i) Ribulose-5-phosphate is acted on by ribulose-5-phosphate epimerase, as shown in NFigure 6.16, which changes the configuration at carbon 3 forming xyluIose-5- phosphate, and also by the enzyme ribose-5-phosphate ketoisomerase, which converts ribulose-5-phosphate to ribose-5-phosphate.

ii) The next step involves the action of the enzyme transketolase. Transketolase with the help of TDP and Mg* transfers carbons land 2 of xylulose-5-phosphate to ribose-5-phosphate forming sedoheptulose-7-phosphate and glyceraldehyde-3- phosphate.

iii) The next step lhvolv the action of the enzyme transaldolase. Transaldolase allows the transfer of a 3-carbon moiety from sedoheptulose-7-phosphate to glyceraldehyde*- osphate to form fructose-6-phosphate and erythrose-4-phosphate.

iv) Transketolase with the help of TPP and Mg^{++} is required again. This time it transfers carbon 1+2 from xylulose-5-phosphate to erythrose-4-phosphate forming fructose- 6-phosphate and glyceraldehyde-3-phosphate.

Thus HMP shunt is not an isolated repetitive cycle, but is integrated with glycolysis. The overall reaction of the HN'IP shunt is as follows:

3 Glucose-6-phosphate + 6NADP+

↓

$3CO_2$ + 6NADPH + $6H^+$ + glyceraldehyde-3-phosphate + 2 glucose-6-phosphate

In order to oxidize glucose completely to CO2 via the HMP pathway, tissues must have the enzymes for converting glyceraldehyde-3-phosphate to glucose-6-phosphate. This involves reversal of giycolysis and the gluconeogenic enzyme fructose- 1,6-bisphosphatase. In tissue > ihat lack this enzyme, glyceraldehyde-3-phosphate follows the normal pathway of glycolysis to pyruvate.

Passage around the cycle oxidises only C-1 of glucose so that six passages around are necessary for the complete oxidation of a molecule of glucose. The pentose phosphates are converted into fructose-6-phosphate, which is isomerized to glucose-6-phosphate to begin the cycle all over again.

How is the HMP pathway regulated? Let's find out next.

6.9.2 Regulation of HMP Pathway

The following factors play an important role in regulation of HMP pathway:

i) The first reaction of this pathway catalysed by glucose-6-phosphate dehydrogenase is the ."rate limiting" step. This is mainly, regulated by the cytoplasmic levels of $NADP^+$ and NADPH.

ii) High carbohydrate content in the diet accelerates the rate of the pathway by activating both the dehydrogenases whereas diabetes mellitus and starvation reverses these reactions.

iii) The HMP shunt isactivated by the increase in $NADP^+$ in the cytoplasm, which in turn, is due to the oxidation (utilimtion) of NADPH by the synthesis

of fatty acids and steroids.

iv) HMP shunt is accelerated due to the stimulation of dehydrogenases by insulin.

v) Thyroid hormone acts in the same way by stimulating glucose-6-phosphate dehydrogenase.

Finally, what is the metabolic significance of the HNfP pathway? Let us find out.

6.9.3 Metabolic Significance of HMP Pathway

Having gone through the HMP pathway, you would have got some idea about the significance of this alternative oxidative pathway for the metabolism of glucose. Let us enumerate the significance one by one

We have seen that CO_2 is the characteristic product in the 1--1WfP pathway, which is not produced in the Embden-Meyerhof pathway. CO_2 produced in this pathway is used for the synthesis of fatty acids and purine bases.

The reduced form of NADP (NADPH) is utilized for the synthesis of fatty acids, cholesterol, steroids and also in the synthesis of amino acids via glutamate dehydrogenase outside the mitochondria. In fact tissues specializing in active lipogenesis — liver, adipose tissue and the lactating mammary glands — also posses an active HMP pathway. The pentose sugars produced in HWP shunt are utilized for the synthesis of nucleic acids and nucleotides.

Skeletal muscle has low activity of glucose-6-phosphate dehydogenase. Yet, like most other tissues it can synthesize ribose-5-phosphate. This is.probably accomplished by a reversal of the shunt pathway utilizing fructose-6-phosphate and glyceraldehyde-3- phosphate and the enzymes transketolase and transaldolase. Both fructose-6-phosphate and glyceraldehyde-3-phosphate are utilized in Embden-Meyerhofpathway for glycolysis. Hence it is not necessary to have a completely functioning pathway for a tissue to synthesize ribose-5-phosphate.

The fragility of erythrocytes is impaired in the absence of NADPH generation due to the deficiency of glucose-6-phosphate dehydrogenase thereby causing haemolytic anaemia when the red blood cells are subjected to certain drugs such as primaquine and sulphonamide. An inverse correlation has been found between the activity of glucose- 6-phosphatedehydrogenase and the fragility ofred cells (i.e. susceptibility to haemolysis). HMP shunt in erythrocytes is of importance due to the generation of NADPH, which maintains the glutathione (G-SH) in the reduced state by glutathione reductase, a flavoprotein containing FAD. Glutathione is a tripeptide (glycine-glutamate-cysteine), which, in the reduced state takes part in redox reactions in cells. In this process, two glutathione molecules combine to give the oxidized form (G-S-S-G). The reduced glutathione then removes H_2O_2 from the erythrocytes by glutathione peroxidase, an enzyme containing selenium

NOTES

as shown in Figure 6.17. fiis reaction is important because accumulation of H_2O_2 may decrease the life-span of erythrocytes by increasing the rate of oxidation of haemoglobin to methaemoglobin.

Glutathione peroxidase is a natural antioxidant present in many tissues. Together, with vitamin E it is part of the body's defense against lipid peroxidation. An association between the incidence of some cancers and low level of blood selenium and glutathione peroxidase activity has been reported.

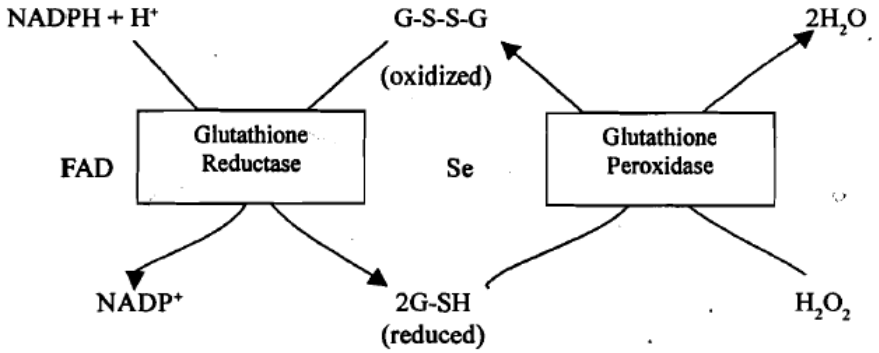

Figure 6.11: Role of NADPH in erythrocytes

A supply of NADPH is critical for the liver microsomal cytochrome P-450 mono oxygenase system which serves to detoxi& drugs and foreign compounds by converting them into soluble forms more readily excreted through the kidney as illustrated in Figure 6.18. HMP pathy;ay becomes an important source of NADPH for this reaction.

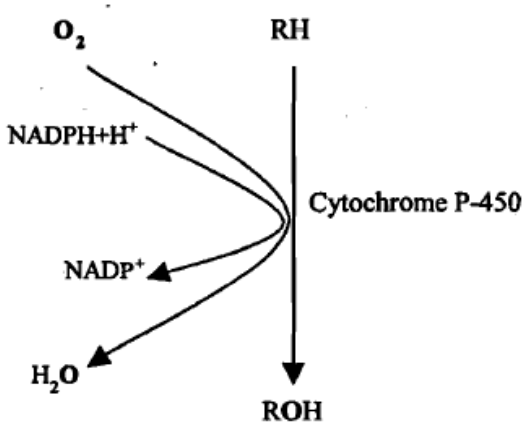

Figure 6.18: Microsomal cytochrome P-4SO mono oxygenase system

Having gone through the discussion above, you would have got a very good idea about the significance of HMP pathway. The HMP pathway or the so-called pentose phosphate pathway, you can now understand is a multifunctional pathway.

HMP pathway, other than the glycolytic pathway, is an alternate oxidative pathway for the metabolism of glucose. Besides glucose, the digestion of foodstuffs

and the utilization of endogenous metabolites cm supply a variety of carbohydrates for glycolysis.

6.10 ENTRY OF OTHER SUGARS INTO GLYCOLYTIC PATHWAY

NOTES

The digestion of foodstuffs and the utilization of endogenous metabolites can supply a variety of carbohydrates for glycolysis. You may already know that the digestion of dietary carbohydrates results in the absorption of monosaccharides such as galactose, fructose and mannose, in addition to glucose. Dietary galactose, fructose and mannose can be converted into glycolytic intermediates and fed into the glycolytic pathway. The metabolic routes for utilizing substrates other than glucose in glycolysis are summarized in Figure 6.19.

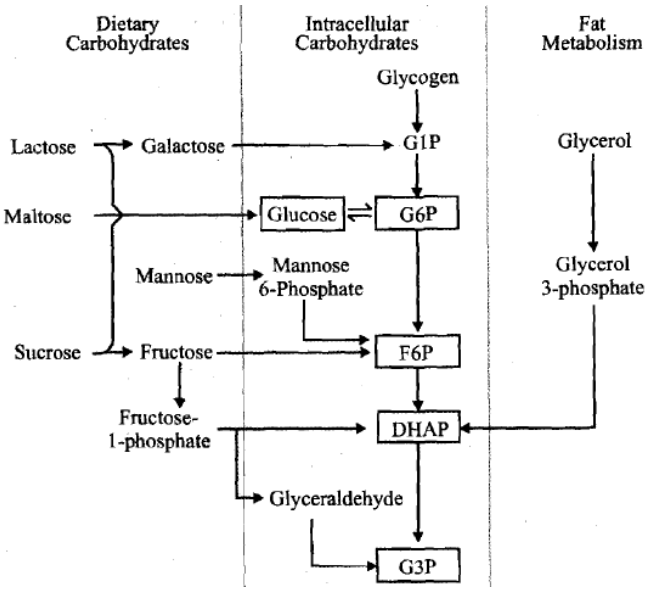

Figure 6.19: Routes for utilizing substrates other than glucose in glycolysis

Let us look at the metabolism of galactose, fructose and mannose in greater details.

Galactose Metabolism

Galactose goes to the liver via portal blood and is phosphorylated by galactokinase to galactose- I -phosphate (GIP) using ATP as a phosphate donor as shown in Figure 6.20. Galactose- I -phosphate reacts with uridine diphosphate glucose to form uridine diphosphate galactose catalyzed by galactose-I-phosphate uridyl transferase.

It is then converted to UDP-glucose catalyzed by UDP-galactose 4-epimerase. Since this would be the well-fed state, the glucose would be incorporated into the glycogen chain through the action of glycogen synthase.

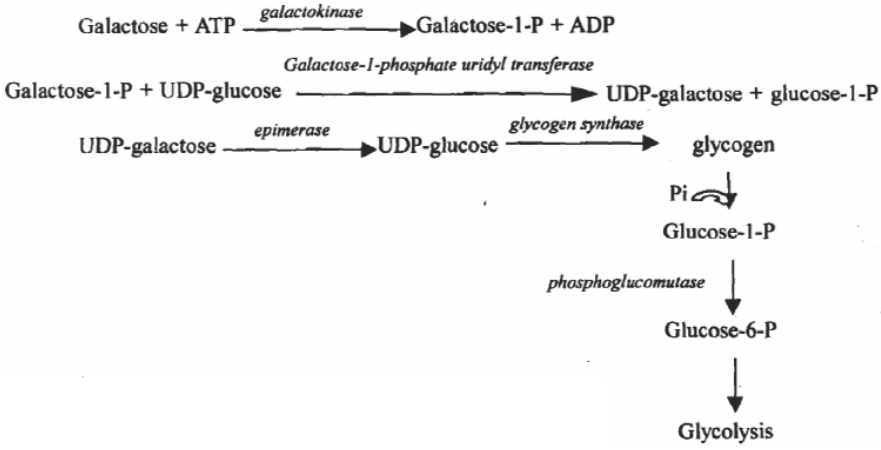

Figure 6.20: Entry of galactose into the glycolytic pathway

Fructose metabolism

The entry of fructose into the glycolytic pathway is illustrated in Figure 6.21. The process starts when fructose absorbed from the diet is taken to the liver. A specific kinase, fructokinase, phosphorylates fructose to fructose-I-phosphate. This is cleaved by Aldolase B, which is found only in the liver, with the formation of dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde is phosphorylated by triokinase to glyceraldehyde-3-phosphate which can enter glycolysis. Dihydroxyacetone phosphate can be isomerized to glyceraldehyde-3-phosphate and enter glycolysis. However, being the well-fed state, the more likely pathway would be for dihydroxyacetone phosphate and glyceraldehyde-3-phosphate to be converted to fructose-1 ,6-bisphosphate by Aldolase A or Aldolase B. Aldolase A, unlike Aldolase B is also found in extrahepatic tissues. Fructose-1,6-bisphosphate through the rest of the gluconeogenic enzyme, ultimately gets incorporated into glycogen chain. Alternatively, if there is a need, glyceraldehyde-3 -phosphate can enter glycolysis.

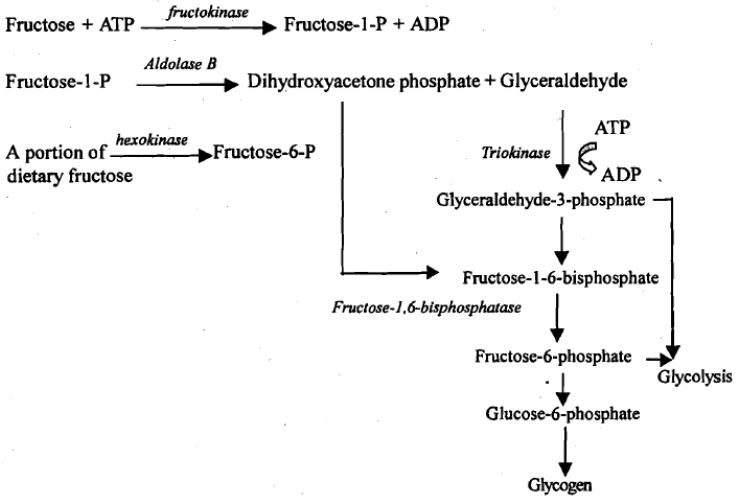

Figure 6.21: Entry of fructose into the glycolytic pathway

Mannose Metabolism

Mannose is phosphorylated by hexokinase forming mannose-6-phosphate. This is isomerized by phosphomannose isomerase to form fructose-6-phosphate which can be incorporated into glycogen chain as shown in Figure 6.22.

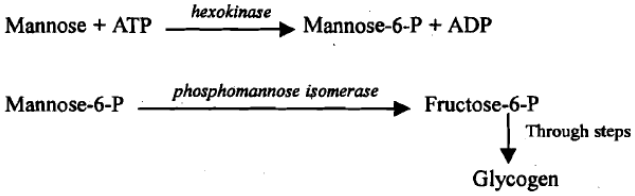

Figure 6.22: Entry of mannose into the glycolytic pathway

To help you recapitulate what you have learnt so far, we suggest, you practice all these reactions with the chemical structures of the intermediates.

Before we end our discussion on this topic, we need to understand that some time the monosaccharides can accumulate in the body and can cause biochemical defects. One such common defect is galactosemia. Let us get to know about this next.

Galactosemia

Inability of conversion of galactose to glucose results in the accumulation of galactose in the blood — known as galactosemia. The biochemical defect usually found in galactosemia is the deficiency of the enzyme galactose-I-phosphate uridyl transferase. Initially, galactose accumulates in the tissues, then in the blood. The major organ damaged by galactose accumulation is liver. Galactose is reduced to the corresponding alcohol called galactitol in the eye which causes cataract.

So it is clear that glucose is the main fuel for the body. Other monosaccharides from the diet are converted to glucose. The level of glucose in the blood is maintained within a specific range in our body. What is this range? How is the blood glucose level regulated in the body? We will -learn about this in the next section.

6.11 REGULATION OF BLOOD GLUCOSE LEVEL

Various levels of regulation are exerted at substrate level, hormonal level, enzymatic level and at organ level on carbohydrate metabolism so as to maintain the blood glucose level at the optimal range between 4.5-5.5 mmol/litre. Events leading to such maintenance include:

a) In the fed state, clearance of blood glucose is mainly by liver via glucokinase. Glucokinase, which is an inducible enzyme, removes most of the blood glucose from circulation after a carbohydrate meal. Further, uptake of glucose also takes place in the extra-hepatic tissues (such as muscle, adipose tissue etc.) favoured by insulin. These two mechanisms regulate

the blood glucose level in the fed state.

b) In the fasting state, glucose release from the liver increases due to the action of glucagon and in the muscle by epinephrine. Glucagon also enhances gluconeogenesis from amino acids and lactate. Both hepatic glycogenolysis and glyconeogenesis contribute to the hyperglycaemic effect of glucagon. Through these mechanisms, the blood glucose level is maintained in the fasting state.

c) The anterior pituitary gland secretes hormones that elevate the blood glucose and therefore antagonize the action of glucose. Two of these major hormones include growth hormone and ACTH (corticotropin).

d) The adrenal cortex secretes the glucocorticoids which increase gluconeogenesis by increasing hepatic intake of amino acids accompanied by increased activity of enzymes of gluconeogenesis, as well as, of transaminases. Glucocorticoids also inhibit utilimion of glucose in extra hepatic tissues. The net effect is one ofincreasing blood sugar level

From the above discussion, it can be seen that insulin is the only hormone decreasing blood glucose level, while all other hormones increase the blood glucose level. You may already be aware of the consequences of elevated blood glucose level. Yes, diabetes mellitus is the resulting metabolic disorder. A brief review follows.

Diabetes mellitus

deficiency of insulin and for defects in insulin action. This anabolic hormone exerts its action on key glycolytic enzymes thus leading to the conversion of glucose to pyruvate as explained under the regulation of glycolysis. On the other hand, insulin suppresses the action of all key gluconeogenic enzymes as shown in Figure 6.7. With respect to glycogen metabolism, the excess glucose is converted to glycogen by activating glycogen synthase thus leading to glycogenesis and inhibiting glycogenolysis. In this metabolic disease-diabetes mellitus, the lack of insulin reverses these actions and the antagonistic hormones (like glucagon, epinephrine, catecholamines, thyroxine etc.) by their concerted efforts on various pathways bring about the hyperglycemic condition.

6.12 ELECTRON TRANSPORT CHAIN

All processes require energy. In living cells, we constantly use energy for a number of biochemical reactions e.g. muscular movements, synthesis ofnew components, transport of ions, secretion and excretion etc. We have already leamt earlier in this unit that ATP is the molecule that supplies the energ for all these processes. Therefore, there is a need for the continuous synthesis of ATP. This section deals with the synthesis of ATP in the mitochondria.

6.12.1 Mitochondrial Electron Transport Chain

Mitochondria houses the electron transport chain (ETC) and the reactions ofoxidative phosphorylation (OP). Hence the mitochondria is called as thepower house of the cell.

Metabolism of carbohydrates, lipids and amino acids yields reducing equivalents such as NADH, $FMNH_2$, $FADH_2$ which undergoes reoxidation. These reduced coenzyrmes in turn each donate a pair of electrons to a specialized set of electron transport chain. U Itimately these electrons are transferred to O_2. As electrons pass through this chain, they lose much of their free energy. Part of this free energy is captured and stored by the formation of ATP from ADP and inorganic phosphate. This process is called as oxidative phosphorylation. We will learn about this later in this section after studying about the components of ETC.

How does the transfer of electron take place? Having read through the carbohydrate metabolism in this unit, surely you should be able to answerthis. Yes, a wide variety of enzymes and coenzymes are involved. Read and find out

6.12.2 Transfer of Electrons

Electron transfer reactions are carried out by a wide variety of enzymes, coenzy•mes [NAD^+, FMN, FAD, coenzyme Q (ubiquinone)], non-heme iron-sulphur centres, cytochromes and metal ions such as Cu and Fe. The mitochondrial electron transport chain consists of four enzyme complexés embedded in the inner mitochondrial membrane plus two free electron carriers. Electrons enter the chain from carrier to carrier arranged in order of increasing redox potential, finally reducing oxygen to water, as you shall see in the next sub-section.

What are the components of the electron transport chain? Let's get to know next.

6.12.3 Components of Electron Transport Chain

The various components of the electron transport chain are illustrated in Figure 6.23.

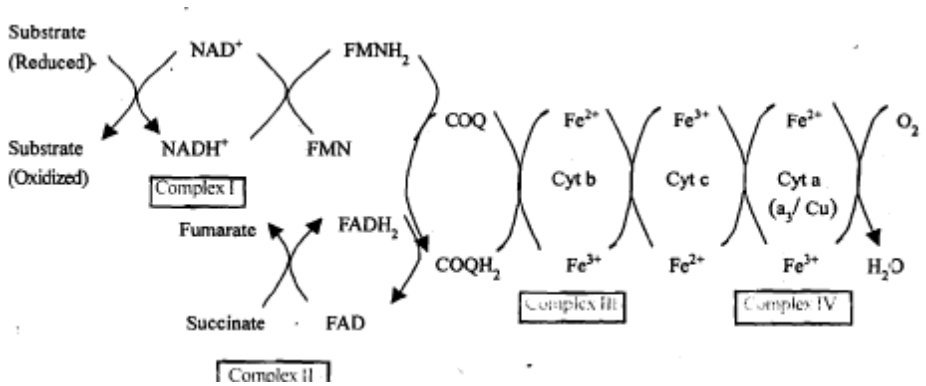

Figure 6.23 : Components of ETC

These components include:

Complex 1 : NADH ⟶ coenzyme Q (NADH coenzyme 0 reductase) contains bound FMN in association with NADH dehydrogenases and Fe-S proteins. The two electrons from NADH first reduce FMN to $FMNH_2$, the Fe-S proteins are reduced next and finally coenzyme Q is reduced to the ubiquinol form

Complex 11: FAD_2 ⟶ coenzyme Q

Not all substrates are linked to the respiratory chain through the NAD-specific dehydrogenases. Some e.g. succinate/fumarate are linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain.

Succinate dehydrogenase and an Fe-S protein make up complex II (succinate:ubiquinone oxidoreductase). The $FADH_2$ component of succinate dehydrogenase reduces the Fe- S centre, which in turn reduces coenzyme Q to ubiquinol.

Complex 111: Coenzyme Q ⟶ cytochrome C (cytochrome reductase)

This complex consists of cytochrome b, an Fe-S protein and cytochrome C (ubiquinone:ferricytochrome C reductase). Electrons are transferred one at a time from COQ with the inner mitochondrial membrane. Coenzyme Q functions as a mediator between the two electron carriers (complex I and II) and one electron carrier (complex Ill and [V). Thus coenzyme Q links the flavoproteins to cytochrome b, the member of the cytochrome chain of lowest redox potential. Q exist in the oxidized quinine or reduced quinol form under aerobic and anaerobic conditions.

An additional component is the iron-sulphur protein (Fe-S; nonheme iron). It is associated with the flavoproteins and with cytochrome b. The sulphur and iron are thought to take part in the oxidoreduction mechanism between flavin and Q, which involved only a single electron change, the iron atom undergoing oxidoreduction between Fe^{2+} and Fe^{3+}.

Complex IV: cytochro me C ⟶ O2(cytochrome oxidase)

The cytochrome C oxidase complex is the last component in the electron transport chain (ferrocytochrome C:oxygen oxidoreductase). It accepts electrons from cytochrome C and catalyzes the four electron reduction of molecular oxygen to water.

$$4e^- + 4H^+ + O_2 \longrightarrow H_2O$$

Electrons flow from Q through the series of cytochrome in order of increasing redox potential to molecular oxygen. The terminal reducing cytochrome (cytochrome oxidase) responsible for the final combination of reducing equivalents with molecular oxygen has a very high affinity for oxygen, allowing the respiratory chain to function at maximum rate until the tissue becomes depleted of02. This is

the only irreversible reaction in the chain and gives direction to the whole process.

6.12.4 Electron Transport Inhibitors

Several compounds, including specific drugs, chemicals and antibiotics have been known to inhibit the electron transfer reactions at specific sites of the electron transport chain, thereby making the ETC non functional. Some ofthese inhibitors are amytal, antimycin A and cyanide. The sites of inhibition are shown in Figure 6.24.

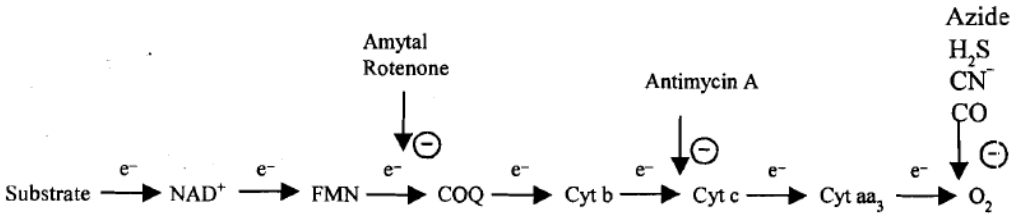

Figure 6.24 : Site specific inhibition of ET

6.12.5 Oxidative Phoshorylation

Oxidative phosphorylation, you already know, is the process by which ADP is phosphorylated by Pi to ATP in the respiratory chain. Oxidative phosphorylation is coupled to oxidation. The phosphorylation reaction is associated with complex V which synthesises ATP utilizing the entry of the proton gradient generated by the electron transport chain from complexes I, Ill and IV. These complexes (I, Ill and IV) are also called as phosphorylation sites I, II and Ill, respectively as illustrated in Figure 6.25. Two electrons are required to reduce one atom ofoxygen to H20. Therefore the oxidation of one molecule of NADH or FADH2 corresponds to the synthesis of three or two ATPs, respectively and to the reduction of atom of oxygen. It is usually stated, the oxidation of NADH or FADH2 occurs with P/O ratio of 3 and 2 respectively.

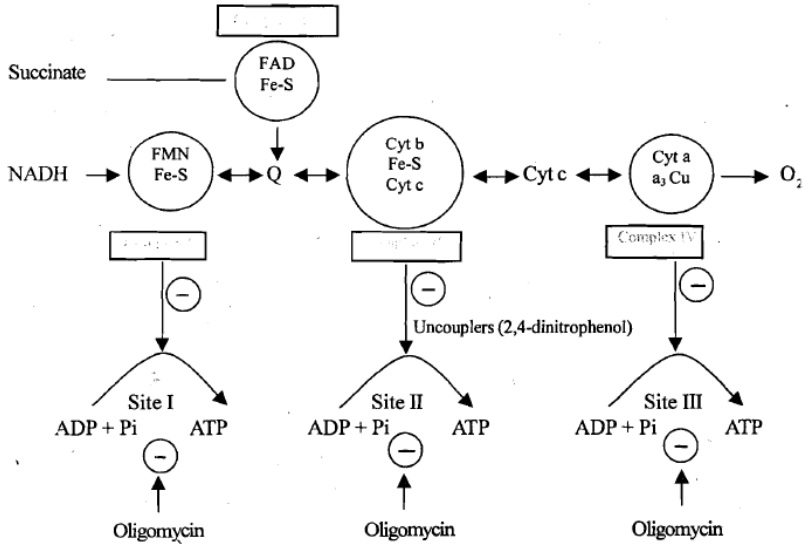

Figure 6.25 : Oxidative phosphorydation sites and action of various inhibitors

6.13 LET US SUM UP

In this unit we studied about carbohydrate metabolism. We learnt that glucose is the principal carbohydrate fuel for the body. Other monosaccharides, such as fructose, galactose, and mannose from the diet are convened to glucose. Glucose in the cell enters any one of the pathways such as glycolysis, glycogenesis, glycogenolysis etc., depending on the cellular requirement of glucose. Excess glucose is converted to fat and stored in adipose tissue. Further, various monosaccharides with atoms ranging from 3 carbons to 7 carbons are all converted to glycolytic pathway intermediates via the HMP shunt pathway. Blood glucose level is maintained within the normal range both during fasting and post-prandial state through the concerted action ofseveral key enzymes and hormones. Several glycogen storage diseases have been characterized due to the deficiency of key enzymes of glycogen metabolism.

The last part ofthe unit focussed on the mitochondrial electron transport. The mitochondrial electron transport chain, we learnt, mediates the reduction of molecular oxygen to water by NADH and FADH2. The transport of electrons through the electron transport chain makes available a significant quantity of free energy which is used in the synthesis of ATP in the process of oxidative phosphorylation.

6.14 GLOSSARY

Glycolysis : degradation of glucose.

Gluconeogenesis : synthesis of glucose from non-carbohydrate source.

Glycogenesis : synthesis of glycogen.

Glycogenolysis : degradation of glycogen.

Electron transport chain : transport of high-energy electrons through a seriesofcarriers in mitochondria.

Oxidative phosphorylation : synthesis of ATP from ADP and Pi during the passage of electrons in the respiratory chain.

6.15 CHECK YOUR PROGRESS

1) List the reactions that need to be circumvented by the special reactions in gluconeogenesis.
2) Enumerate the major substrates for gluconeogenesis.
3) What are anaplerotic reactions?

UNIT

7

LIPID METABOLISM

NOTES

STRUCTURE

7.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

explain how fatty acids are oxidized for the production of enery,
describe the synthesis of fatty acids,
discuss the metabolism of cholesterol,
relate the cholesterol and lipoprotein metabolism to hyperlipidemia, and
discuss the significance of eicosanoids in human nutrition
discuss the metabolism of phospholipids
discuss the metabolism of triacylglycerols
Issues relate to cholesterol

7.2 INTRODUCTION

Lipids are a heterogeneous group of organic compounds. The major dietary lipids for humans are animal and plant triacylglycerols, sterols and membrane phospholipids.

Here, in this unit, we shall focus on their metabolism. The process of lipid

metabolism involves synthesis and degradation of the lipid stores. It also involves the production of the structural and functional lipids characteristic of individual tissues. This unit gives an overview of lipid metabolism at the cellular level, which would provide enough background for understanding the aberrations with regard to lipid metabolism and its related diseases.

The first part of the unit, section 7.2 — Lipid Metabolism I — focuses on fatty acid metabolism i.e. issues/reactions related to degradation and the synthesis of fatty acids— saturated, unsaturated — in our body.

The second part of the unit, section 7.3 — Lipid Metabolism I! — looks at the metabolism ofneutral fats, phospholipids, cholesterol etc.

In addition, you will also find information regarding hyperlipoproteinemias, ketosis. What do these terms mean? Read and find out in this unit

7.3 LIPID METABOLISM - I

You learnt earlier that the lipids are absorbed through the intestine. As these mo lecules are oils, solubilization (emulsification) ofdietary lipids is accomplished via bile salts that are synthesized in the liver and secreted from the gall bladder.

The emulsified fats are then degraded by pancreatic lipases. These enzymes, secreted into the intestine from the pancreas, generate free fatty acids and a mixture of mono- and diacylglycerols from dietary triacylglycerols.

Following absorption ofthe products ofpancreatic lipase by the intestinal mucosal cells, the resynthesis of triacylglycerols occurs. The triacylglycerols are then solubilized in lipoprotein complexes (complexes of lipid and protein) called chylomicrons. Triacylglycerols synthesized in the liver are packaged into VLDLs and released into the blood directly.

Chylomicrons from the intestine are then released into the blood via the lymphatic system for delivery to the various tissues for storage or production ofenergy through oxidation. The triacylglycerol components of VLDLs and chylomicrons are hydrolyzed to free fatty acids and glycerol in the capillaries of adipose tissue and skeletal muscle.

7.3.1 Oxidation of Fatty Acids

Fatty acids released from chylomicrons and VLDL are transferred across cell membranes by passive diffusion, which depends upon the concentration gradient. Their oxidation consists of: (a) activaUon, (b) transport into the mitochondrial matrix, and (c) reactions of ß-oxidation. Let us next learn about these three stages.

a) *Activation of fatty acids*

Fatty acids cannot undergo oxidation in the form in which they are normally present in the body. They must be converted into a form which can be catabolized. This process is called activation and fatty acid is converted into an active intermediate. Fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria. This activation is catalyzed byfatty acyl CoA synthetase. At least three acyl CoA synthetases, each specific for a particular size (length) of fatty acid are known. Let us see what are these.

1) Acetyl CoA synthetase: This acts on acetate and low molecular weight fatty acids,

2) Medium chain acyl CoA synthetase: This acts on fatty acids with 4-11 carbon atoms, and

3) Acyl CoA synthetase: This acts on fatty acids with 6 to 20 carbon atoms.

The activity of acetyl CoA synthetase in muscles is restricted to the mitochondrial matrix. The medium-chain acyl CoA synthetase occurs only in liver mitochondria, where medium chain fatty acids (obtained from digestion ofdietary triacylglycerols and transported by the portal blood) are metabolized. Acyl CoA synthetase, the major activating enzyme, occurs on the outer mitochondrial membrane surface and in endoplasmic reticulum.

Activation of fatty acids results in the formation of an ester between the carboxyl (COOH) group of the fatty acid and SH group of the coenzyme A, forming fatty acid ester of coenzyme A. The overall reaction of activation is:

$$\underset{\text{(fatty acid)}}{RCOO^- + ATP + CoASH} \rightleftharpoons \underset{\text{(fatty acyl CoA)}}{R - \overset{\overset{O}{\|}}{C} - CO\ SCoA} + AMP + PP$$

The reaction favours the formation of fatty acyl CoA, as the pyrophosphate formed is hydrolyzed by the enzyme pyrophosphatase: PP_1,+ $H_2O \rightarrow + 2P_1$

Thus, activation of a fatty acid molecule requires expenditure of two high-energy 196 phosphate bonds.

b) *Transport of acyl CoA to mitochondrial matrtix*

You learnt above that the activation of fatty acids takes place largely in the cell cytoplasm. However, the enzyme required for oxidation is present in the mitochondria. The activated long chain fatty acids, therefore, must be transported into the mitochondria. However, the mitochondrial membrane is impermeable (not allowing passage) to fatty acyl CoA ester. Hence, the transport of acyl CoA is achieved by carnitine (y-ß-hydroxy-y- trimethylammonium butyrate). Two carnitine acyl transferases are involved in acyl CoA transport: carnitine palmitoyl

NOTES

transferase I (CPT I), located on the outer surface of the inner mitochondrial membrane and carnitine palmitoyl transferase II (CPT II) located on the inner surface.

The activated long chain fatty acyl CoA ester reacts with carnitine in the presence of carnitine palmitoyl transferase I. COA is released and the fatty acid forms complex with carnitine called acyl-carnitine. This is able to penetrate the inner mitochondrial membrane and gain access to ß-oxidation systems of enzymes. Here, another enzyme carnitine-acylcarnitine translocase acts as an inner membrane exchange transporter. Thus, acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine, as shown in Figure 7.1. This carnitine is generated when acylcarnitine reacts with CoA to form once again fatty acyl CoA, catalyzed by carnitine palmitoyl transferase II, located on the inside of the mitochondrial membrane. Once inside the mitochondrion, the fatty acyl-CoA is a substrate for the ß-oxidation machinery, as discussed in the next step. The role of carnitine in transport of fatty acids into the mitochondrial matrix is given below in Figure 7.1.

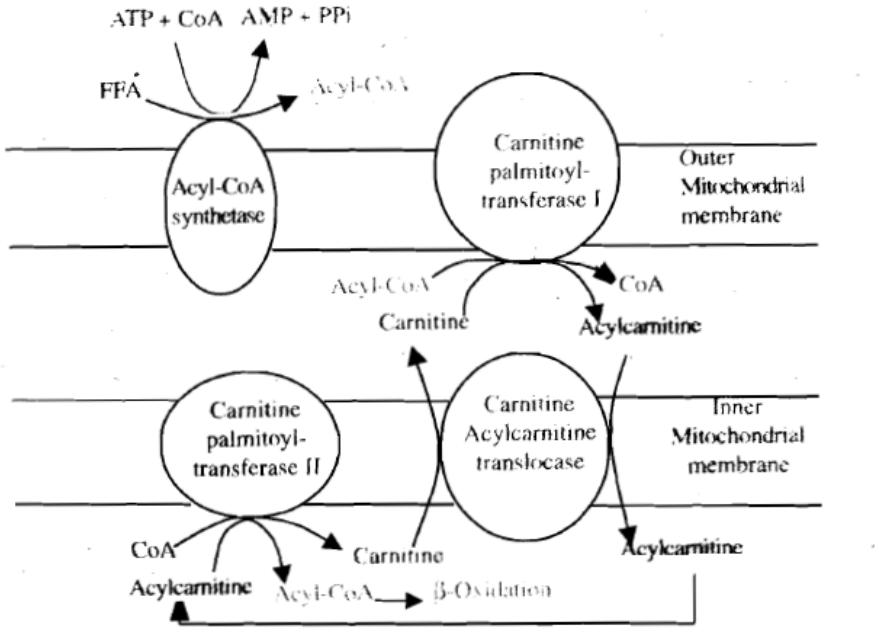

Figure 7.1 : Carnitine cycle

c) *ß-oxidation*

The majorpathway for fatty acid oxidation is ß-oxidation. The process of fatty acid oxidation is termed as ß-oxidation since it occurs through the sequential removal of 2- carbon units (as acetyl CoA) by oxidaiion at the ß-carbon position (between a(2) and ß(3) carbon atoms) of the fatty acyl-CoA molecule. The reactions take place entirely in mitochondrial matrix.

Oxidation of a saturated acyl CoA with an even number of C atom to acetyl CoA requires a repeated, sequential action of the following four enzymes and the steps are highlighted herewith and in Figure 7.2:

i) Acyl CoA dehydrogenase

ii) Enoyl CoA hydratase

iii) ß-hydroxy acyl CoA dehydrogenase

iv) Acetyl CoA acyltransferase (ß-ketothiolase or thiolase).

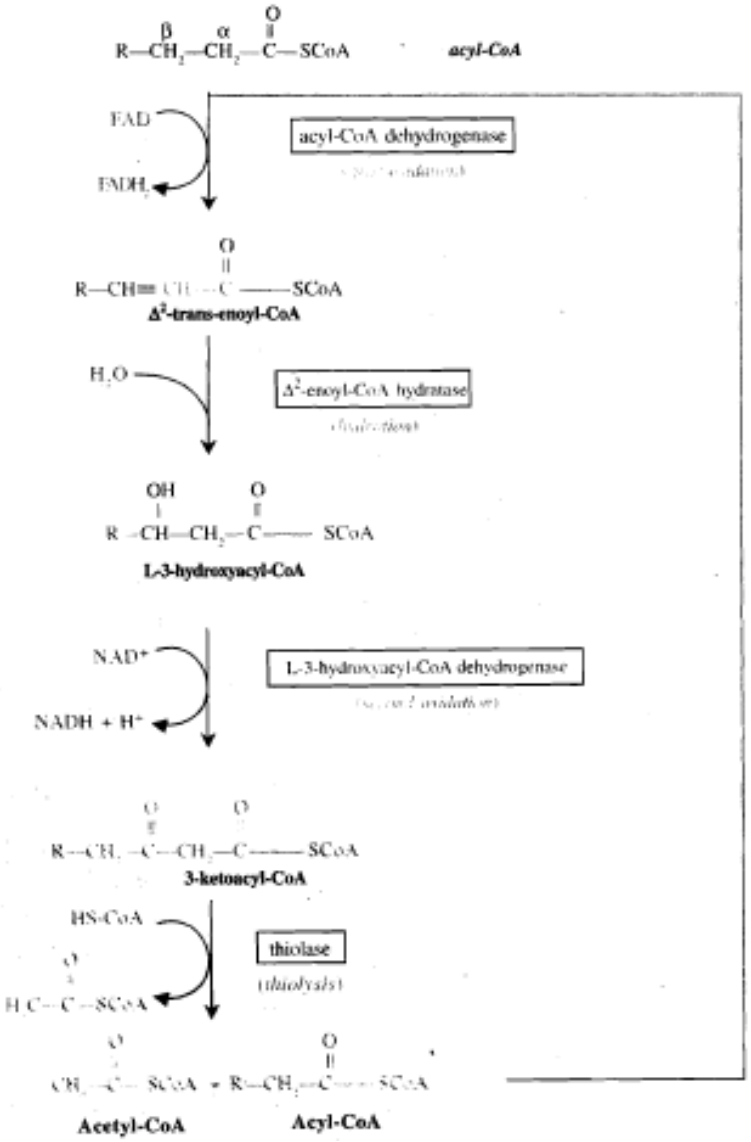

Figure 7.2 : ß-oxidation of fatty acids

i) Acyl CoA dehydrogenase dehydrogenates acyl CoA (i.e. removes two H^+) at the and C atoms. This causes unsaturation to give a, unsaturated acyl CoA (or A2 unsaturated acyl COA). The dehydrogenases are flavoprotein and contain a tightly bound molecule of flavin adenine dinucleotide (FAD as the coenzyme). The electrons of the FADH2 via another flavoprotein are transferred to the respiratory chain to give 2 ATP molecules. The A2

double bond formed has a trans geometrical configuration. The double bonds naturally occurring in fatty acids are in cis form.

ii) Enoyl-CoA hydratase hydrates the A2 unsaturated acyl CoA. This enzyme has broad specificity and can act on a, (or A2) unsaturated CoA in trans or cis configuration. The product formed is L (+)ß-hydroxyacyl CoA. When the trans double bond is hydrated, the D-isomer is formed with cis double bond.

iii) ß-hydroxyacyl CoA dehydrogenase oxidizes ß-hydroxyacyl CoA by an NAD+ - linked reaction that is absolutely specific for L-stereoisomer. The electrons from the NADH generated are passed on to NADH dehydrogenase of the respiratory chain and finally 3 ATP molecules are formed

iv) Acetyl CoA acyl transferase (or thiolase) catalyzes a thiolytic cleavage (i.e. involving SH group) and gives acetyl CoA and acyl CoA, which is shortened by 2 C atoms.

The entire sequence of the oxidation of fatty acid, right from the activation stage to p- oxidation is given in Figure 7.3. The shortened fatty acyl CoA from one cycle is further oxidized in successive passes until it is entirely converted to acetyl CoA.

it is important to note that a majority of natural lipids contain an even number of carbon atoms. A small proportion that contain odd numbers, upon complete lb-oxidation, yield acetyl-CoA units plus a single mole of propionyl-CoA. The propionyl-CoA is converted, in an ATP-dependent pathway, to succinyl-CoA. The succinyl-CoA can then enter the citric acid cycle for further oxidation.

Before we conclude, let us look at the energetics of the ß-oxidation process, discussed above. Table 7.1 presents the outcome. As is evident, 2 mole equivalents ofATP are used during the activation of the fatty acid. On the other hand, the electrons of the FADH2 are transferred to the respiratory chain to give 2 ATP molecules. The electrons from the NADH generated are passed on to the respiratory chain and finally 3 ATP molecules are formed. Oxidation of acetyl CoA gives 12 moles of ATP (each acetyl CoA in citric acid cycle gives 3 NADH and 1 $FADH_2$ and 1 GTP for total 12 ATP).

Table 7.1 : Energetics of ß-oxidation

Reaction	Moles of ATPgained /lost
Activation reaction	-2
First dehydrogenation (FAD)	+2
Second dehydrogenation (NAD)	+3
Oxidation of acetyl CoA	12

Let us understand this by taking an example of palmitic acid (C 16). Palmitoyl CoA will give 8 acetyl CoA molecules (one acetyl CoA gives 12 ATP, hence 8 molecules will give 96 ATP) and will undergo P oxidation 7 times (since 2 aceiyl CoA molecules

are formed in the last cycle). Activation step is only once. The overall ATP yield therefore is:

Activation step	..	-2×1	=	-2
β-oxidation	..	7×5	=	35
Acetyl CoA	..	8×12	=	96
				129

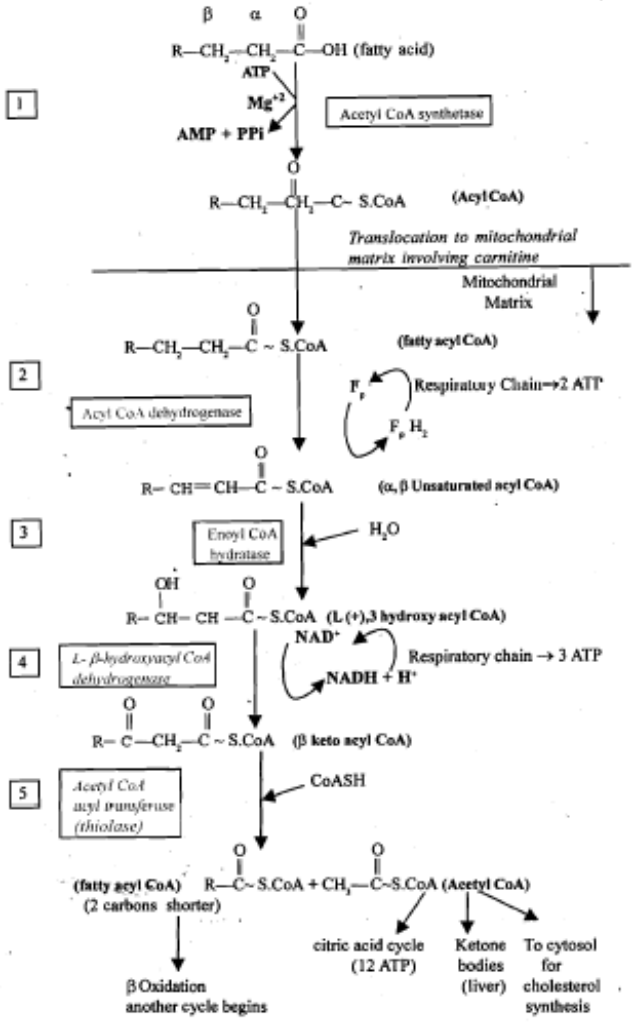

Figure 7.3 : Fatty acid activation, transport and ß-oxidation

Complete oxidation of one molecule of palmitic acid gives 129 ATP molecules. Similarly, the net result of the oxidation of one mole of oleic acid (an 18-carbon fatty acid) will be 146 moles of ATP (2 mole equivalents are used during the activation of the fatty acid). With our discussion above, we have come to the end of our study on fatty acid oxidation.

Next, we shall learn about the oxidation of unsaturated fatty acid.

7.3.2 Oxidation of Mono and Poly Unsaturated Fatty Acids

The oxidation of unsaturated fatty acids is essentially the same process as for saturated fats, as discussed above, except when a double bond is encountered. In such a case, the bond is isomerized by a specific enoyl-CoA isomerase and oxidation continues. In the case of linoleate (linoleic acid), the presence of the C-12 unsaturation results in the formation of a dienoyl-CoA during oxidation. This molecule is the substrate for an additional oxidizing enzyme, the NADPH requiring 2,4-dienoyl-CoA reductase.

Thus, oxidation of unsaturated fatty acids require A3 cis or trans A2 trans enoyl CoA isomerase and NADPH dependent 2,4 dienoyl CoA reductase, in addition to the enzymes of ß-oxidation. Hence, these two enzymes are also referred to as additional or auxillary enzymes.

To help you understand the process and the role of the auxiliary enzymes in the oxidation process of unsaturated fatty acids, we have included the oxidation of oleic acid and linoleic acids— two of the unsaturated fatty acids here in Box l. We have presented these oxidation reactions only to help your understanding on the subject and not primarily for examination purposes. This is extra reading material. Do not get bogged down by these reactions. In fact you should practice these reaction using linolenic acid (C-18; A 9, 12, 15) and arachidonic acid (C-20, 5,8, I l, 14) also.

Box 1 : Oxidation of Unsaturated Fatty Acids

Oxidation of unsaurated fatty acids given in Figures 7.4 and 7.5 are involved herein. In Figure 7.4, you can see that oleic acid (C18) has a double bond between carbon 9 and 10. All naturally-occurring double bonds are in cis configuration. Usual 3 turns ofß-oxidation results in the formation of3 molecules of acetyl CoA and oleic acid becomes a C_{12} fatty acid, with the original cis double bond now being between carbons 3 and 4, i.e. it is & cis enoyl CoA. This is an inactive substrate in'ß-oxidation. So another enzyme A_3 cis (or transh A_2 trans enoyl CoA isomerase converts the cis double bonds to trans (which is required in ß-oxidation) and puts the double bond in positions 2-3. Now A2 trans enoyl CoA is formed and it goes through five more tums ofß-oxidation forming 6 acetyl CoA molecules.

Next, let us look at the oxidation oflinoleic acid. As evident in Figure 7.5, linoleic acid has 18 carbon atoms, with 2 double bonds in positions 9-10 and 12-13, respectively. It goes through three turns of ß-oxidation forming 3 acetyl CoA molecules and a C_{12} fatty acid with 2 cis double bond in 3-4 and 6-7 positions. This is acted upon by the auxillary enzyme AJ cis (or trans) trans enoyl CoA isomerase forming trans- A6-cis dienoyl CoA. Now one more turn of ß-oxidation takes place, removing one molecule of acetyl CoA and forming A6-cis enoyl COA. This is acted upon by acyl CoA dehydrogenase and an extra trans double bond is introduced in 2-3 position forming A2 trans-A4-cis dienoyl CoA. Now a second auxiliary enzyme A2 trans-A4cis dienoyl CoA reductase, utilizing NADPH, reduces one double bond and forms As-trans enoyl CoA, while the configuration (trans) of the double bond is

suitable for ß-oxidation position 3-4 is not suitable. So the first auxiliary enzyme, A3 cis (or A2 trans enoyl CoA isomerase changes the position and forms A2 trans enoyl CoA. This goes through four more turns of ß-oxidation forming 5 more acetyl CoA molecules. Thus with the help of these two auxillary enzymes, linoleic acid can be oxidized completely to 9 molecules of acetyl CoA. In this way, all unsaturated fatty acids can undergo ß-oxidation.

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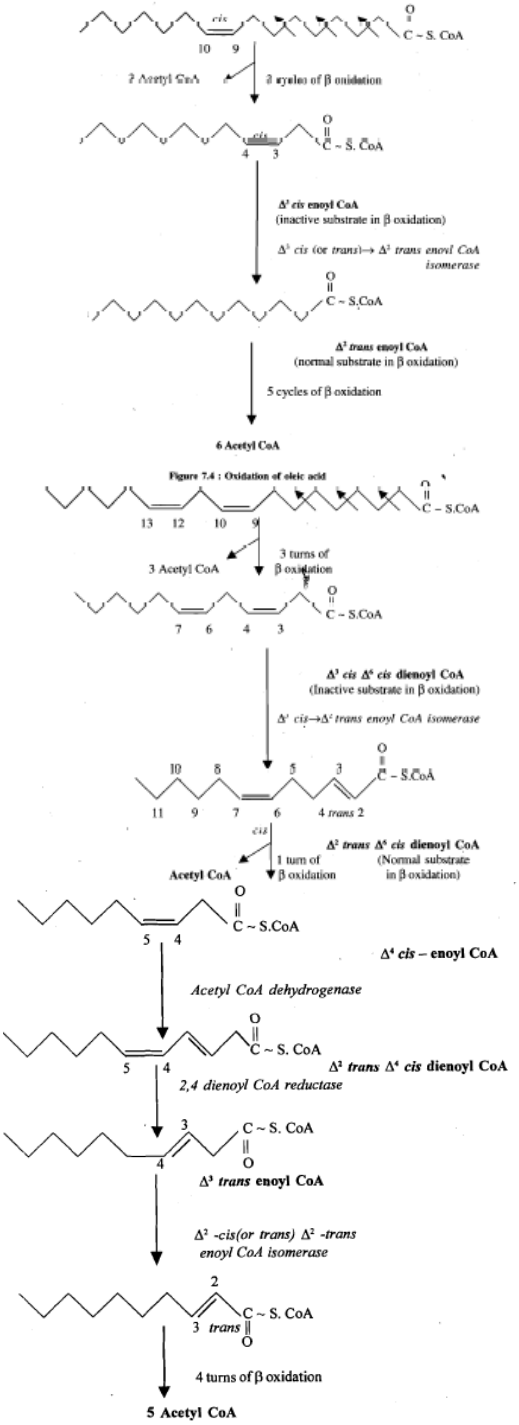

Figure 7.5 : Oxidation of linoleic acid

So we have seen that the oxidation of fatty acids yields acetyl CoA, which is a major source of useful metabolic energy. Now, what is the metabolic fate of acetyl CoA? Read and find out in the next section. But, first let us recapitulate what we have learnt so far.

STUDENT ACTIVITY - 1

1) List the three steps involved in the oxidation of fatty acids? Give the enzymes involved in the process of activation of fatty acids.

.....

.....

.....

2) Name the enzymes involved in the transfer of fatty acyl CoA ester? Briefl discuss the role of carnitine in transfer of fatty acids.

.....

.....

.....

3) Whatdoyou understand bytheterm ß-oxidation?Highlighttherole of4enzymes and steps involved.

.....

.....

.....

4) How many molecules ofATP are obtained from a ß-oxidation of 1 molecule of stearic acid (Cl 8)?

.....

.....

.....

5) How is the oxidation of poly unsaturated fatty acids (oleic, linoleic) different from oxidation of fatty acids?

.....

.....

.....

Having studied the oxidation of fatty acid, next we shall learn about the synthesis of, fatty acids.

7.3.3 Lipogenesis — Synthesis of Fatty Acids

You would notice that acetyl CoA can react "reversibly" in the degradation or synthesis of lipids. Above, we saw that fatty acids are degraded to acetyl CoA. Interestingly, the formation of lipids too starts with acetyl CoA. Let us see how?

NOTES

When there is an oversupply of dietary carbohydrates (CHOs), the excess CHOs are converted to triacylglycerols (you already know that triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified. In other,words, fatty acids are stored primarily in the adipose tissue as triacylglycerol). Further individuals on low fat diets also convert glucose to triacylglycerol, which is stored. This involves the synthesis of fatty acids from acetyl CoA and the esterification of fatty acids in the production of triacylglycerol. The process is called 'lipogenesis'. Infact, the sequence of reactions involved in the formation of lipids is known as lipogenesis. Lipogenesis is not simply the reversal of the fatty acid degradation, but does starts with acetyl CoA and does build up by the addition oftwo carbons units.

The fatty acid synthesis occurs in the cytoplasm in contrast to the degradation (oxidation), which you may recall reading earlier, occurs in the mitochondria. The major lipogenic tissues are the intestine, liver and adipose tissue. During lactation, the mammary gland also becomes a major site for lipogenesis and places a heavy demand on a continuing supply of glucose for the synthesis ofmilk lipids.

The metabolic reactions involved in the synthesis of fatty acid are illustrated step by step in Figure 7.6. The synthesis occurs in the cytosol from acetyl CoA. This is also called de novo (afresh) synthesis.

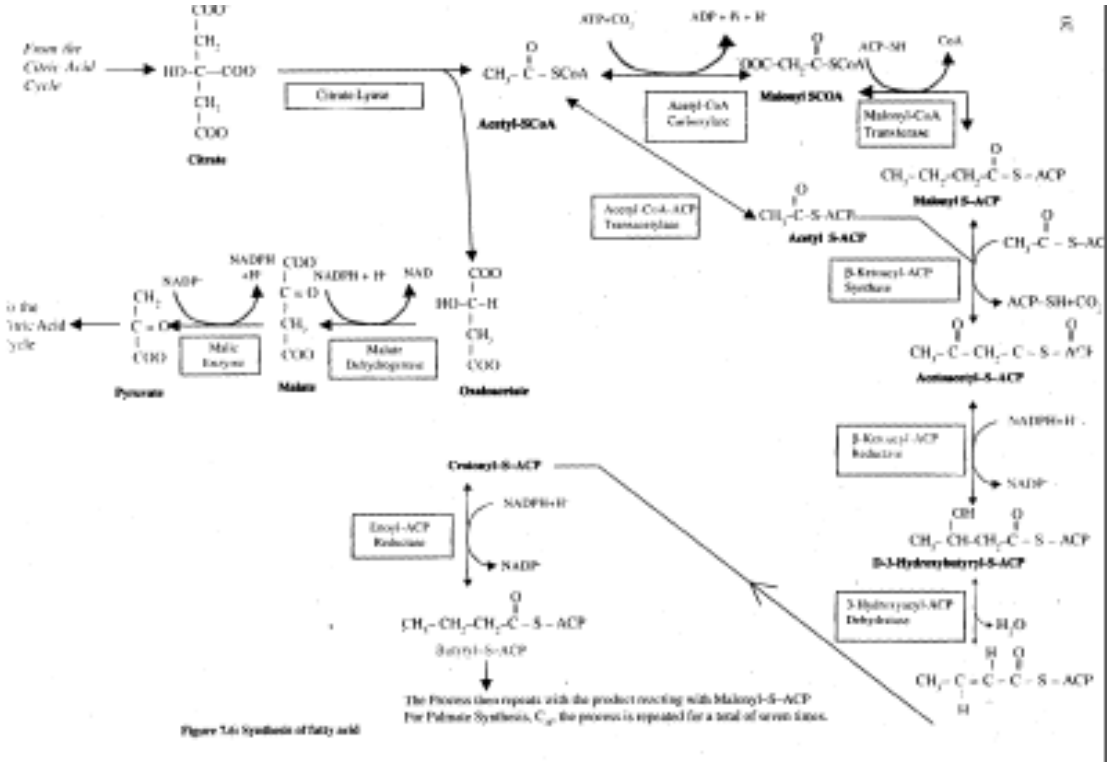

Figure 7.6: Synthesis of fatty acid

When glucose is abundant and the amount of citrate in the mitochondrial matrix exceeds the demand by the citric acid cycle, the excess citrate is transported out of the mitochondria into the cytosol by tricarboxylate translocase. Here, as you can see in Figure 7.6, the citrate is cleaved by-citrate Iyase to provide the acetyl group for fatty acid synthesis. Besides acetyl CoA, NADPH is also required. The NADPH necessary for fatty acid synthesis derives from the conversions of malic enzyme, glucose-6-P dehydrogenase, gluconate-6-P dehydrogenase and NADP-dependent isocitrate dehydrogenase.

The key regulating enzyme of lipogenesis is acetyl-CoA carboxylase. It catalyzes the synthesis of malonyl-CoA from acetyl-CoA and COY In the formation ofmalonyl CoA via acetyl CoA carboxylase, biotin is tightly bound to the enzyme as a prosthetic group and acts as a carrier ofa carboxyl group that is transferred to acetyl CoA. The formation of malonyl CoA signals the beginning ofthe synthesis of fatty acid.

The synthesis of fatty acids from acetyl-CoA and malonyl-CoA is carried out by fatty acid synthase, FAS. Actually, many of the enzymes for the fatty acid synthesis are organized into a multienzyme complex called fatty acid synthase. The sequence of reactions catalyzed by this enzyme, as presented in Figure 7.6, can be represented by the following seven reactions.

In the first reaction, acetyl CoA is added to a cysteine-SH group of the condensing enzyme (CE) domain:

$$\text{acetyl CoA + CE-cys-SH} \rightarrow \text{acetyl-cys-CE + CoASH}$$

Mechanistically, this is a two-step process, in which the group is first transferred to the ACP (acyl carrier peptide), and then to the cysteine-SH group of the condensing enzyme domain.

In the second reaction, malonyl CoA is added to the ACP sulfhydryl group:

$$\text{malonyl CoA + ACP-SH} \rightarrow \text{malonyl ACP + CoASH}$$

This -SH group is a part ofa phosphopantethenic acid prosthetic group of the ACP.

In the third reaction, the acetyl group is transferred to the malonyl group with the release ofcarbon dioxide:

$$\text{malonyl ACP + acetyl-cys-CE} \rightarrow \text{beta-ketobutyryl-ACP + } CO_2$$

In the fourth reaction, the keto group is reduced to a hydroxyl group by the beta-ketoacyl reductase ,activity.

$$\text{beta-ketobutyryl - ACP + NADPH + H+} \rightarrow \text{beta-hydroxybutyryl-ACP + } NADP_+$$

In the fifth reaction, the beta-hydroxybutyryl-ACP is dehydrated to form a trans-monounsaturated fatty acyl group by the beta-hydroxyacyl dehydratase activity:

$$\text{beta-hydroxybutyryl- ACP} \rightarrow \text{2-butenoyl-ACP + } H_2O$$

In the sixth reaction, the double bond is reduced by NADPH, yielding a saturated fatty acyl group two carbons longer than the initial one (an acetyl goup was converted to a butyryl group in this case):

$$\text{2-butenoy1-ACP+ NADPH+ } H^+ \rightarrow \text{butyryl-ACP + NADP+}$$

The butyryl group is then transferred from the ACP sulfhydryl group to the CE sulfhydryl: Lipid [etabolism

$$\text{butyryl - ACP + CE-cys-SH} \rightarrow \text{ACP - SH + butyryl-cys-CE}$$

This is catalyzed by the same transferase activity as was used previously for the original acetyl group.

The butyryl group is now ready to condense with a new malonyl group (third reaction above) to repeat the process.

When the fitty acyl group becomes 16 carbons long, a thioesterase activity hydrolyses it, forming free palmitate:

$$\text{palmitoyl - ACP + } H_2O \rightarrow \text{palmitate + ACP-SH}$$

Go through these reactions carefully. Initially, they might seem a bit tough, but if you follow the sequence as presented in Figure 7.6, you will understand the process of synthesis better.

So you notice, the primary fatty acid synthesized by FAS is palmitic acid. Once an acetyl group and a malonyl group are bound to the fatty acid synthase, seven rounds of enzymatic reactions proceed for the synthesis of palmitic acid, which is then released from the complex. The overall reaction is as follows:

$$\text{Acetyl CoA + 7 Malonyl CoA + 14 NADPH + 14 } H^+$$

$$\rightarrow \text{Palmitic acid + 8CoASH + 14}NADP^+ + 6H_2O + 7CO_2$$

Palmitate is then released from the enzyme and can then undergo separate elongation and/or unsaturation to yield other fatty acid molecules. We shall learn about this next.

So we have seen that in the opposite of fatty acid degradation, which is located within the mitochondria, de novo synthesis of fatty acids takes place within the cytosol. The primary fatty acid synthesized by FAS is palmitic acid. Palmitic acid can be converted to other unsaturated fatty acids. Let us see how.

Synthesis of other unsaturated fatty acids

Palmitic acid may be converted to stearic acid (18:0) by elongation ofthe carbon chain. Desaturation of stearic acid produces oleic acid (C, 8 : 1 A 9). The enzymes are located in the mitochondria and endoplasmic reticulum and can use fatty acid of varying chain lengths and degrees of unsaturated as substrates. Desaturation occurs in the ER membranes as well and in mammalian cells involves 4 broad

specificityfatty acyl-CoA desaturases (non-heme iron containing enzymes). These enzymes introduce unsaturation at C4, C5, C6 or C9. Since these enzymes cannot introduce sites of unsaturation beyond C9 they cannot synthesize either linoleate (18:20 12) or linolenate (18:30' 12'15). These fatty acids must be acquired from the diet and are, therefore, referred to as essential fatty acids. Linoleic is especially important in that it is required for the synthesis of arachidonic acid. As we shall encounter later, arachindonate is a precursor for the eicosanoids (the prostaglandins and thromboxanes). It is this role of fatty acids in eicosanoid synthesis that leads to poor growth, wound healing and dermatitis in persons on fat free diets. Also, linoleic acid is a constituent ofepidermal cell sphingolipids that function as the water permeability barrier in the skin.

Before we end our study on fatty acid degradation and synthesis, let us recapitulate the salient featur&s of the two processes. Table 7.2 gives the comparison of fatty acid synthesis and degradation. This will help you understand the two processes better.

Table 7.2 : Comparison of fatty acid synthesis and degradation

	Synthesis	Degradation
Greatest flux through pathway	After CHO rich meal	In starvation
Hormonal state favouring pathway	High insulin / glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Primarily cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (Mitochondria to cytosol)	Carnitine (cystôsol to mitochondria)
Oxidation/reduction cofactors	NADPH	NAD^+, FAD
Two C donor/product	Malonyl CoA : donor of one acetyl group	Acetyl CoA : Product
Activator	Citrate	
Inhibitor	Fatty acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA inhibits carnitine acyltransferase
Product of pathway	Palmitate	Acetyl CoA

Next, we shall focus on a family of compounds called eicosanoids. What are eicosanoids? What is their role in the body? You may recall reading about them in Unit 2 sub-section 2.3.5. We suggest you look up this section again now. So then what do you find? Yes, eicosanoids are derived from polyunsaturated fatty acids. We shall learn about their metabolism next.

7.3.4 Metabolism of Eicosanoids

You already know now that prostaglandins and the related compounds such as thromboxanes and leukotrienes (collectively known as eicosanoids) are extremely

potent compounds that elicit a wide range of physiologic responses. These compounds have extremely short half life and are produced in very small amounts. They have been compared to hormones in terms of their actions, but they differ from the true hormones in that they are formed in almost all tissues rather than in specialized glands. They generally act locally rather than after transport in the blood to distant sites of action. Prostaglandins are metabolized to inactive products at their site of synthesis and are not stored to any appreciable extent. Let us see how these eicosanoids are synthesized in our body, starting with prostaglandins. Here, we are not going into the details of the reactions involved in the synthesis of these eicosanoids. As dietitians, it is only important for us to understand that eicosanoids are derived from essential fatty acids (EFAs).

A) *Synthesis of Prostaglandins*

The dietary precursor of the prostaglandins is the essential fatty acid linoleic acid. It is converted to its immediate precursor of the prostaglandins — 20 C, PUFA containing 3, 4 or 5 double bonds. Arachidonic acid is the precursor of the predominant classes of prostaglandins.

B) *Synthesis of Leukotrienes*

Arachidonic acid is converted to a variety of hydroperoxy acids by a separate pathway involving a family of lipoxygenases. For e.g. neutrophils contain 5-lipoxygenase, which converts arachidonic acid to 5-hydroxy 6, 8, I l, 14 eicosatetraenoic acid (5 HPETE). 5 HPETE is converted to a series of leukotrienes.

STUDENT ACTIVITY - 2

1) With reference to lipogenesis, answer the following:

   a) Definition oflipogenes

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   b) Key regulating enzyme

   .....

   .....

   c) Conversion of Malonyl CoA to ß-hydroxybutyryl ACP

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   .....

2) Comment on the statement, 'fatty acid synthesis is simply a reversal of fatty acid degradation'.

   .....

   .....

3) How is palmitate converted into oleic acid?

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4) What do you understand by the term eicosanoids? Where are these derived from? Discuss the synthesis of prostaglandins.

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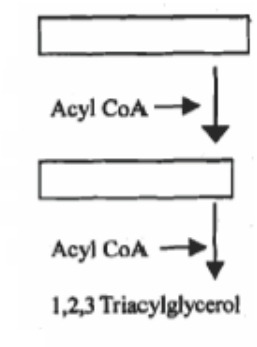
7.4 LIPID METABOLISM – II

Earl ier in section 7.2, we got to know about the fate of fatty acids in our body and also how they are synthesized. Now, in this section we shall focus on the metabolism Of lipids such as triacylglycerol, cholesterol, phospholipids etc. You have already learnt about the structure and properties of these compounds in Unit 2, section 2.2 and 2.3. Do look up this unit once again, as it will help you understand their metabolism better. We start with the metabolism oftriacylglycerol.

7.4.1 Metabolism of Triacylglycerols

The synthesis of triacylglycerol takes place in the endoplasmic reticulum. In liver and adipose tissue, fatty acids in the cytosol obtained from the diet or from de novo synthesis ofpalmitic acid become inserted into the endoplasmic reticulum (ER) membrane. Fatty acids, you learnt earlier, are stored for future use as triacylglycerols in all cells, but primarily in adipocytes of adipose tissue. Triacylglycerols constitute molecules of glycerol to which three fatty acids have been esterified. The fatty acids incorporated into triacylglycerols are activated to acyl-CoAs through the action of acyl-CoA synthetases. This is the same kind of reaction, which you have seen earlier in activation of fatty acid, prior to oxidation. A membrane bound acyl CoA transferase then esterifies two molecules ofacyE-CoA with glycerol 3-phosphate, to forrnphosphatidic acid i.e. 1 ,2- diacylglycerol phosphate. Thus phosphatidic acid contains glycerol with a fatty acid each in carbon 1 and 2 and phosphate group in carbon 3. The phosphate is then removed by phosphatidic acidphosphatase to yield 1 ,2-diacylglycerol, the substrate for addition of the third fatty acid. Phosphatidic acid phosphatase releases phosphate and in the membrane, 1 ,2-diacylglycerol is esterified with a third molecule of fatty acid.

In the intestine, iacylglycerol synthesis also occurs in the ER membrane, but the starting material is 2-monoacylglycerol (which is glycerol esterified with a fatty acid at carbon 2) as can be seen in Figure 7.7.



In the liver and intestine, triacylglycerol is packaged into lipoproteins, which are then secreted into the circulation.

Next, let us learn about the synthesis ofphospholipids.

7.4.2 Synthesis of Phospholipids

Phospholipids are synthesized by etherification of an alcohol to the phosphate of phosphatidic acid (I ,2-diacylglycerol 3-phosphate). Most phospholipids have a saturated fatty acid on C-1 and an unsaturated fatty acid on C-2 of the glycerol backbone. The most commonly added alcohols (serine, ethanolamine and choline) also contain nitrogen that may be positively charged, whereas, glycerol and inositol do not. The major classifications of phospholipids are: Phosphatidylcholine (PC), Phosphatidylserine (PS), Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE) and Phosphatidylinositol (PI).

Phosphatidylcholine, a major phospholipid constituent ofmembranes and lipoproteins is synthesized de novo in liver cells. The synthesis occurs in the ER and is linked, through 1,2-diacylglycerol, with the synthesis of triacylglycerol. Three compounds specifically involved in the synthesis of phosphatidyl choline are: a) choline, b) choline phosphate and c) cytidine diphosphatidyl choline (OP-choline). The synthesis of phosphatidyl choline (with the pathway and the enzymes involved) is given in the Figure 7.8. As can be seen, choline is activated first by phosphorylation and then by coupling to CDP prior to attachment to phosphatidic acid. PC is also synthesized by the addition of choline to CDP-activated 1,2-diacylglycerol.

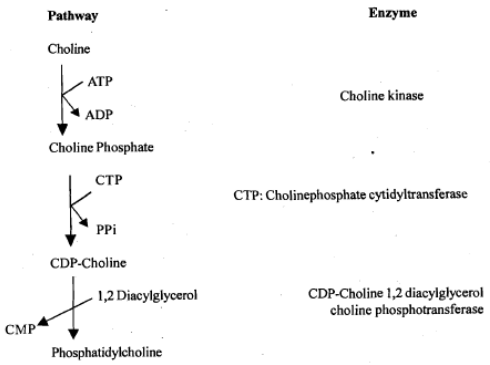


Figure 7.8 : Synthesis of phosphatidylcholine (PC)

A third pathway to PC synthesis, involves the conversion of either PS or PE to PC as shown in Figure 7.9. The conversion of PS to PC first requires decarboxylation of PS to yield PE, this then undergoes a series of three methylation reactions utilizing S- adenosylmethionine (SAM) as a methyl group donor.

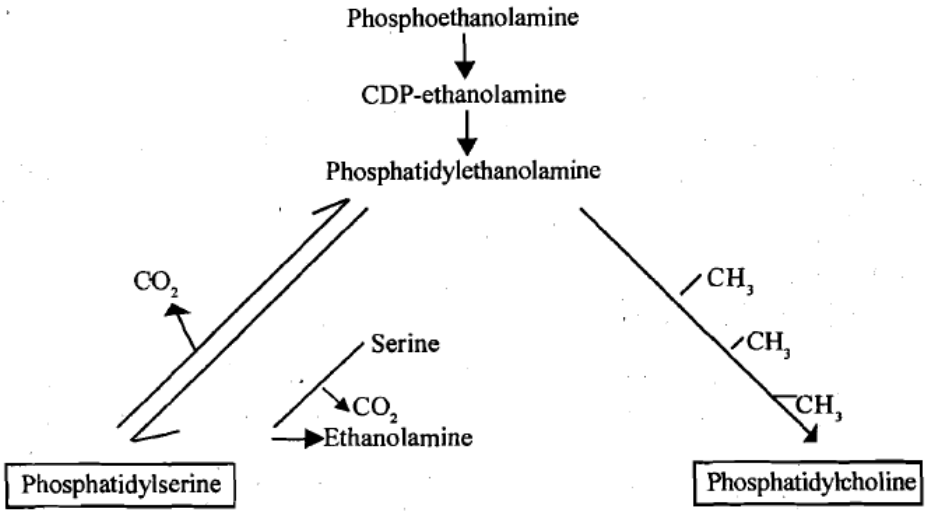


Figure 7.9 : Synthesis of phosphatidylserine (PS) and phosphatidylcholine (PC) from phosphoethanolamine (PE)

Phosphatidylserine arises by an exchange of the ethanolamine residue of phosphatidylethanolamine for a seryl gioup. Decarboxylation of the serine of phosphatidylserine reforms phosphatidylethanolamine. Three successive methylation reactions convert phosphatidylethanolamine to phosphatidylcholine. S-Adenosyl methionine is the methyl group donor.

We move on next to the metabolism of cholesterol.

7.4.3 Metabolism of Cholesterol

Cholesterol, as you may already know, is involved in two major biological processes a) It is a structural component ofcell membranes, and b) Steroid hormones, vitamin I), (cholecalciferol) and the bile salts are derived from the parent compound.

Cholesterol is synthesized de novo in the liver and the intestinal epithelial cells and is also derived from dietary lipids. De novo synthesis of cholesterol is regulated by the amount ofcholesterol and triglyceride in the dietary lipid. Let us learn how it is synthesized.

A) *Cholesterol Biosynthesis in Liver and Intestinal Epithelium*

The biosynthesis of cholesterol, a complex molecule with 27 carbon atoms, starts with the two-carbon atom compound acetyl-CoA which is converted to isopentenyl pyrophosphate (an isoprene derivative with five carbon atoms) and then squalene (30 carbon atoms), which is finally cyclized to cholesterol. It involves 32 different enzymes, some of which are soluble in the cytosol and others of which are bound to the ER.

The process has five major steps, which are listed herewith:

1) Acetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)

2) HMG-CoA is converted to mevalonate

3) Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2

4) IPP is converted to squalene (through a series of steps)

5) Squalene is converted to cholesterol.

The pathway of cholesterol synthesis is presented in the Figure 7.10.

For those of you who would want to know a bit more of each of these steps as illustrated in Figure 7.10, the following description will be useful.

The acetyl-CoA utiliæd for cholesterol biosynthesis is derived from an oxidation reaction (eg, fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis. Acetyl-CoA can also be derived from cytoplasmic oxidation of ethanol. All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor. Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of HMG-CoA. Unlike the HMG- CoA formed during ketone body synthesis in the mitochondria, this form is synthesized in the cytoplasm. However, the pathway and the necessary enzymes are the same as those in the mitochondria. Two moles of acetyl-CoA are condensed in a reversal of the thiolase reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole ofacetyl- CoA are converted to HMG-CoA by the action of HMG-CoA synthase. HMG-CoA is converted to mevalonate by HMG-CoA reductase, HMGR (this enzyme is bound in the endoplasmic reticulum, ER). HMGR absolutely requires NADPH as a cofactor and two moles ofNADPH are consumed during the conversion ofHMG-CoA to mevalonate. The reaction catalyzed by HMGR is the rate limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls. You will learn more about this later in the section on regulation of cholesterol synthesis.

Mevalonate is then activated by three successive phosphorylations, yielding 5-pyrophosphomevalonate. In addition to activating mevalonate, the phosphorylations maintain its solubility, since otherwise it is insoluble in water. After phosphorylation, an ATP-dependent decarboxylation yields isopentenyl diophosphate, IDP, an activated isoprenoid molecule. Isopentenyl diophosphate is in equilibrium with its isomer, dimethylallyl diophosphate, DMDP. One molecule ofIDP condenses with one molecule

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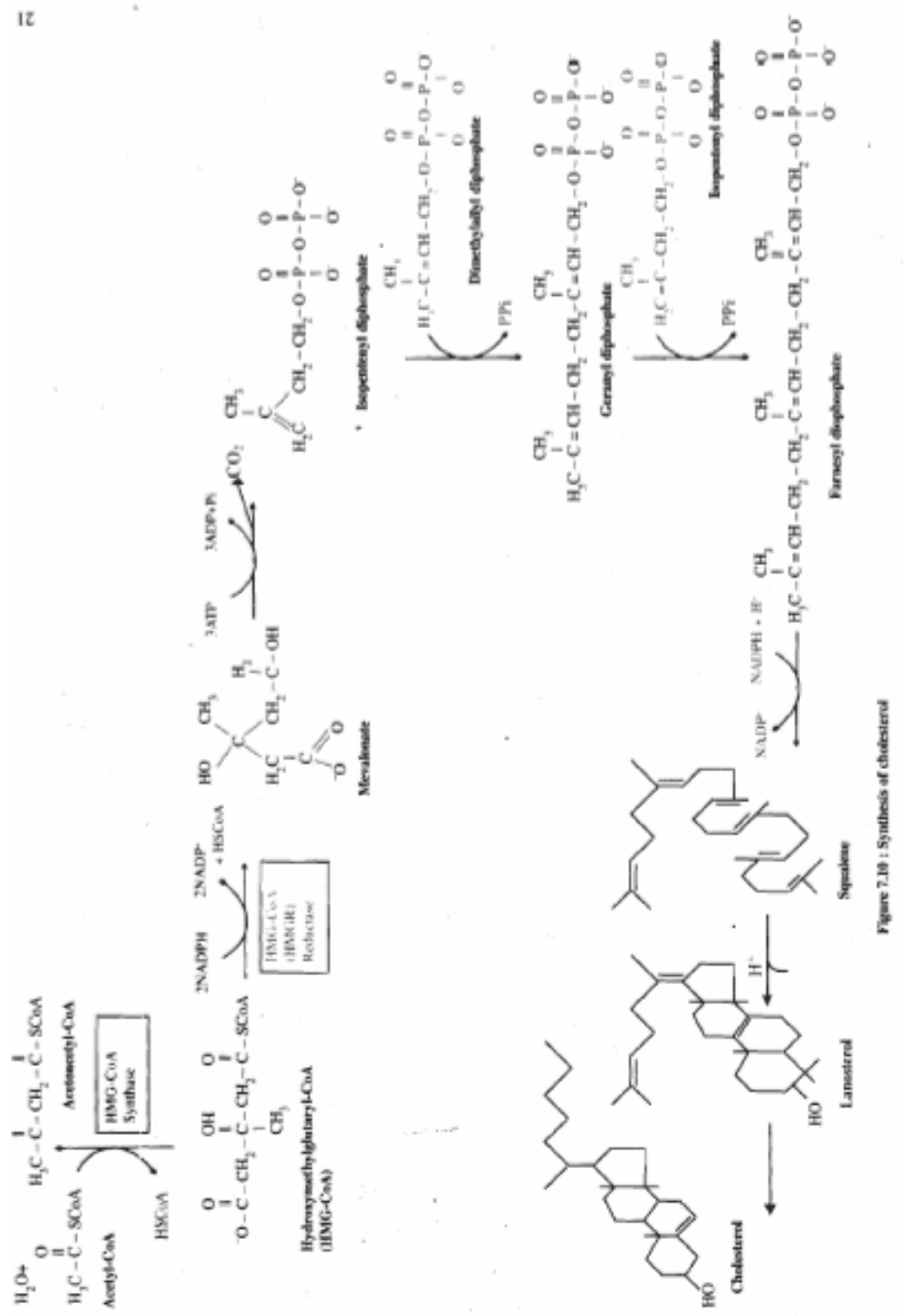

Figure 7.10 : Synthesis of cholesterol

Of DMDP to generate geranyl diphosphate, GDP. GDP further condenses (to combine) with another IDP molecule to yield farnesyl diphosphate, FDP. Finally, the NADPH- requiring enzyme, squalene synthaze catalyses the head-to-tail condensation of two molecules of FDP, yielding squalene (squalene synthase also is tightly associated with the endoplasmic reticulum). Squalene undergoes a two step cyclisation to yield lanosterol. By the term cyclimtion we mean, changing an

open-chain hydrocarbon to a closed ring. The first reaction is catalyzed by squalene monooxygenase or squalene epoxidase. This enzyme uses NADPH as a cofactor to introduce molecular oxygen as ari epoxide at the 2,3 position of squalene. Through a series of 19 additional reactions, lanosterol is converted to cholesterol.

B) *Regulation of Cholesterol Synthesis*

The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms. Of these three mechanisms, regulation of HMG CoA reductase (HMGR) activity is the primary means for controlling the level of cholesterol biosynthesis. HMG CoA reductase is an intrinsic membrane protein of the ER. The enzyme's active site extends into the cytosol. 1--1WfGR is the rate limiting enzyme in cholesterol synthesis and is subject to different types of metabolic control, which include:

a) Feedback inhibition: Cholesterol is a feedback inhibitor of HMG CoA reductase, thus decreasing further cholesterol synthesis.

b) Hormonal regulation: HMG CoA reductase activity is controlled hormonally. Glucagon favours the formation of the inactive (phosphorylated) form of HMG CoA reductase and bence decreases the rate of cholesterol synthesis. In contrast, insulin favours formation of the active (unphosphorylated) form of 1--1WfG CoA reductase and results in an increase in the rate of cholesterol synthesis.

c) Sterol-mediated regulation of transcription: The synthesis of cholesterol is also regulated by the amount of cholesterol taken up by the cells during lipoprotein metabolism. Chylomicron remnants intemalized by liver cells, and LDL internalized by cells ofliver and peripheral tissues, provide cholesterol, which causes a decrease in de novo cholesterol synthesis. You have already studied about chylomicrons and LDL in Unit 2. Chylomicron and IDL are lipoprotein complexes. Yon will read in details about these complexes in the next section on lipoprotein synthesis.

d) Inhibition by drugs: Lovostatin and mevastatin are reversible competitiveinhibitors of HMG CoA reductase. They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia. Finally, let us look at the degradation of cholesterol in our body.

C) *Degradation of Cholesterol*

The ring structure of cholesterol cannot be metabolized to C02 and H20 in humans. Rather, the intact sterol ring is eliminated from the body by:

a) conversion to bile acids which are excreted in the faeces.

b) secretion of cholesterol into the bile, which transports it to intestine for elimination is modified by bacteria before excretion

NOTES

Figure 7. I I summarizes the sources of liver cholesterol and routes by which cholesterol leaves the liver.

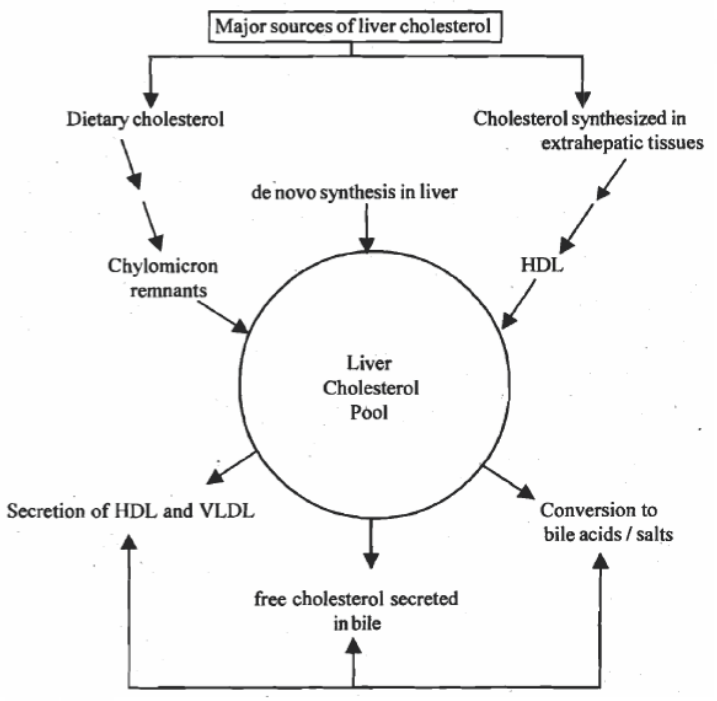

Figure 7.11 : Major routes by which cholesterol leaves liver

STUDENT ACTIVITY - 3

1) What is the site of synthesis of triacyglycerol? Graphically represent the steps of conversion of fatty acyl CoA to triacyglycerol.

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2) How are phospholipids classified? How are these synthesized

.....

.....

3) Enumerate the five main steps involved in cholesterol biosynthesis

.....

.....

4) How is the biosynthesis of cholesterol regulated by the amount of cholesterol in the diet?

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Having studied about cholestrol metabolism, we move on next to the study of lipoprotein metabolism.

7.4.4 Lipoprotein Metabolism

NOTES

Lipoproteins, as we already know, are the compounds of protein that carry fats and fat-like substances, such as cholesterol, in the blood. You may recall reading earlier about these compounds in Unit 5. The principle lipids carried by lipoprotein particles are triacylglycerols and cholesterol (free or esterified), obtained either from the diet or novo synthesis. Let us learn a bit more about these plasma proteins.

A) *Plasma Lipoproteins — An Introduction*

The plasma lipoproteins are molecular complexes of lipids and specific proteins called apolipoproteins. In fact, lipoproteins are composed ofa neutral lipid core (containing triacylglycerol in cholesteryl esters or both) surrounded by a shell of apolipoproteins (apoproteins), phospholipids and non-esterified cholesterol-all oriented so that their polar portions are exposed on the surface of the lipoprotein. This makes the particle soluble in aqueous medium. What are apolipoproteins? You have seen that various types of proteins are present along with different types of lipids in the lipoproteins. These proteins are specifically referred to as apolipoproteins. You have already come across this concept earlier while studying about enzymes in Unit 4. Do you recall reading about apoproteins? Yes, we learnt that many enzymes consist of the protein molecule along with the non-protein molecule. The protein molecule is referred to as apoenzyme. Apolipoprotein and apoenzymes are also called by a general term apoprotein, indicating that a non-protein portion is also associated with it.

These dynamic particles, the lipoproteins — are in constant state of synthesis, degradation and removal from the plasma. Lipoproteins function both to keep lipids soluble as they transport them in the plasma, and to provide an emcient mechanism for delivering their lipid contents to the tissues. In humans, the delivery system is less perfect than in other animals, and as a result, humans experience a gradual deposition of lipids— especially cholesterol in tissues. This is potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels— a condition known as atherosclerosis about which you may recall studying in the course "Applied Physiology" in Unit 4.

Do you recall the lipoproteins highlighted in Unit 5? For your information, Table 7.3 here presents the different lipoproteins and their composition. You would notice that the different lipoproteins are classified based on their size and density.

Table 7.3 : Composition ofthe plasma lipoprotein (%)

Plasma Lipoproteins	Triacylglycerol	Protein	Phospholipid	Cholesterol
Chylomicrons	90	2	3	5
VLDL	60	5	15	20
LDL	8	20	22	50
HDL	5	25	30	40

The chylomicrons are the lipoprotein particles lowest in density and largest in size, and contain the most lipid and the smallest percentage of protein as can be seen in Table 7.3. Chylomicrons function to deliver dietary triacylglycerols to adipose tissue and muscle and dietaj cholesterol to the liver.

VLDLs and MDLs are successively denser, having a higher content of protein and a lower content of lipid. HDL particles are the densest of the plasma lipoproteins.

The apolipoproteins associated with lipoprotein particles have a number of diverse functions. It:

a) serves as structural components of the particles,

b) provides recognition sites for cell-surface receptors, and

c) serves as activators or coenzymes for enzymes involved in lipoprotein metabolism.

Apolipoproteins are divided by structure and function into classes A to H, with most classes having subclasses, for example, apoA-I and apoC-II.

Having got a basic insight into the structure, composition of lipoproteins, we shall now move on to read about the metabolism of these compounds starting with chylomicrons.

B) *Metabolism of Chylomicrons*

Chylomicrons are assembled in intestinal mucosal cells and carry dietary triacylglycerol, cholesterol and cholesteryl esters (plus additional lipids made in these cells) to the peripheral tissues.

The particle released by the intestinal mucosal cell is called a "nascent" chylomicron and contains apolipoprotein B-48 (apoB-48 as shown in the Figure 7.12). Chylomicrons leave the intestine via the lymphatic system and enter the blood circulation. When it reaches the plasma, the nascent chylomicron is rapidly

modified, receiving apoE and apoCII apolipoproteins (from plasma HDLs) which is required for the.activation of lipoprotein lipase.

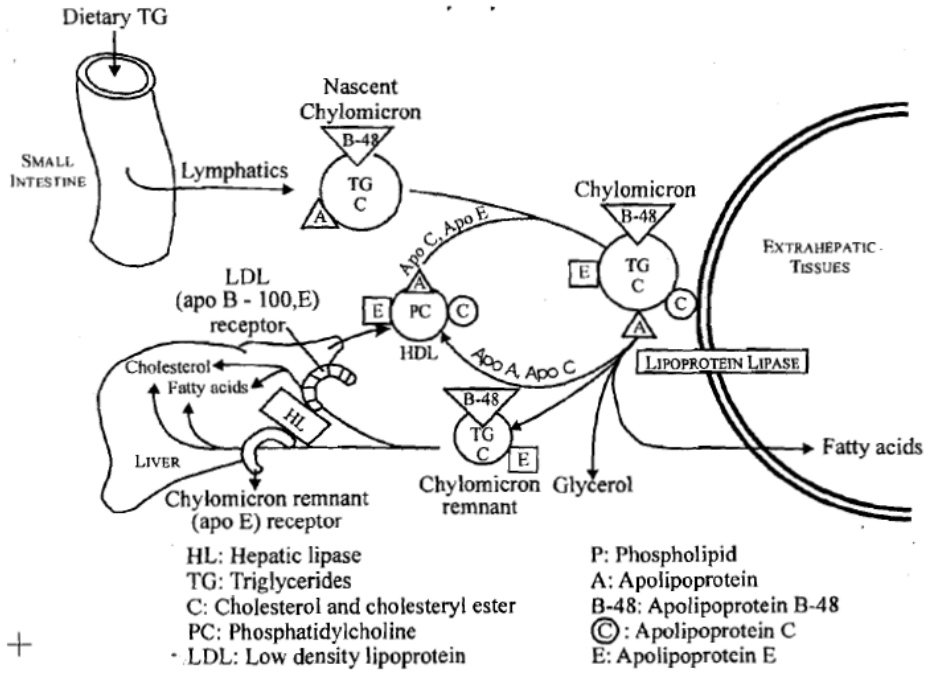


Figure 7.12 : Metabolism of Chylomicrons.

In the capillaries of adipose tissue and muscle, the fatty acids of chylomicrons are removed from the triacylglycerols by the action of lipoprotein lipase (LPL), which is found on the surface of the endothelial cells of the capillaries. Lipoprotein lipase is an extracellular enzyme that hydrolyses triacylglycerol into two monoacylglycerol and two fatty acids as indicated in the Figure 7.12. The apoC-II in the chylomicrons activates LPL in the presence of phospholipid. The free fatty acids then enter the cells passively down a concentration gradient and the glycerol backbone of the triacylglycerols is returned via the blood, to the liver and kidneys. Patients with a deficiency of lipoprotein lipase or apoC-II show a dramatic accumulation of triacylglycerol-rich lipoproteins in the plasma, for example, type I hyperlipidemia (familial hyperchylomicronemia).

As the chylomicron circulates and the triacylglycerol in its core is degraded by lipoprotein lipase, the particle decreases in size and increases in density, since it has lost a considerable amount of its lipid component. In addition, the C apoproteins are returned to the HDLs (from which they were originally obtained). The remaining particle left is called a "remnant". Chylomicron remnants-containing primarily cholesterol, apoE and apoB-48 are then delivered to, and taken up by the liver through interaction with the chylomicron remnant receptor. The recognition of chylomicron remnants by the hepatic remnant receptor requires apoE. Chylomicron remnants bind to these receptors and are taken into the cells by endocytosis. The endocytosed vesicle then fuses with a lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are

hydrolytically degraded, releasing amino acids, free cholesterol and fatty acids. The cholesterol released from the chylomicron regulates the rate of de novo cholesterol synthesis in the liver by causing a decrease in cell content of HMG CoA reductase, which you learnt earlier is the key enzyme in cholesterol synthesis, as well as by inhibiting the enzyme.

C) *Metabolism of Very Low Density Lipoproteins (VLDL)*

VLDLs are produced in the liver. They are composed predominantly oftriacylglycerols (TG), cholesterol and cholesteryl esters (C) and their function is to carry this lipid from the liver to the peripheral tissues. There, the triacylglycerol is degraded by lipoprotein lipase, as discussed, for chylomicron degradation. The process involved, thereafter is illustrated in Figure 7.13 and the process includes:

1) Release of VLDL: VLDLs are released from the liver as nascent VLDL particles containing apolipoproteins B-100 and A-I. They must obtain apoC-II and apoE from circulating HDL as shown in Figure 7.13. As with chylomicrons, apoC-II is required for activation of lipoprotein lipase.

2) Modification ofcirculating VLDL: Next, as VLDLs pass through the circulation, their structure is altered. Fatty acids and glycerol are removed by lipoprotein lipase, causing the VLDL to decrease in size and Become denser to form intermediate density lipoproteins (IDL). Surface components, including the C and E apolipoproteins are transferred to HDL. Finally, cholesteryl esters are transferred from HDL to VLDL in an exchange reaction that concomitantly transfers triacylglycerol or phospholipid from VLDL to the HDL. This exchange is accomplished by cholesteryl ester transfer protein.

3) Production ofLDLfrom VLDL in plasma: After these modifications, the VLDL has been converted in the plasma to LDL. An intermediate-sized particle, the intermediate density lipoprotein (IDL) as shown in Figure 7.13, is observed during the transition from VLDL to LDL in the plasma. IDLs can also be taken up by cells through receptor-mediated endocytosis.

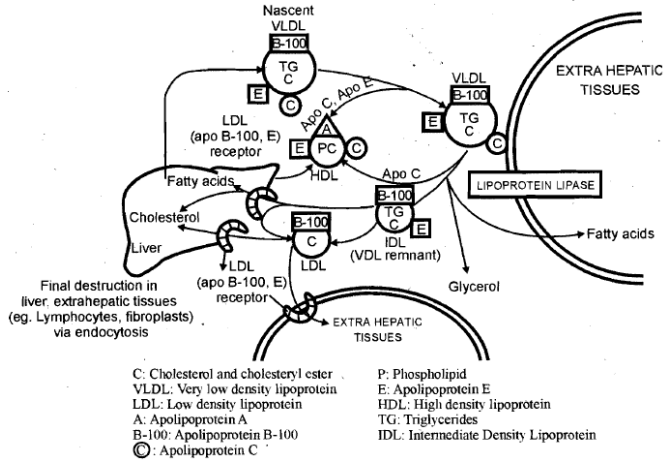


Figure 7.13: Metabolism of VLDL

Next. let us look at the metabolism of LDL.

D) *Metabolism of Low Density Lipoproteins (LDL)*

LDL, as seen earlier, contains much less triacylglycerol than its VLDL predecessors, and has a high concentration of cholesterol and cholesteryl esters. The primary function of LDL particles is to provide cholesterol to the peripheral tissues. How do they do that? In fact, a multistep process is involved in the metabolism of LDL. We shall not go into the details of these steps, but certainly look at the mechanism involved in simple terms.

LDL particles provide cholesterol to the peripheral tissues by depositing free cholesterol on the membranes of cells as they come in contact with the cell surface and by binding to receptors on cell-surface membranes that recognize apolipoprotein B-lOO. LDL receptors are negatively charged glycoprotein molecules that are clustered in pits on cell membranes. The intracellular side of the pit is coated with the protein clathrin, which stabilizes the shape of the pit. After binding, the LDL is internalized as intact particles by endocytosis. The vesicle containing the LDL rapidly loses its clathrin coåt and fuses with other similar vesicles, forming larger vesicles called endosomes. The pH of the contents of the endosome falls allowing separation of the LDL from its receptor. The receptors then migrate to one side of the end some, whereas the LDLs stay free within the lumen of the vesicle.

The receptors can be recycled, whereas, the lipoprotein remnants in the vesicle are degraded by lysosomal (hydrolytic) enzymes, releasing cholesterol, amino acids, fatty acids and phospholipids. These compounds can be recycled by the cell. The number of receptors for lipoproteins vary according to the availability of these lipoprotein particles and according to the needs of the cell. For example, if there is a large amount of a particular circulating plasma lipoprotein, the number of cell-surface receptors for it decreases, frequently termed "down-regulation". Conversely, if cells are starved for cholesterol, they increase the number of cell-surface receptors, i.e.

Lastly, we come to the metabolism of HDL.

E) *Metabolism of High Density Lipoproteins (HDL)*

HDL particles are synthesized in the liver and are released into the bloodstream by exocytosis. HDL performs a number of important functions as you may have realized while reading through the earlier sections. These include:

a) HDL serves as a circulating reservoir ofapolipoprotein - apoC-II (the apolipoprotein that is transferred to VLDL and chylomicrons).

b) It is an activator of lipoprotein lipase, removing free (unesterified cholesterol from extrahepatic tissues and esterifying it, using the plasma enzyme phosphatidylcholine cholesterol acyltransferase (PCAT — also known as LCAT, where "L" stands for lecithin).

c) It transfers cholesteryl esters to VLDL and LDL in exchange for triacylglycerol, and d) It carries cholesteryl esters to the liver, where the HDL is degraded and cholesterol is released.

NOTES

A brief discussion of the functions and metabolism of HDL follows. The metabolism of HDL is illustrated in Figure 7.14.

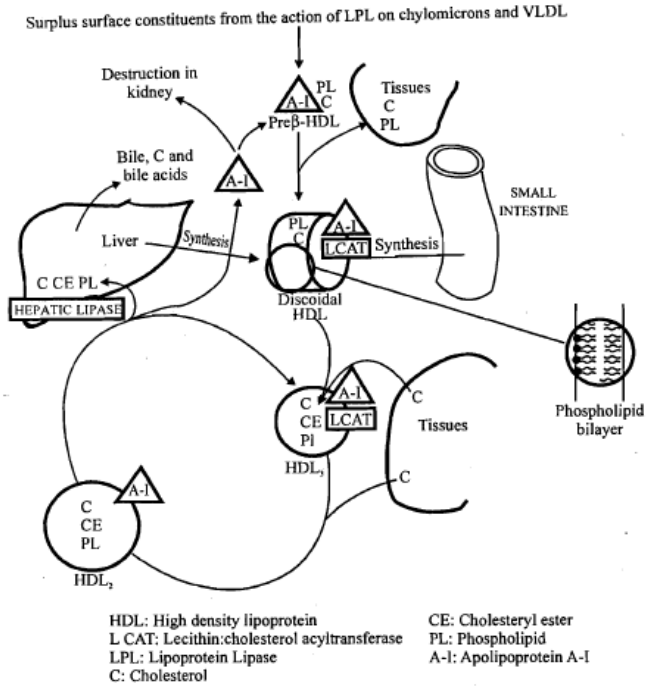


Figure 7.14 : Metabolism of HDL

1) HDL as a reservoir of apolipoproteins: HDL particles not only serve as the source of apolipoproteins required for the proper metabolism of other plasma lipoproteins, but also take back most of these proteins before the chylomicron remnants and LDLs bind to their cell-surface receptors and are endocytosed.

2) HDL uptake of free cholesterol: Newly secreted HDL are disc-shaped particles as shown in the Figure 7.14, containing predominantly unesterifie cholesterol, phospholipids and a number of apolipoproteins including apoE, apoA and apoC. They are rapidly converted to spherical particles as they accumulate cholesterol. HDL particles are excellent acceptors ofuncsterifie cholesterol from the surface of cell membranes and from other circulating lipoproteins.

3) Esterification of free cholesterol: Once free cholesterol is taken up by the HDL, it is immediately esterified by phosphatidyl cholesterol acyl transferase (PCAT), a plasma enzyme synthesized by the liver, which is activated by apoA-I of the HDL. Plasma levels ofapoA-I are increased by modest alcohol intake. The fatty acid from carbon 2 of phosphatidylcholine is transferred directly to the cholesterol, leaving lysophosphatidylcholine. The resulting cholesteryl ester is so hydrophobic that it is effectively "trapped" in the HDL and can no longer be transferred to a membrane. The only mechanism for removing it from HDL in the plasma is through transfer to VLDL by the

cholesteryl ester transfer protein, where it ultimately remains in the LDL until that particle is taken up by a cell. About two-thirds of the cholesterol in the plasma is esterified with fatty acid. In liver disease, a decreased concentration of plasma cholesteryl esters is observed. This may be due to either a deficiency in phosphatidylcholine production or a lack of PCAT.

4) Fate of IIDLs: Spherical HDL particles are taken up by the liver by receptor- mediated endocytosis and the cholesteryl esters are degraded. The cholesterol thus released by the action of enzyme hepatic lipase can be repackaged in lipoproteins, converted into bile acids, or secreted into the bile for removal from the body.

With the metabolism of HDL, we come to the end of our discussion on metabolism of lipoproteins. We have seen in this section, the fate of the different lipoproteins. Serum lipoprotein levels are maintained in the body. What happens when the levels of these lipoproteins are elevated in the body? Read the next section and find out.

7.5 HYPERLIPOPROTEINEMIAS

The term hyperlipoproteinemia describes a group of disorders in which serum lipoprotein levels are elevated. These disorders are classified into six types, depending on which lipoproteins are abnormally elevated and are summarized in Table 7.4. Each hyperlipoproteinemia is not a single disease but a group of disorders marked by the same lipoprotein abnormality, and each includes some primary (genetically transmitted) disorders and some secondary disorders. When a disorder of lipid metabolism occurs secondary to a particular' disease (e.g. type IV hyperlipoproteinemia secondary to uncontrolled diabetes), treatment of the underlying illness will frequently correct the lipid abnormality. Similarly, when a primary lipid disorder is aggravated by exogenous obesity, alcohol or glucocorticoids, the elimination of the aggravating factor will make diet therapy easier. Diet also affects the development of hyperlipoproteinemias. The dietary factors causing an increase in plasma lipoproteins in a great many people are obesity and high intake of foods rich in cholesterol and saturated fats.

Table 7.4 : Types of hyperlipopmteinemia

Type	Triglyceride	Total Cholesterol	LDL Cholesterol	Raised Lipoprotein
I	+++	+	N	Chylomicrons
II a	N	++	++	LDL
II b	++	++	++	LDL/VLDL
III	++	+	N	IDL and chylomicron remnants
IV	++	N/+	N	VLDL
V	++	+	N	VLDL / chylomicrons

N= Normal; + = slightly raised; ++ = moderately raised; +++ = extremely raised

A brief review follows:

NOTES

a) **Type I** hyperlipoproteinemia is an uncommon pattern marked by elevated chylomicrons. Cholesterol is normal and triglycerides are markedly elevated, usually greater than 1000 mg/dl. Among the primary disorders producing this pattern are familial lipoprotein lipase deficiency and apolipoprotein C II deficiency.

b) **Type II** a hyperlipoproteinemia is marked by high LDLwith norma,l VLDL. Plasma cholesterol levels are high but triglycerides are normal. The genetic disorder associated with this pattern is familial hypercholesterolemia, in which there is an autosomal dominant pattern of inheritance. The biochemical defect is a deficiency of LDL receptors. This pattern is also seen secondary to nephrotic syndrome, Cushing's syndrome and hypothyroidism.

c) **Type IlI** b hyperlipoproteinemia is a common pattern characterized by increases in VLDL and LDL. Both cholesterol and triglyceride levels are elevated. Familial combined hyperlipoproteinemia, also called familial multiple lipoprotein-type hyperlipidemia, is a disorder in which individuals with type II a, type II b and type IV hyperlipoproteinemias are found in the same family. Type 11b hyperlipoproteinemia can be seen secondary to nephrotic syndrome, Cushing's disease and hypothyroidism. Primary type II b hyperlipoproteinemia can be aggravated by exogenous obesity or glucocorticoids.

d) **Type III** hyperlipoproteinemia is marked by a reduced electrophoretic mobility of VLDL. In this, cholesterol and triglycerides are both elevated, frequently to about the same level— for example, cholesterol and triglycerides may both be 400 mg/dl.The primary form of this disorder is called familial dysbetalipoproteinemia. These patients accumulate a partially degraded VLDL (beta VLDL).
e) Type IV, a common pattern of hyperlipoproteinemia, is marked by elevated VLDL with high cholesterol and triglycerides.

1) The primary disorders associated with this pattern are familial multiple lipoprotein-type hyperlipidemia, and the mild form of familial hypertriglyceridemia.

2) In other associated disorders, elevated VLDL is common secondary to diabetes and uremia, and is also associated with hypopituitarism and nephrotic syndrome. Alcohol, glucocorticoids, oestrogens and exogenous obesity may aggravate an already elevated VLDL in patients with primary hyperlipidemia but they seldom induce hyperlipidemia in normal individuals.

f) **Type IV**, a rare pattern, is marked by elevated chylomiprons and VLDL. Both cholesterol and triglycerides are high.

1) Theprimary disorders with the pattern are familial lipoprotein lipase deficiency, apolipoprotein C II deficiency, and the more severe form of the familial hypertriglyceridemias.

2) Type V hyperlipoproteinemia may be seen secondary to the same disorders as type IV, it is most commonly seen secondary to poorly controlled diabetes.

Our reading on this topic would not be complete without some information about the diagnosis of these disorders. We shall learn about this next.

Diagnosis of Hyperlipoproteinemia

We have already seen how hyperlipoproteinemia is classified based on the elevated levels of lipoproteins such as chylomicron, LDL or VLDL etc. Next, we shall learn why the diagnosis of hyperlipoproteinemia is important and when to do it. Some information of the diagnosis mechanism is also included.

1) Because of the high association of hyperlipidemia with coronary heart disease, it is generally recommended that serum cholesterol and triglycerides be measured periodically, especially in young adults. If there is a history of hyperlipoproteinemia or premature coronary artery disease in the family, children should be tested as well.

   a) Timing of measurement: The serum cholesterol level is relatively unaffected by eating, but a recent meal can cause marked elevation of triglycerides. Triglycerides should only be measured after a 12 to 14-hour fast. Serum lipids are determined when the patient is maintaining a steady weight and has been on his usual diet for several weeks.

   b) Repeat measurements: Before making a firm diagnosis, fasting lipids should be measured two or three times at 2 to 3-week intervals.

2) The presence of chylomicrons can be determined by refrigerating the plasma at $4^{O}C$ overnight. If chylomicrons are present, they will form a creamy layer on top of the plasma. The presence of chylomicrons in plasma drawn after a 12-hour fast is indicative of type I or type V hyperlipoproteinemia. Fasting chylomicronemia is usually seen only with fasting triglyceride levels of greater than 1000 mg/dl.

3) The implications of elevated serum cholesterol depend on the lipoprotein, with which it is associated. As noted above, the risk of coronary artery disease is highly associated with elevated LDL cholesterol. A marked increase in VLDL may result in some increase in cholesterol in addition to an increase in triglyceride. VLDL contains about I mg of cholesterol for every 4 mg of triglyceride. HDL cholesterol is easily determined by precipitating LDL and VLDL with phosphotungstic acid and magnesium chloride. The nonprecipitated cholesterol is HDL. LDL cholesterol can then be calculated :

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \frac{\text{triglycerides}}{5}$$

4) Most patients with hyperlipidemia can be assigned to a specific hyperlipoproteinemia on the basis of total cholesterol, triglycerides, HDL cholesterol and the refrigerator test for chylomicrons. Isoelectric focusing of apolipoproteins is necessary to establish the diagnosis of Cll apolipoprotein deficiency and familial dysbetalipoproteinemia (type III hyperlipoproteinemia).

Finally, we shall end our discussion on lipid metabolism by learning about ketosis. What is ketosis? Ketosis is the body's survival system. Let'S get to know about this system in our body.

7.6 KETOSIS

Being in ketosis means our body has burned a large amount of fat in response to the fact that it did not have sufficient glucose available for energy needs. Under everyday conditions, the carbohydrates we eat are converted to glucose, which you already know is the body's primary source of energy. Whenever our intake of carbohydrates is limited, for a long enough period of time, we will reach a point where our body draws on its alternate energy system i.e. the fat stores for fuel.

The condition called ketosis, means our body burns fat and turns it into a source of fuel called ketone bodies. Ketone bodies are produced whenever body fat is burned. When you burn a larger amount of fat that is immediately needed for energy, the excess ketone bodies are discarded in the urine. Let us next see how ketone bodies are formed?

Acetyl CoA is oxidized to C02 via citric acid cycle, as given in carbohydrate metabolism. Only in liver mitochondria, the acetyl CoA can be converted to ketone bodies, i.e. acetoacetate, acetone and 3-hydroxybutyrate. The ketone bodies are water soluble lipid fuels that are continuously released from the liver. When carbohydrate is plentiful and glucose is readily available to the tissues, the amount of circulating ketone bodies is low. When large amounts of triacylglycerols are being hydrolyzed in adipose tissue, in response to an increase in whole body energy demand, the rate of oxidation of fatty acid increases in the liver and other tissues. In the liver these increases ketogenesis and thus increases the ketone body concentration in the circulation. Normally, some acetoacetate is converted to 3-hydroxybutyrate. Further, acetoacetate and hydroxybutyrate are valuable fuels for skeletal and cardiac muscle. It is estimated that they supply 10 per cent of the daily energy requirement ofthese tissues. The breakdown of ketone bodies by the peripheral tissues is galled ketolysis. When the process of ketogenesis exceeds ketolysis, ketosis or ketoacidosis occurs. Here we must differentiate ketosis from ketoacidosis. Ketosis we have seen is our body's natural survival system. Ketoacidosis, on the other hand, is a life-threatening condition most often

associated with uncontrolled insulin-deficient 7Ype I diabetes. In Type I diabetes, the absence of insulin leads to a toxic build-up of blood glucose andan extreme breakdown of fat and muscle tissue. The presence of insulin keeps ketone bodies production in check so that a mild, beneficial ketosis is achieved.

7.7 LET US SUM UP

In this unit, lipid metabolism atcellular level has been discussed at length. First we have seen how fatty acids are used for the production of energy. Secondly, the body has the capacity to synthesize a variety of fatty acids except the essential fatty acid. Higher fatty acids i.e. eicasonoids have important biological functions. The significance of cholesterol in biologic processes along with their synthesis and regulation has been looked into. Lastly, we have seen the transport of lipoproteins and its relation to hyperlipoproteinemia. Six types of hyperlipoproteinemia are present. Ketosis occurs when the process of ketogenesis exceeds the process of ketolysis.

7.8 GLOSSARY

Apolipoprotein : the protein component of lipoproteins.

Atherosclerosis : a thickening and narrowing of the walls of the large and medium sized blood vessels caused by the invasion of lipids, primarily cholesterol and other materials, into the intemal or inner layer to form plaque.

Condensation : a chemical change in which two molecules combine to form a larger molecule with elimination ofa small molecule e.g. H20.

Endocytosis : process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.

Endoplasmic Reticulum : membrane network within the cytoplasm of cells involved in the synthesis, modification and transport of cellular materials.

Esterification : the process of converting an acid into an alkyl or aryl derivative and consists of the reaction of an acid with an alcohol in the presence of a trace of mineral acid as catalyst or the reaction of an acyl chloride with an alcohol. It can also be accomplished by enzymatic processes.

Exocytosis : a process of cellular secretion or excretion in which substances contained in vesicles are discharged from the cell by fusion of the vesicular membrane with the outer cell membrane.

Head-to-tail condensation : a chemical change in which two molecules combine head-to tail to form a larger molecule with elimination of a small molecule e.g. H20 is called head-to-tail condensation.

Infarction : an area ofcoagulation necrosis in a tissue due to local ischemia resulting from obstruction of circulation to the area.

Ketoacidosis : a life-threatening condition most often associated with uncontrolled IDDM.

Ketone bodies : water-soluble lipid fuels that are continuously released from the liver.

Ketosis : burning or utilization of a large amount of fat in response to decreased glucose availability for energy needs.

Leukotrienes : compounds derived from arachidonic acid and are linear oxidation products found in leukocytes; contain a conjugated triene double bond arrangement.

Prostaglandins : C-20 unsaturated hydroxy acids with a substituted cyclopentane ring and two aliphatic side chains. It is one of the extremely potent compounds that elicit a wide range of physiologic responses.

Thromboxanes : compounds that cause the aggregation of platelets that is involved in the formation of a blood clot. Thromboxanes have an oxygen atom incorporated into a cyclopentane ring which produces a six membered ring.

7.8 CHECK YOUR PROGRESS

1) What do you mean by the term 'hyperlipoproteinemia ? How are these Classified
2) Explain how free cholesterol is esterifie
3) How can you determine the presence of chylomicrons, HDL and IDL in the plasma?

4) Define the following terms

   a) Lipoproteins

   b) Apolipoproteins

5) What is ketosis? Is it same as ketoacidosis?

6) What are ketone bodies? How are these produced? Name three ketone bodies.

NOTES

UNIT

8

AMINO ACID AND NUCLEOTIDE METABOLISM

STRUCTURE

8.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

explain how amino acids are catabolized in the body,

describe the synthesis of urea,

discuss how a-keto acids are used for the production of energy,

explain how non-essential amino acids are synthesized in the body,

relate the specialized products formed from amino acids with their biological functions,

describe how purines and pyrimidines are synthesized in the body,

8.2 INTRODUCTION

We already studied about the digestion, absorption and transport of proteins and amino acids. We learnt that proteins do not immediately diffuse into the surrounding tissues of the blood stream, but first undergo a series of steps of biochemical reactions in the digestive tract. These reactions reduce the protein into its individual amino acids which are rapidly absorbed into the blood stream. Amino acids contain nitrogen, which cannot be stored, and therefore amino acids, which are in excess of biosynthetic needs of the cell, are degraded. This involves the removal ofa-amino groups (amino groups attached to carbon atom next to the

carboxyl carbon) by two processes. What are these processes? How are amino acids catabolized in the body? What are the specialized products forrned from amino acids and what are their biological functions? These are a few aspects related to amino acid metabolism discussed in this unit.

The second part of the unit focuses on nucleotide metabolism. Nucleotides are important for being precursors ofnucleic acids (DNA, RNA). Here in this unit we will learn how the purine and pyrimidine bases found in the nucleotides are synthesized and degraded. However, to understand the metabolism of nucleotides, it is essential to be thoroughly familiar with the nomenclature and the essential structures of the purine bases-adenine and guanine; the pyrimidine bases-cytosine, uracil and thymine; the nucleosides and deoxynucleosidesderived from these; and the nucleotide and deoxynucleotide derivatives. We have already studied about these aspects earlier in Unit 2. We strongly suggest that you must revisit Unit 2, section 2.8 before reading this unit.

8.3 AMINO ACIDS METABOLISM

Nitrogen enters the body through a variety of compounds present in the food, the most important being amino acids present in dietary proteins. The primary role of amino acids is in the synthesis of tissue proteins and other biosynthetic reactions. Amino acids contain nitrogen, which cannot be stored, and therefore amino acids, which are in excess of biosynthetic needs of the cell, are degraded. This involves the following:

a. the removal of a - amino groups (amino groups attached to carbon atom next to the carboxyl carbon) by two processes called and oxidative deamination, forming ammonia and the corresponding a-keto acids, and

b. the conversion of ammonia to urea in the Urea Cycle. A portion ofthe free ammonia is excreted in urine, and the remaining is excreted in urine after getting converted to urea.

The carbon skeletons (structure left after the removal of amino group) of the a-keto acids are converted to common intermediates of energy producing metabolic. pathways. Lastly, the body has the capacity to produce certain specialized products from amino acids. A summary of nitrogen and amino acid metabolism is presented in Figure 8.1.

Amino acids contain nitrogen, which cannot be stored, and therefore amino acids, which are in excess of biosynthetic needs of the cell, are degraded. This involves the removal ofa-amino groups (amino groups attached to carbon atom next to the carboxyl carbon) by two processes

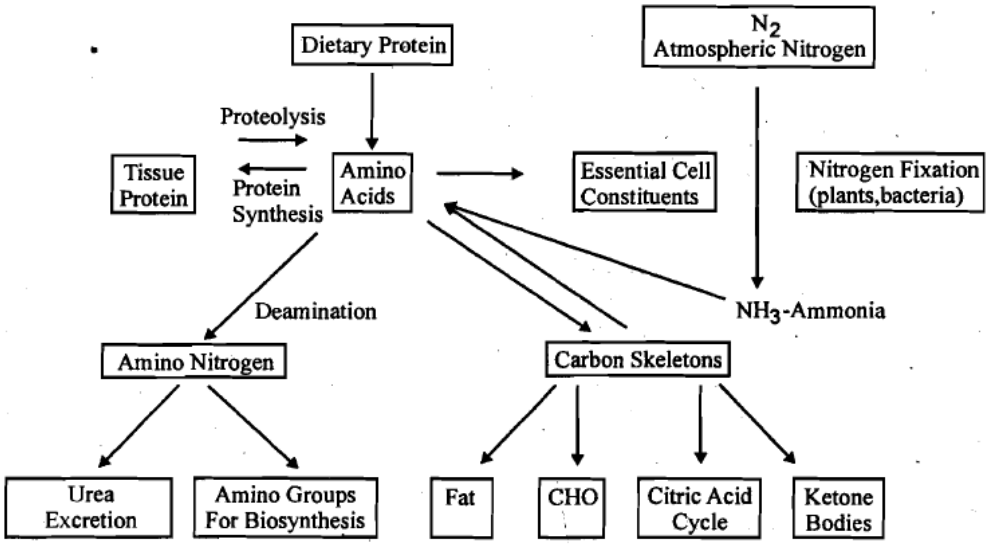


Figure 8.1: Nitrogen and amino acid metabolism

The amino acids that are released by the hydrolysis of dietary and tissue-protein, mix with other amino acids distributed throughout the body. This is called as amino acid pool. The amino acid pool contains 100 g of amino acid and is very small in comparison to .the amount of protein in the body. The concentration of free amino acids in the extracellular fluids is significantly lower than that within the cells of the body. The movement of amino acids from the extracellular space to the interior of the cells is by active transport mechanism which requires carrier molecule and energy from ATP.

The first step in the catabolism of all amino acids is the removal of a-amino group. Once removed, nitrogen can be incorporated into other compounds or excreted.

We learnt earlier in this unit that the removal of the a-amino group can be achieved by the two processes, transamination and oxidative deamination. We shall get to know about these processes next.

8.3.1 fransamination Reaction

Most common amino acids can be converted into the corresponding keto acid by transamination. In this reaction, there is a transfer of a-amino grouß of amino acid to keto acid (keto group present next to the carboxyl group) —R-CO-COOH. As a result, the amino acid becomes a-keto acid and the keto acid is convened into amino acid, as shown in Figure 8.2.

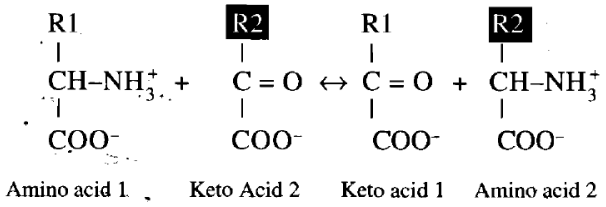
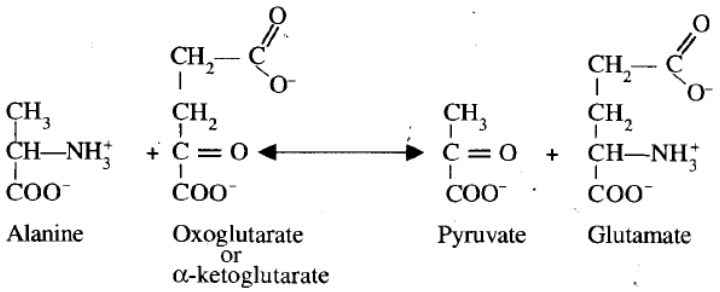


Figure 8.2 : Transamination reaction

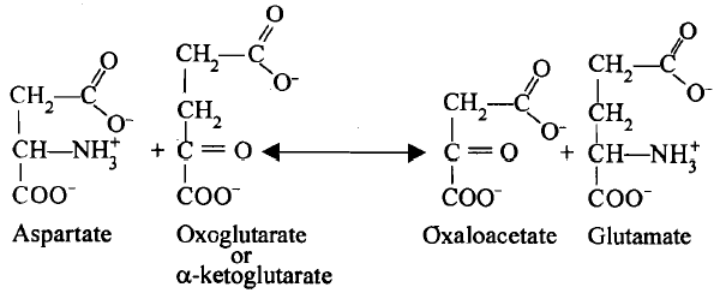
a-ketoglutarate plays a significant role in amino acid metabolism by accepting the amino groups from other amino acids thus forming glutamate. The glutamate formed can be oxidatively deaminated (oxidized coupled with removal of ammonia) or can be used as an amino group donor in the synthesis of non essential amino acids. The reaction of transamination is catalyzed by aminotransferases (transaminases). The two most important transferases are:

a) *Alanine aminotransferase*

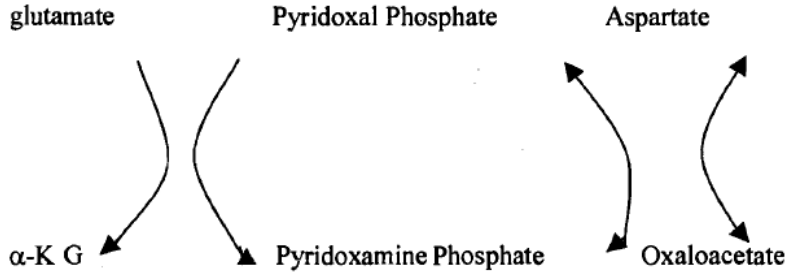


b) *Aspartate aminotransferase (AST)*

Asparate + a-ketoglutarate □ oxaloacetate + glutamate



There are at least 13 different aminotransferases. All aminotransferases require the coenzyme pyridoxal phosphate (derived from vitamin B6). You have already learnt about coenzymes, particularly about pyridoxal phosphate in Unit 4. Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an a-keto acid and regenerates back the coenzyme. The details of transamination reactions are given in the section on biochemical role of vitamins in Unit 10 later. Thus you can see that by transamination, one amino acid can be converted into another amino acid as shown herewith.

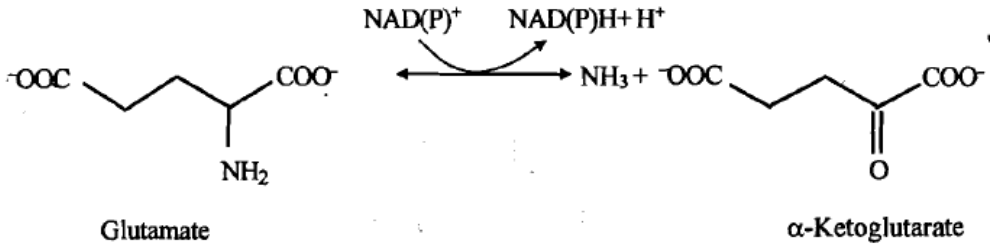


8.3.2 Deamination Reaction

Deamination is aprocess by which N of amino acid is removed as ammonia (NH3. These reactions occur primarily in liver and kidney. The reaction is catalyzed by the following two enzymes:

a) *Glutamate dehydrogenase*

Glutamate, as we have just seen above, is the major end product of transamination reactions. Further breakdown of glutamate occurs through the process of oxidative deamination. This is catalyzed by the enzyme L-glutamate dehydrogenase to form a-- iminoglutaric acid, which on addition of a molecule of water forms NH, and aketoglutarate. (This enzyme requires NAD(P) provided by vitamin B,(niacin) and is present in the mitochondrion). It is a reversible reaction as shown herewith :



$$\text{Glutamate} + \xrightarrow[NAD(P)^+]{H_2O} \alpha\text{-Ketoglutarate} + NH_3 + NAD(P)H + H^+$$

b) *Amino acid oxidase*

D-amino acids present in the diet are efficiently metabolized by the liver by the enzyme amino acid oxidase. Amino acid oxidases are of two types. D-amino acid oxidase (breaks down D-amino acid) and L-amino acid oxidase (which acts on L-amino acids). D-amino acid oxidase requires FAD (provided by vitamin B2) as the cofactor. It liberates NH3 and a-keto acids, which can enter the general pathway of amino acid metabolism. However, the tissue proteins contain L-amino acids. These are catabolized by L-amino acid oxidases of liver and kidney which uses FMN (provided by vitamin B2) as the coenzyme and once again as earlier, liberates NH3 and (I-keto acids. However, the activity of L-amino acid oxidase in the body is very little and hence this type of oxidative deamination is not the major pathway of amino acid catabolism. Then, how are the amino acids broken down? Primarily by the transamination process. The amino acids are converted to glutamate as you have already learnt and then the glutamate is catabolized by L-glutamate dehydrogenase. The activity of this enzyme is very high in the body.

The discussion so far centered on the removal of amino groups. The end product formed being ammonia and the corresponding a-keto acids. What happens to this ammonia in the body? The next section focuses on the conversion of ammonia into urea. Let us see how this is done.

8.3.3 Urea cycle

From our discussion above, it is clear that the amino group of all amino acids is ultimately convened to ammonia (NH3). Ammonia is highly toxic to the nervous system. Hence, it must be removed. How is this done? Basically, ammonia combines with CO2 to form urea, which is not toxic to the body. Hence, one of the major end products of protein metabolism is urea.

Urea is the major disposal form of amino groups derived from amino acids and accounts for 90% of the nitrogen containing compounds of urine. One of the nitrogen of the urea molecule is supplied by free NH3 and the other one by aspartate. Glutamate is the immediate precursor of both ammonia and aspartate nitrogen. The carbon and oxygen of urea are derived from COT Urea is produced by the liver and is then transported in the blood to the kidneys for excretion in the urine. The steps involved in converting ammonia to urea include:

1) Ammonia + CO2 + 2ATP ⟶ Carbamoyl-Phosphate

2) Carbamoyl-P + Ornithine ⟶ Citrulline

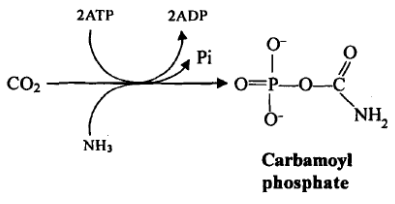
3) Citrulline + Aspartate ⟶ Fumarate + Arginine

4) Arginine ⟶ Urea + Ornithine

Most of our nitrogenous waste comes from the breakdown of amino acids. This occurs by deamination. Deamination of amino acids results in the production of ammonia (NH,) as we learnt above. Ammonia is an extremely toxic base and its accumulation in the body would quickly be fatal. However, liver contains a system of carrier molecules and enzymes which quickly converts the ammonia (and carbon dioxide) into urea. This is called the 'urea cycle'. This entire sequence of urea cycle is discussed below, along with the enzymes involved in the synthesis of urea.

Carbamoyl Phosphate Synthetase (CPSI)

In the first step, the mitochondrial enzyme Carbamoyl Phosphate Synthetase I converts the ammonia produced by glutamate dehydrogenase into carbamoyl phosphate. See Figure 8.3 which illustrates the entire urea cycle. The formation of carbamoyl phosphate requires 2 molecules of ATP and takes place in the matrix of mitochondria. The ammonium may come from glutamate (as learnt earlier by the action of L-glutamate dehydrogenase) or in free form (as NH^3) from blood, and the HCO_3 comes from respiration (CO^2 is hydrated to form carbonic acid, H_2CO_3 which ionizes to $H^{++}HCO$-). NH^3 (as NHi, ammonium ion) directly takes part in this reaction. Hence, this reaction is also called ammonia-fixation reaction



Carbamoyl phosphate

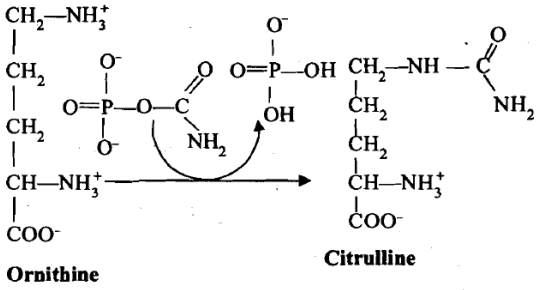
$$NH_4^+ + HCO_3^- + 2\ ATP \rightarrow NH_2\ COOPO_3^{2-} + 2\ ADP + Pi$$
(carbamoyl phosphate)

Pi is inorganic phosphate. ATP, as usual, functions as a magnesium-ATP complex.

Carbamoyl phosphate is next transferred to ornithine. Let us learn about this in the next step.

Ornithine Carbamoyl Transferase

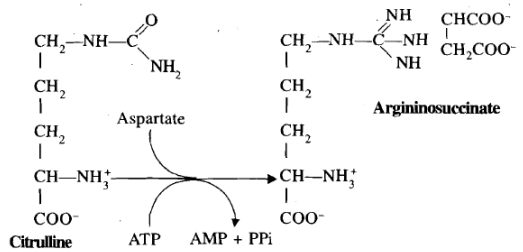
In this reaction, carbamoyl moiety is transferred to ornithine to generate citrulline. Ornithine transcarbamoylase (OTC) catalyzes the condensation of ornithine with transported to the cytosol, where the remaining reactions of the cycle take place. Citrulline leaves the mitochondria by the same transport system that facilitates ornithines' entry from the cytoplasm.



Once in the cytosol, citrulline condenses with aspartate, as can be seen in the next step. Please note the enzymes for the remaining three steps are located in the cytoplasm (cytosol) of the cell. Hence, the citrulline formed in the mitochondria is transferred across the mitochondrial membrane. The next three enzymes functioning in the cytosol are as follows:

Argininosuccinate Synthetase

In a 2-step reaction catalyzed by the cytosolic enzyme argininosuccinate synthetase, citrulline and aspartate are condensed to form argininosuccinate. This reaction involves the addition ofAMP (from ATP) to the amido carbonyl ofcitrulline, forming an activated intermediate on the enzyme surface (AMP-citrulline), and the subsequent addition of " aspartate to form argininosuccinate as shown herewith.



Citrulline + Aspartate + ATP → Argininosuccinate + AMP + PPi

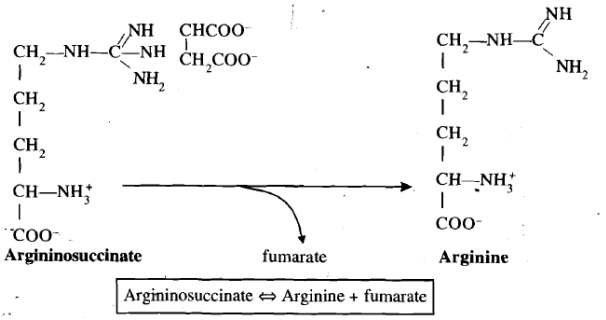
Inorganic pyrophosphate (PPi) consists of 2 phosphate groups. It is very unstable and is hydrolyzed by inorganic pyrophosphatase. This means breakdown of 2 phosphate groups releases energy for the reaction. Hence, this may be considered as a utilization of 2 ATP molecules.

$$PPi + H20 \longrightarrow 2Pi$$

Elimination of fumarate from argininosuccinate then yields arginine, which is the next step in the urea cycle.

Argininosuccinate Lyase

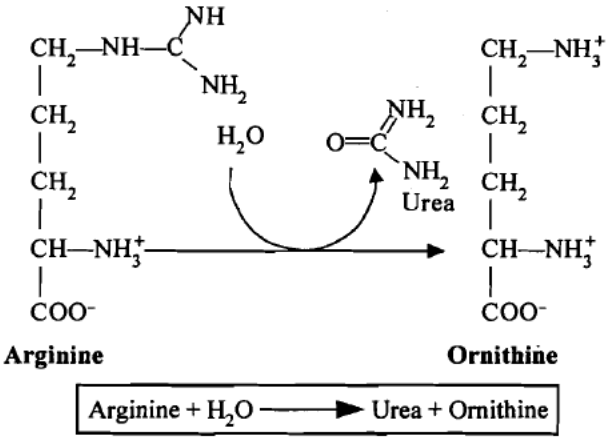
Arginine and fumarate are produced from argininosuccinate by the cytosolic enzyme argininosuccinate Iyase (also called argininosuccinase). It reversibly catalyzes the cleavage of argininosuccinate to fumarate and arginine.



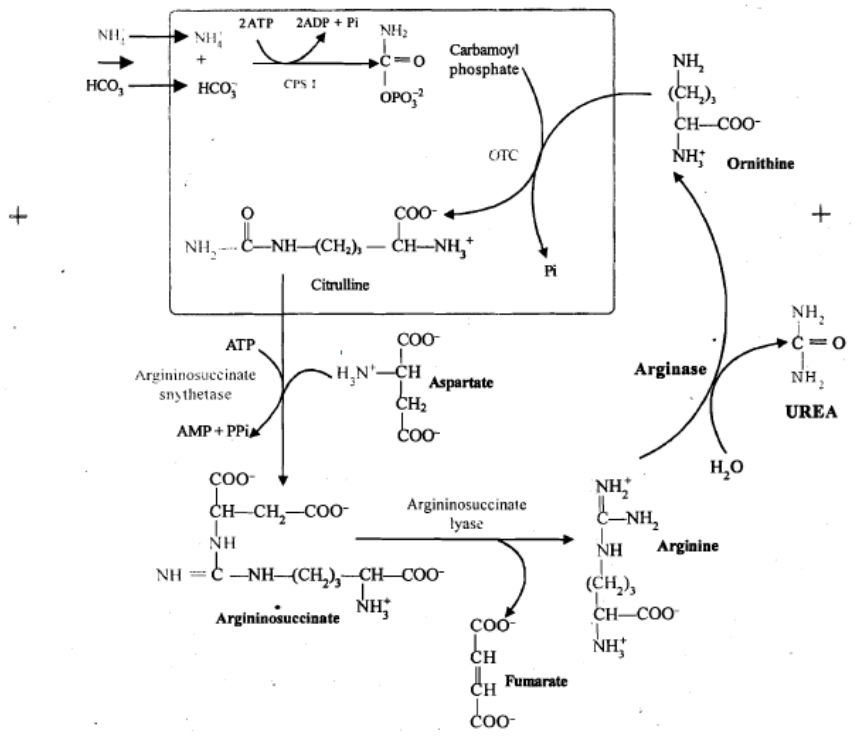
This reaction also supplies arginine for protein synthesis. Ihe fumarate, generated via the action of argininosuccinate Iyase, is reconverted to aspartate for use in the argininosuccinate synthetase reaction earlier.

Arginase

In the final step of the cycle, the cytosolic enzyme arginase cleaves arginine (from the diet or from protein breakdown), generating urea and ornithine. The enzyme arginase cleaves urea from arginine, regenerating cytosolic omithine, which can be transported to the mitochondrial matrix for another round of urea synthesis. Hence, this last enzyme of urea cycle catalyzes the hydrolytic cleavage of arginine to urea and ornithine. Ornithine, as you can see, is regenerated to be used again.



The urea passes via a transport protein into the blood and is carried to the kidneys where it enters the glomerular filtrate, from which it is excreted in the urine. The entire urea cycle is presented in Figure 8.3.



CPS-I is carbamoyl phosphate synthetase-I, OTC is ornithine transcarbamoylase.

Figure 8.3: The urea cycle

With our discussion on the urea cycle, we come to an end of our study about the catabolism of ammo acids.

8.3.4 Metabolism of Carbon Skeletons of Amino Acids

The catabolism of 20 amino acids found in proteins invol ves, as seen above, the removal of a-amino group. This is followed by the breakdown of the remaining product, which is referred to as the carbon (C) skeletons. The catabolism of C skeletons results in the formation of seven different products. These are oxaloacetate, a-ketoglutarate, pyruvate, fumarate, acetyl CoA, acetoacetyl CoA and succinyl CoA. Table 8.1 gives the products formed by the amino acids.

Table 8.1 The products formed by the amino acids.

Product formed	Amino acid forming the product
Oxaloacetate (OAA)	Asparagine, Aspartate
α-ketoglutarate (α-KG)	Glutamine, Glutamate, Proline, Arginine, Histidine
Pyruvate	Alanine, Serine, Glycine, Cystine, Threonine
Fumarate	Phenylalanine, Tyrosine
Succinyl CoA (Succ-CoA)	Methionine, Valine, Isoleucine, Threonine
Acetyl CoA or acetoacetyl CoA	Leucine, Isoleucine, Lysine, Tryptophan

These products can enter the various metabolic pathways as shown in Figure 8.4.

Gluconeogenic and Ketogenic Amino Acids

(Potential Carbon Fate)

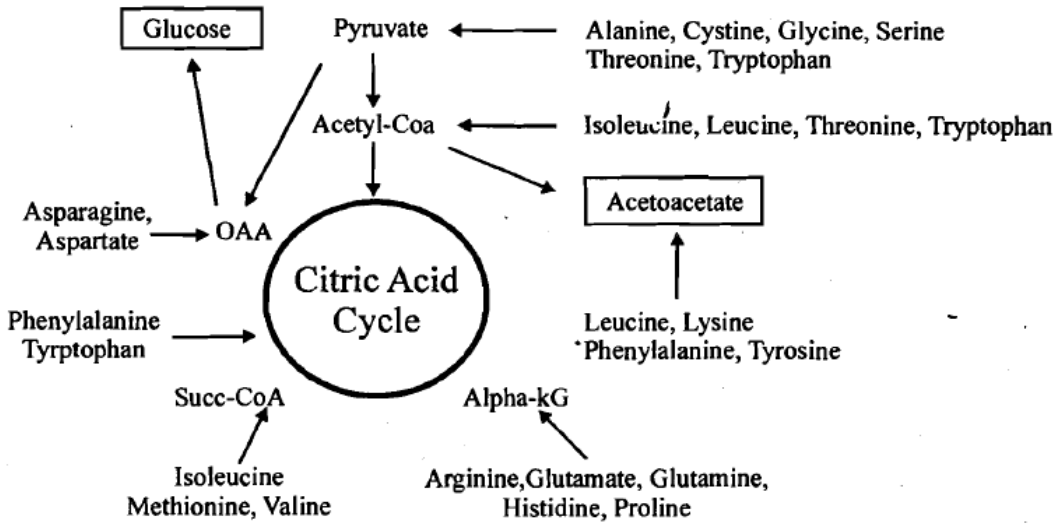


Figure 8.4 : Metabolic pathways of products of carbon skeleton

The 20 amino acids can be classified as either glucogenic or ketogenic amino acids. Some fall into both categories. Let us understand the terms glucogenic and ketogenic in relation to amino acid metabolism.

Ketogenic and Glucogenic Amino Acids

Amino acids can be classified as ketogenic or glucogenic, according to the nature of their metabolic end products as described herewith:

a) Ketogenic: Amino acids whose catabolism yields either acetoacetate or one of its precursors — acetyl CoA or acetoacetyl CoA — are termed ketogenic. This is because acetoacetate is commonly referred to as ketone body. The ketogenic amino acids are degraded to intermediates that can be utilized in the formation of ketone bodies. These products are: acetyl CoA and acetoacetyl CoA.

b) Glycogenic: Amino acids whose catabolism yields pyruvate or one of the intermediates ofthe citric acid cycle are termed 'glucogenic' or 'glycogenic'. This is because these intermediates can act as substrates for gluconeogenesis (synthesis ofglucose) and therefore can give rise to the net formation of glycogen in liver and muscle. The glucogenic amino acids are degraded to intermediates that can be utilized in the formation of glucose. These products are: pyruvate, oxaloacetate, succinyl CoA, a-ketoglutarate and fumarate.

Table 8.2 : Glucogenic and ketogenic amino acids

Glucogenic		Glucogenic + Ketogenic	Ketogenic
Alanine	Proline	Tyrosine	Leucine
Asparagine	Serine	Isoleucine	Lysine
Aspartate	Arginine	Phenylalanine	
Cysteine	Histidine	Tryptophan	
Glutamate	Methionine		
Glutamine	Threonine		
Glycine	Valine		

Next, what is the fate of the products (as highlighted in Table 8.1 earlier) formed by the catabolism of the carbon skeletons? How are these products further metabolized? Let's find out. Oxaloacetate, a-ketoglutarate, fumurate and succinyl CoA are intermediates of the citric acid cycle as you may have noticed in Figure 8.4. Hence they can be oxidized to C02 and H20, or if the blood glucose level is low, they can undergo gluconeogenesis to form glucose. Similarly, pyruvate can go through the reversal of glycolysis and form glucose or get converted to acetyl CoA which can be further oxidized to C02 and H20 in the citric acid cycle. Thus, the nutritional state of the individual (well-fed or starvation) will determine the intermediary metabolic pathway taken by these products. Acetoacetyl CoA is oxidized to acetyl CoA. Acetyl CoA is further oxidized to C02 and H20 in the citric acid cycle.

With this, we have completed the full circle of amino acid catabolism. To summarize what we have learnt so far : the potentially toxic nitrogen of amino acids is eliminated via transaminations, deamination and urea formation, the carbon skeletons are generally conserved as carbohydrate, via gluconeogenesis, or as fatty acid via fatty acid synthesis pathways. In this respect, amino acids fall into three categories: glucogenic, ketogenic or glucogenic and ketogenic. Glucogenic amino acids are those that give rise to a net production of pyruvate or TCA cycle intermediates; such as a-ketoglutarate or oxaloacetate, all of which are the precursors to glucose via gluconeogenesis. Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetyl CoA or acetoacetyl CoA, neither of which can bring about net glucose production.

A small group of amino acids comprised of isoleucine, phenylalanine, threonine, fryptophan and tyrosine give rise to both glucose and fatty acid precursors and are thus characterized as being glucogenic and ketogenic. Finally, it should be recognized that amino acids have a third possible fate. During times of starvation, the reduced carbon skeleton is used for energy proauction, with the result that it is oxidized to CO_2 and H_2O. So far we have studied about the catabolism of proteins, amino acids. Next we shall study about the anabolic activity i.e. biosynthesis of amino acids. In fact, the next few sections focuses on the synthesis Of amino acids and specialized products from amino acids, formation of biogenic amines from

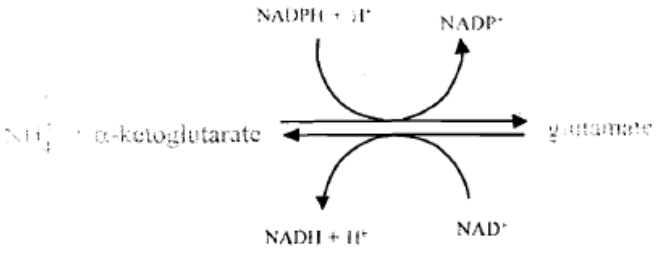
various amino acids and lastly, the non-protein functions of amino acids. We start with biosynthesis of nonessential amino acids.

8.3.5 Biosynthesis of Nonessential Amino Acids

This label 'nonessential' may mislead some people into believing that we don't need it. But, in essence, "non essential" as you may already know, means only that the body can synthesize this amino acid. It does not mean "unimportant." The nonessential amino acids can be synthesized in sufficient amounts from the intermediates of metabolism or from essential amino acids. These synthetic reactions are given next.

a) Synthesis from a-keto acids

Alanine, aspartate and glutamate are synthesized by the transfer of an amino group to the corresponding (I-keto acids which are pyruvate, oxaloacetate and a-ketoglutarate, respectively, as discussed in transamination reaction earlier in sub-section 8.2. I. Glutamate and aspartate are synthesized from their widely distributed a-keto acid precursors by the simple I-step transamination reactions. The former is catalyzed by glutamate dehydrogenase and the latter by aspartate aminotransferase.



Reactions of glutamate dehydrogenase

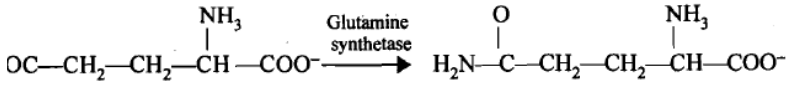
There are 2 main pathways to the production of muscle alanine: directly from protein degradation and via the transamination of pyruvate by glutamate-pyruvate aminotransferase as shown herewith

glutamate + pyruvate <.....> α-ketoglutarate + alanine

b) Synthesis by amidation (introduction of NH 3 into COOH group)

Glutamine, asparagine, proline, serine, glycine, cysteine and Wrosine is formed by amidation. Let us learn how.

i) Glutamine is synthesized from glutamate by glutamine synthetase. NH3 combines with glutamate to forn glutamine catalyzed by the enzyme glutamine synthetase as presented in the reaction below:

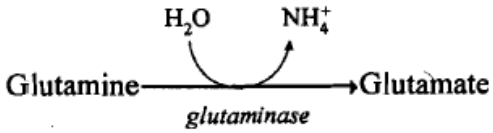


L-glutamate L-glutamine

In this reaction, NH_3 is substituted in the carboxyl (COOH) group of glutamate

giving rise to the amide ($CONH_2$) group, thus glutamine is the amide of glutamate. In this way, glutamine synthase fixes NH_3 as glutamine. Hence, this reaction is also called 'ammonia- fixation reaction'. You may recall reading about this earlier in the urea cycle. Note, glutamine is the transport form of NH_3.

The glutamine synthetase reaction is also important in several respects. First, it produces glutamine, one of the 20 major amino acids. Its role is to carry ammonia to and from various tissues but principally from peripheral tissues to the kidney, where the amide nitrogen is hydrolyzed by the enzyme glutaminase as can be seen in the reaction below. This process regenerates glutamate and free ammonium ion (NH_4^+) which is excreted in the urine.



Note that, in this function, ammonia arising in peripheral tissue is carried in a nonionizable form which has none of the neurotoxic or alkalosis-generating properties of free ammonia.

ii) Asparagine is formed from aspartate by asparagine synthetase.

iii) Proline: Proline is formed from glutamate.

iv) Serine, glycine and cysteine are formed as discussed herewith:

1) Serine: It is formed from 3-phosphoglycerate which is an intermediate of glycolysis.

2) Glycine: It is synthesized from serine. The main pathway to glycine isa l- step reaction catalyzed by serine hydroxymethyltransferase. This reaction involves the transfer of the hydroxymethyl group from serine to the cofactor tetrahydrofolate (THF), producing glycine. In fact serine and glycine are readily interconvertible.

3) Cysteine: It is formed from methionine. In fact the sulfur for cysteine synthesis comes from the essential amino acid methionine. Thus while methionine is an essential amino acid, cysteine is a nonessential amino acid.

v) Tyrosine: It is formed from phenylalanine by the enzyme phenylalanine hydroxylase. Tyrosine is produced in cells by hydroxylating the essential amino acid phenylalanine. This relationship is much like that between cysteine and methionine. Half of the phenylalanine required goes into the production of tyrosine; if the diet is rich in tyrosine itself, the requirements for phenylalanine are reduced by about 50%. Note, tyrosine can be readily synthesized in the body while phenylalanine cannot be synthesized and must be provided in the diet.

After the study of the biosynthesis of nonessential amino acids, we move on to learn about the synthesis of specialized products from amino acids. Amino acids function as precursors for many nitrogen containing compounds. Which are these compounds and what is the role of amino acids in their formation? Let's find out. But, first let us check how much we have grasped from our study so far.

8.3.6 Synthesis of Specialized Products from Amino Acids

In addition to serving as building blocks for proteins, amino acids are precursors of many nitrogen containing compounds that have important biological functions. These a)

Synthesis of porphyrins - Porphyrins are the cyclic compounds that readily bind metal ions, usually Fe^{2+} or Fe^{3+}. The most common metalloporphyrin in humans is heme, which is the prosthetic group for the following compounds:

Haemoglobin - You may already know that haemoglobin is present in the red blood cells (RBC) and transports oxygen from lungs to tissues.

Myoglobin - It is present in muscles and stores oxygen as a reserve against oxygen deprivation.

Cytochromes - They take part in oxidation-reduction reactions since they contain iron which can accept electrons to exist in the ferrous (Fe^{2+}) state or give up electrons to exist in ferric (Fe^{3+}) state. Thus several cytochromes bp c1 , c; a and a3 are present in the mitochondrial respiratory chain acting as carriers of electrons. You shall read more about this later in Unit 11.

Catalase - It is an enzyme found in blood, bone marrow, mucous membrane, kidney and liver. The function is to destroy the highly toxic hydrogen peroxide (H_2O_2) formed during various oxidation reactions in the body to the non-toxic water (H_2O)

$$2\ H_2O_2 \longrightarrow 2H_2O+O_2$$

Tryptophanpyrrolase - It is an enzyme which catalyses the first step i the degradation of tryptophan, which is an oxidative reaction requiring oxygen. Hence, this enzyme is also called tryptophan oxygenase.

b) **Synthesis of creatine** — Creatine phosphate, the phosphorylated derivative of creatine is found in muscles. It is-a high energy compound that can reversibly donate a phosphate group to ADP to form ATP. It is synthesized from glycine.

c) **Synthesis of histamine**— Histamine is a powerful vasodilator (causing relaxation of the smooth muscle of the vascular system) and is forrned by the decarboxylation of the amino acid histidine. It is secreted by mast cells (present in the connective tissue) as a result of allergic reactions or trauma. It is a chemical messenger • that mediates a wide range of cellular responses.

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d) **Synthesis of serotonin** — It is also called 5-hydroxytryptamine and is synthesized from tryptophan (the aromatic amino acid) and involves decarboxylation reaction. Serotonin functions as a neurotransmitter.

e) **Synthesis of catecholamines** — Dopamine, norepinephrine (noradrenalin) and epinephrine (adrenalin) are biologically active amines (containing amino groups) that are collectively termed as catecholamines. They are classified as hormones. Dopamine and norepinephrine function as neurotransmitters in the brain and the autonomic nervous system. Norepinephrine and epinephrine are synthesized in the adrenal medulta (the inner, reddish-brown portion of the adrenal glands that synthesizes, stores, and releases epinephrine and norepinephrine). The catecholamines are synthesized from tyrosine (tyrosine norepinephrine —Y epinephrine).

f) **Synthesis of melanin** — Pigment melanin is also synthesized from tyrosine in a multi-step pathway.

8.3.7 Decarboxylation Reaction and Biogenic Amines

What are biogenic amines? Any of a group of naturally occurring, biologically active amines, such as norepinephrine, histamine and serotonin, that act primarily as neurotransmitters and are capable of affecting mental functioning and of regulating blood pressure, body temperature and other bodily processes are called biogenic amines. Amines are usually formed by the decarboxylation of amino acids. Decarboxylation is the reaction by which C02 is removed from COOH group of an amino acid. As a result, an amine is formed. The reaction is catalyzed by the enzyme dicarboxylase, which requires pyridoxal phosphate (vitamin B 6) as a coenzyme. Biogenic amines formed from various amino acids and their biologic importance is given in Table 8.3.

Amino acids	Amine	Biologic importance
Tyrosine	Tyramine	• Increases blood pressure (Vasoconstriction) • Contracts uterus
Tryptophan	Tryptamine	• Tissue hormone: a derivative of 5-OH Tryptamine (Serotonin) • Vasoconstriction
	5-methoxy Tryptamine (Melatonin)	• Increases blood pressure • Hormone of pineal gland
Histidine	Histamine	• Vasodilator, decreases blood pressure • HCl↑ • Pepsin ↑
Serine	Ethanolamine	• Forms choline by three methylations • Constituent of phospholipids like cephalin
Threonine	Propanolamine	• Constituent of vitamin B_{12}
Cysteine	β-mercaptoethanolamine	• Constituent of coenzyme A

Aspartic acid	β-alanine	• Constituent of pantothenic acid (coenzyme A) • As a constituent of dipeptide carnosine and anserine (they activate myocin, the muscle protein, ATP-ase activity and also enhance copper uptake)
Glutamic acid	γ-amino butyric acid (GABA)	• Presynaptic inhibitory neuro-transmitter in brain • Forms a bypass in citric acid cycle (GABA-shunt)
3,4,di-OH-phenylalanine (DOPA)	Dopamine	• Precursor of the hormones epinephrine and norepinephrine
Cysteine	Taurine	• Constituent of bile acid, taurocholic acid

Next, in our study of amino acid metabolism, we shall look at the non-protein functions of amino acid. Yes, the non-protein functions of amino acids. The well-known function of amino acid is as the building block or the basic unit of proteins. Additionally, amino acids peiform other functions as well without being a part of the protein molecule. Let us learn about these functions next.

1) Immunity: Amino acids ar involved in giving immunity by mainlining the vulnerable surfaces of the body in such a way so as to resist infections. Most of the external agents that cause damage to the human system enter through either the a) lung or b) gastrointestinal tract. Both these organs are protected by the mucus membrane, which offers resistance against the invasion of microorganisms and has the ability to stop the growth of microorganisms. The amino acid which is of importance and found in the mucous membrane is threonine. The mucus protein synthesis is reduced in Protein Energy Malnutrition (PEN'). It is estimated that about 60% of the adult requirements for threonine is involved in maintaining the mucus protein synthesis to optimum. If threonine is deficient in diet, then the mucus membrane has a compromised immune system, where suffcient immunity is not provided against the invasion of foreign organisms by the mucus membrane.

2) Acute phase proteins (fighting functions): When there is an infection in the body, the acute phase proteins are released by the liver in higher amounts into the blood circulation, which will provide immunity against these infections, e.g. serum ferritin is an acute phase protein. During infections, the ferritin levels go up.

3) Synthesis of Glutathione (GSH): Glutathione is a tripeptide consisting of three amino acids: (l) Glutamine (2) Cysteine and (3) Glycine. This is a very important agent in inactivating free radicals like peroxides, superoxide anion, fatty acids etc. If these free radicals are not removed by glutathione, then they damage the cell membrane and alter the cell membrane permeability. Thus, GSH prevents the release of free radicals. If

superoxide anion (Oi) (it is generated when a single electron is transferred to oxygen) has access to lipids, it can oxidize poly unsaturated fitty acids (PUFA) in cell membrane and one PUFA oxidized can trigger the oxidation ofother PUFAs, thus giving rise to free radical chain reaction. PUFA can take part in immediate chain reactions and cause damage. So the free radicals have to be scavenged or quenched (destroyed). Many of the age-related functions like loss of mental functions, memory occurs because of the oxidative damage. Also, cardio vascular disease (CVD), cancer etc. may be caused earlier in individuals with oxidative damage. Glutathione is an antioxidant. Erythrocyte glutathione is reduced in children with kwashiorkor. It is also lower in low birth weight infants.

4) Glutamine, which is an amide ofglutamic acid, as you learnt earlier, is needed for the regulation of protein turnover in muscle. It is also needed for muscle function. During trauma and infections, glutamine levels are lower and provision of glutamine helps in recovery. As already discussed earlier, glutamine is also the transport form of NH3.

5) Taurinederived from cysteine isa ß-amino acid. Taurine provides resistance against the peroxides that are formed. So, some toxic products can be inactivated by taurine. The levels oftaurine in breast milk are higher, providing a protective function from free radicals.

6) Creatine is a compound that chiefly arises from glycine, arginine and a source of methyl group and is catabolized to creatinine. Creatine is present as creatine phosphate in muscle. The function ofcreatine is to provide quick eneru to muscles. Some of these functions are impaired in PEM.

7) Nitric oxide (NO) is the metabolic product of arginine. It has gained importance as a lot of functions have been discovered, involving virtually every tissue ofthe body. They are:

   maintenance of vascular tone and blood pressure

   inhibition ofadhesion (sticking together), activation and aggregation of platelets

   involvement in the higher level cognitive ftmctions, and pa.rticipation in immunoprotection.

It appears that NO acts through macrophages, moves towards the place, where the organism is present and then brings about macrophage killing. Nitric oxide stimulates Amino Acid and macrophage killing. The level of arginine required to produce level of nitric oxide should Nucleotide metabolism be such that it will provide the most benefits e.g. DHEA ($_3$ fatty acid).

8.4 NUCLEOTIDE METABOLISM

The nucleotides i.e. ribonucleoside and deoxyribonucleoside phosphate are essential

for all cells. Without them, DNA and RNA cannot be produced, with the result the cells cannot proliferate and proteins cannot be synthesized. Fufiher, nucleotides serve as camers of activated intermediates in the synthesis of carbohydrates, lipids and proteins. They are also structural components of a number of essential coenzymes such as coenzyme A, FAD, FMN, NAD^+ and $NADP^+$. Nucleotides also play an important role as "energy currency" of the cell. Lastly, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism by either inhibiting or activating key enzymes.

We learnt earlier in Unit 2 that nucleotides consist of a nitrogenous base which may be either a purine (adenine or guanine) or a pyrimidine (cytosine or uracil or thymine) base, The purine and pyrimidine bases found in the nucleotides can be synthesized de novo or can be obtained through salvage pathways and reuse those we already have. The salvage pathways, in fact, are a major source of nucleotides for synthesis of DNA, RNA and enzyme co-factors. We shall learn about purine and pyrimidine synthesis next.

8.3.1 Purine Nucleotide Synthesis - De Novo Synthesis

Look at Figure 8.5. This is the structure ofpurine ring. The atoms ofthe purine nucleotide ring are contributed by a number ofcompounds including amino acids such as aspartic acid, glycine and glutamine, CO_2 and derivatives of tetrahydrofolate as can be seen in Figure 8.5.

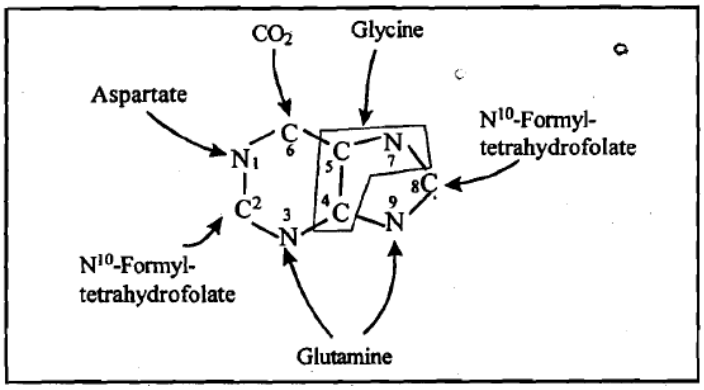

Figure 8.5 : Origin of atoms in the purine ring structure

The purine ring is formed by a series of reactions, as presented in Figure 8.5, that add the donated carbons and nitrogen to a preformed ribose 5-phosphate, which is synthesized by the HMP shunt, about which you may recall reading in Unit 6 on carbohydrate meubolism.

The major site of purine synthesis is in the liver. Synthesis of the purine nucleotides begins with 5-phosphoribosyl-l-pyrophosphate (PRPP) and then follows a series of reactions and leads to the first fully formed nucleotide, inosine 5'-monophosphate (IMP) and AMP and GMP are subsequently derived from IMP. The purine base of IMP is hypoxanthine. The de-novo pathway starting from PRPP to the formation of IMP is diagrammed in Figure 8.6. Let us learn about each of these reactions one

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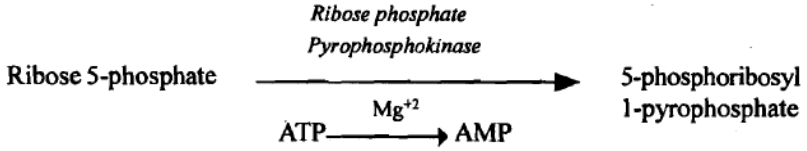
by one.

1) *Synthesis of 5-phosphoribosyl-l-pyrophosphate (PRPP)*

PRPP is synthesized from ATP and ribose-5-phosphate. The reaction is catalyzed by the enzyme ribose 5-phosphate pyrophosphokinase. The enzyme is activated by Pi and inhibited by purine nucleoside and triphosphates.



2) *Synthesis of 5' - phosphoribosylamine*

5 '-phosphoribosylamine is synthesized from PRPP and glutamine. In this, the amide group of glutamine replaces the pyrophosphate group attached to Cl of PRPP. The enzyme catalyzing the reaction is glutamine phosphoribosyl pyrophosphate amidotransferase. The enzyme is inhibited by the purine 5' nucleotidesAMP, GMP and IMP, which are the end products of this pathway (feedback inhibition). This is the committed step in purine nucleotide biosynthesis.

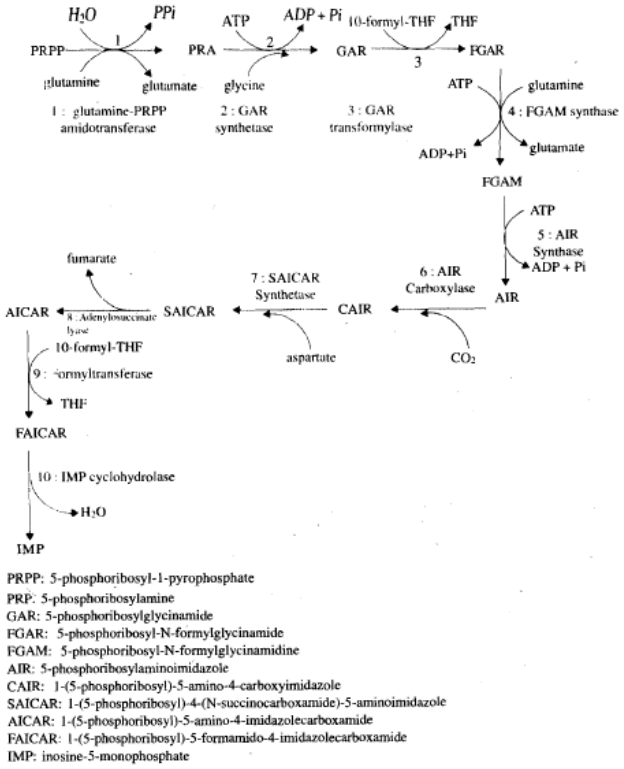
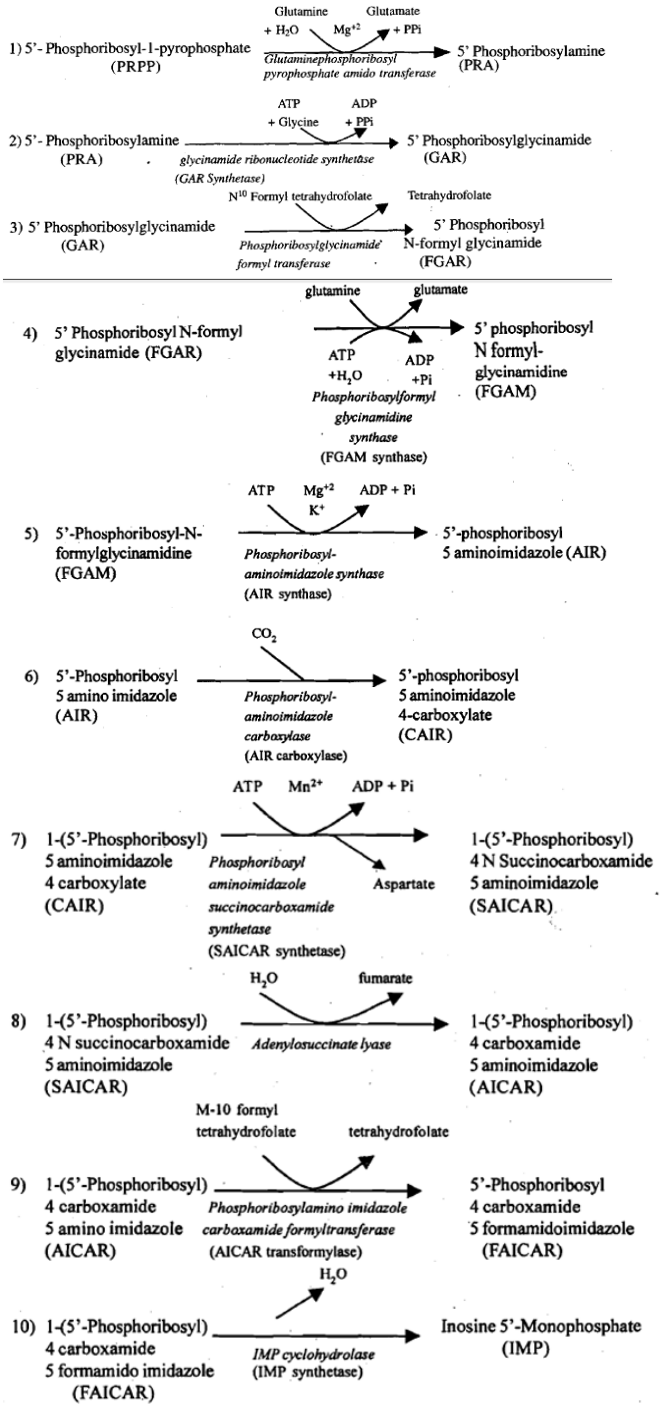


Figure 8.6 : Pathway for synthesis of purine nucleotide

3) *Synthesis of inosine monophosphate*

This pathway requires 4 ATP molecules as an energy source. In all, 10 steps are involved in the purine nucleotide biosynthesis as shown in Figure 8.6 and



Earlier Figure 8.6 illustrated the formation of IMP, the first fully formed nucleotide.

IMP can then become either AMP or GMP as discussed in the next step. It is important for us to understand that nucleotides are important for reasons besides being precursors of nucleic acids. Most of them provide energy used to drive

NOTES

biochemical reactions. fact, IMP represents a branch point for purine biosynthesis, because it can be converted into either ANfP or GMP through two distinct reaction pathways. We shall learn about this next.

4) *Conversion of IMP to AMP and GMP*

It is clear that besides being precursors of nucleic acids, most nucleotides provide energy used to drive biochemical reactions. You have frequently heard ATP referred to as the "universal energy currency" of the cell. ATP is a nucleotide and it is also called a nucleoside triphosphate. Adenosine monophosphate (AMP) indicates that a single phosphate is in ester linkage to the hydroxyl groups of an adenosine molecule. Guanosine monophosphate (GMP) would indicate that a phosphate is in ester linkage to the hydroxyl group of a guanosine.

The conversion of IMP to either GMP or AMP utilizes a two-step, energy-requiring pathway. The synthesis of AMP requires GTP as an energy source, whereas, the synthesis of GMP requires ATP. The first reaction in each pathway is inhibited by the end product of that pathway as shown in Figure 8.7.

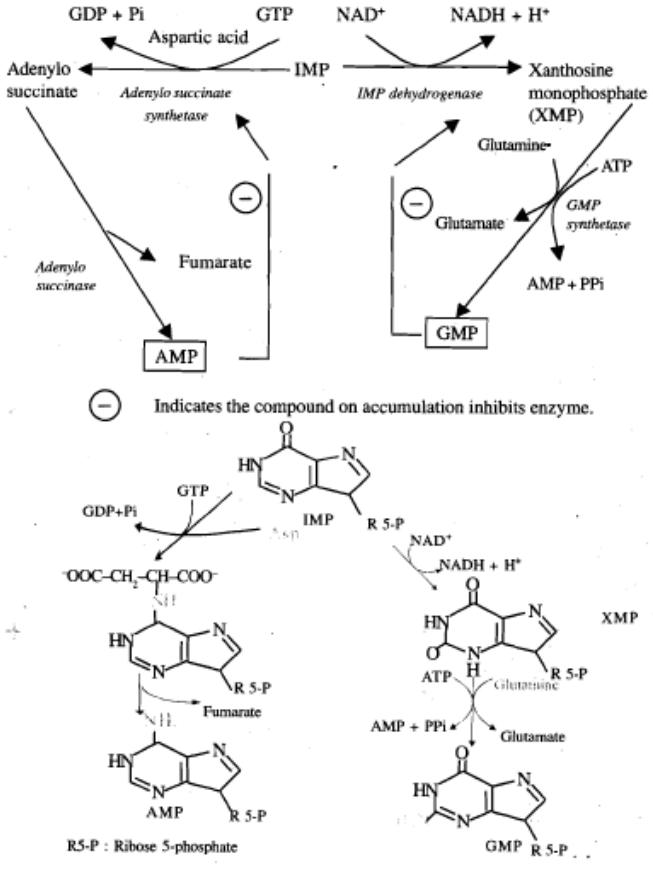


Figure 8.7 . • Conversion of IMP to AMP and GMP

5) **Interconversion of nucleotides: Conversion of nucleoside monophosphate (NMP) to nucleoside diphosphates (NDP) and triphosphates (NTP)**

You already know that nucleotides with one phosphate are also calred nucleoside monophosphates (NMP), those with two phosphates are nucleoside diphosphates ODP) and those with three phosphates are nucleoside triphosphates (NTP). ATP, you learnt in the above section, is a nucleotide and it is also called a nucleoside triphosphate.

NDP are synthesized from the corresponding NMP by base specific nucleoside monophosphate (NMP) kinases. These kinases do not discriminate between ribose or deoxyribose in the substrate. ATP is the source of transferred phosphate.

$$AMP + ATP \xrightarrow{\textit{adenylate kinase}} 2\ ADP$$

$$GMP + ATP \xrightarrow{\textit{guanylate kinase}} GDP + ADP$$

Adenylate kinase is particularly active in liver and muscle, where the turnover of energy from ATP is high. Its function is to maintain equilibrium among AMP, ADP and ATP.

$$2\ ADP \longrightarrow AMP + ATP$$

NDP and NTP are interconverted by *nucleoside diphosphate kinase*.

$$GDP + ATP \Leftrightarrow GTP + ADP$$

$$CDP + ATP \Leftrightarrow CTP + ADP$$

The discussion above focused on the de novo synthesis of purine nucleotides, starting from PRPP leading to IMP and finally to GMP and AMP. Before we end our study on purine synthesi we would also like to look at the inhibitors ofpurine synthesis. These are discussed next.

Inhibitors of purine synthesis

Some inhibitors ofpurine synthesis are specific for inhibiting the youth of rapidly dividing microorganisms for e.g. PABA analogues— sulfonamides. Sulfonamides are structural analogs of PABA that competitively inhibit bacterial synthesis of folic acid. Since purine synthesis requires THF as a coenzyme, the sulpha drugs slow down this pathway in bacteria. Humans cannot synthesize folic acid, and must rely on external sources of this vitamin. Therefore sulfa drugs do not interfere with human purine synthesis.

Folic acid analogues: Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by dihydrofolate reductase (reaction 9 of purine synthesis). These drugs limit the amount oftetrahydrofolate available for use in purine synthesis, and thus slow down DNA replication in mammalian cells. These compounds are therefore useful in treating rapidly growing cancers, but are toxic to all dividing cells.

You may recall reading earlier that there is yet another salvage pathway for purine synthesis. Let us leam about this pathway next.

8.4.2 Salvage Pathway for Purines

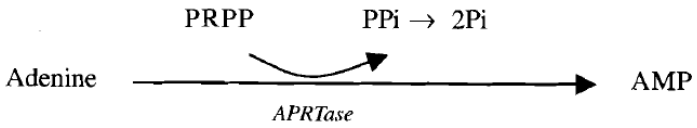
Purines that result from the normal turnover of cellular nucleic acids (i.e. from the degradation of nucleic acid) or those that are obtained from the diet and not degraded can be reconverted into NTP and used by the body. This is referred to as the "salvage pathway ' for purines. As expected, this would be a very short pathway since purines are already available. The free purine bases—adenine, guanine and hypoxanthine-can be reconverted to their corresponding nucleotides by phosphoribosylation. Following two key transferase enzymes are involved in the salvage of purines:

a) Adenine phosphoribosyl transferase (APRTase); and

b) Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) Amino Acid and

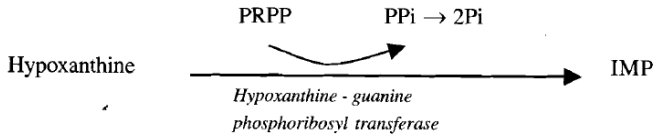
Both the enzymes utilize phosphoribosyl pyrophosphate (PRPP) as the source of the ribose-5-phosphate group. The release of pyrophosphate and further hydrolysis to inorganic phosphate makes these reactions irreversible. A deficiency of HGPRT causes the Lesch-Nyhan Syndrome.

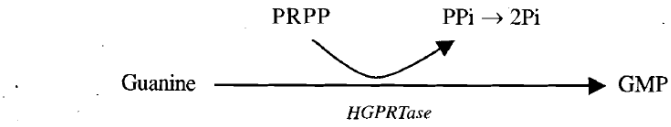
Adenosine phosphoribosyltransferase (APRTase) catalyzes the following reaction:



Thus ribose-5-phosphate is transferred from PRPP to the purine base hypoxanthine to form the nucleotide, IMP (inosine monophosphate).

Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) catalyzes the following reactions:





When guanine adds on ribose-5-phosphate, GMP is formed while adenine gives AMP as you may have noted above. Salvage pathways are important, in those tissues which cannot synthesize purines and purine nucleotides. For example, the human brain has a low level of the enzyme PRPP glutamyl amido transferase, while erythrocytes cannot synthesize 5-phosphoribosyl amine. Hence, liver the major site of synthesis, provides purines and purine nucleotides from salvage pathway in these tissues. This concept i.e. the salvage pathway, you would agree is like that of 'recycled paper'.

With the understanding of salvage pathway, we come to an end of our study about purine synthesis. After synthesis, it is the turn of the degradation of nucleotides. In the next sub-section, we shall learn about the degradation process of purine nucleotides.

8.4.3 Degradation of Purine Nucleotides

Purines are degraded to uric acid, a relatively water insoluble compound in the acid form and only slightly more soluble as the anion. Most of purine degradation takes place in all tissues ; the last steps, however, the oxidation of hypoxanthine and xanthine to uric acid, are restricted to the liver.

The end product of purine catabolism in humans is uric acid, which is itself a purine. The steps involved in degradation of purine to uric acid are diagrammatically presented in Figure 8.8 and a summary is presented herewith.

AMP Inosine → Hypoxanthine→ Xanthine Uric acid

GMP → Guanosine → Guanine Xanthine → Uric acid

The steps in uric acid synthesis include:

1) An amino group is removed from AMP to produce IMP or from adenosine to produce inosine.

2) IMP and GMP are converted into their nucleoside forms.

3) Purine nucleoside phosphorylase converts inosine and guanosine into theirrespective purine bases, hypoxanthine and guanine.

4) Guanine is deaminated to form xanthine.

5) Hypoxanthine is oxidized byxanthine oxidase to xanthine, which is finall oxidized to uric acid.

Uric acid is excreted in the urine. Chronic elevation ofuric acid in blood occurs in 3% of the population. What is the outcome? Gout results from excess uric acid in various fluids. It is somewhat insoluble. One result is arthritic pain in joints due to deposition in cartilaginous tissue. The big toe is most susceptible.

Gout is characterized by hyperuricemia, with recurrent attack of acute arthritic joint inflammation caused by the deposition of uric acid crystals. Primary gout is due to an inborn error of metabolism, such as overproduction of uric acid. Secondary gout may be caused by other diseases such as cancer, chronic renal insufficiency, HGPRTase deficiency etc.

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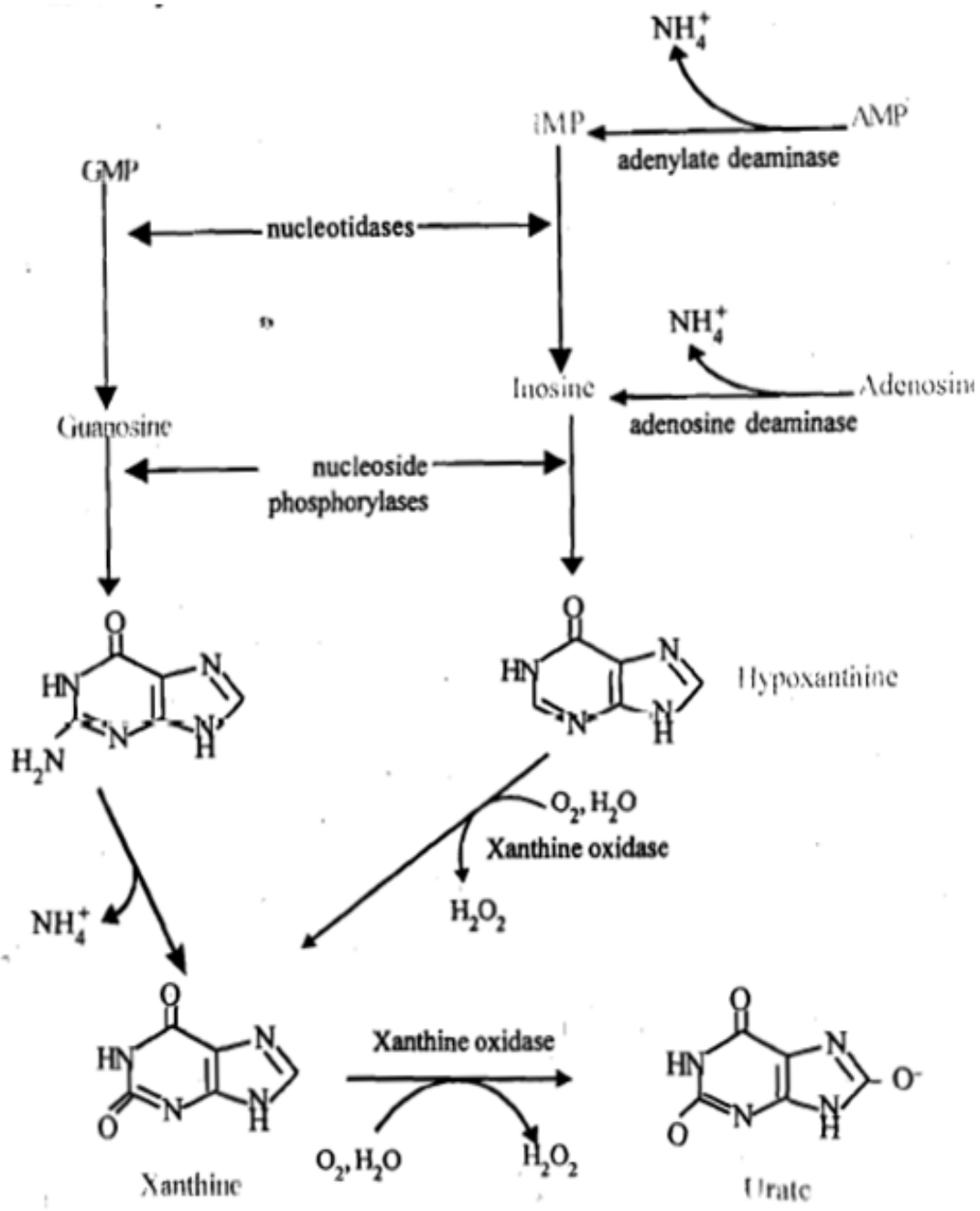

Figure 8.8 : Steps in uric acid synthesis

8.4.4 Pyrimindine Synthesis

The synthesis of pyimidines is much simpler. Only two molecules contribute to the pyrimidine skeleton, carbamoyl phosphate and aspartate as can be seen in the structure of the pyrimidine ring in Figure 8.9. The sources of the C and N atoms of viz. the pyrimidine ring are glutamine, C02 (from which carbamoyl phosphate is synthesized) and aspartic acid. Here, the pyrimidine base is first synthesized to which ribQse-5-P is added. You would realize that this is different from purine synthesis, where the purine nucleus is built on ribose-5-P.

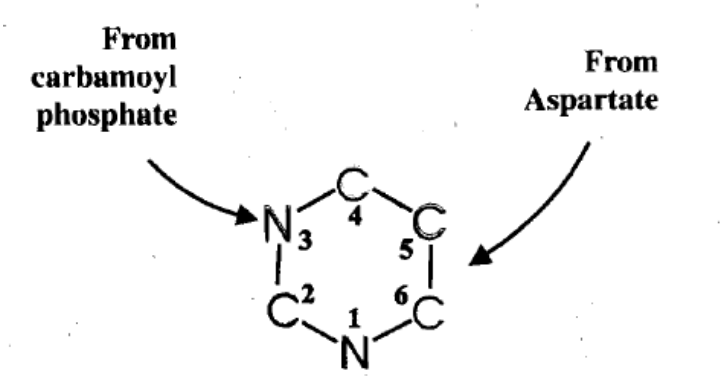

Figure 8.9 : Origin of atoms in the pyrimidine ring

Let us get to know about the pyrimidine synthesis and the steps involved next.

1) *Synthesis of carbamoyl phosphate*

As mentioned above, the pyrimidine skeleton is synthesized from carbamoyl phosphate and aspartate. The synthesis of carbamoyl phosphate from glutamine, C02 and two ATPs occurs in the cytoplasm ofthe cell, catalyzed by carbamoyl phosphatase synthetase II (CPS II). This is the committed step (rate-limiting) in pyrimidine biosynthesis. The CPS II does not require biotin. It is inhibited by UTP and activated by ATP and PRPP. You may recall reading about CPS I), earlier in sub-section 8.2.3. The differences between carbamoyl phosphate synthetase I and II are given herewith.

	CPS 1	CPS 11
Cellular location	Mitochondria	Cytosol
Pathway	Urea cycle	Pyrimidine synthesis
Source of N	Ammonia	Y-Amide group of glutamine

2) *Synthesis of orotic acid*

From carbamoyl phosphate and aspartate, carbamoyl aspartate is formed by the action of aspartate transcarboxylase. The pyrimidine ring is then closed through a loss of a molecule of water by dihydroorotase, to form dihydroorotate. This is then oxidized by dihydroorotate dehydrogenase to produce orotic acid as shown in Figure 8.10. At this point, the pyrimidine ring is synthesized. The 3 enzymes:

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CPS II, aspartate transcarbamoylase and dihydroorotase are all domains (regions) .0f the same polypeptide chain which facilitates the ordered synthesis of the compound. That is, the protein is a multienzyme complex.

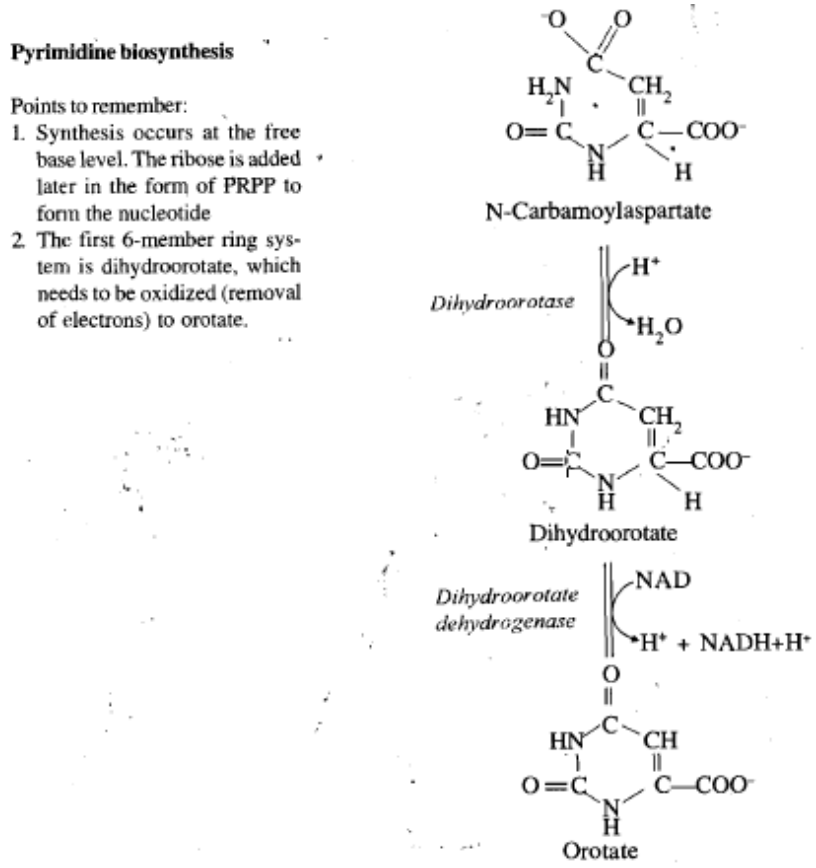

Figure 8.10 : Synthesis of orotate

3) Formation of pyrimidine nucleotide

The first pyrimidine nucleotide, as seen in the last step, is orotate. Once the pyrimidine ring is synthesized, it is converted to the nucleotide orotidine-5' -monophosphate (OMP), which involves addition of ribose and phosphate. PRPP (you recall reading about it earlier in sub-section 8.3.1) is the rjbose-5-phosphate donor. The reaction is irreversible. However, OMP is not a pyrimidine present in nucleic acid. Hence, OMP is converted to UMP by orotidylate decarboxylase by removing acidic carboxyl group. Thus UMP (uridine monophosphate) has uracil as the pyrimidine base. Figure 8. I I illustrates these steps in the pyrimidine synthesis. Deficiency of orotate phosphoribosyl transferase and orotidylate decarboxylase, results in accumulation of orotic acid which is excreted in the urine. This condition is called orotic aciduria.

Besides UMP, nucleic acids also contain 2 other pyrimidine nucleotides which are CMP (cytidine monophosphate having cytosine) and TMP (thymidine monophosphate with thymine). So next, we shall see how CMP is synthesized.

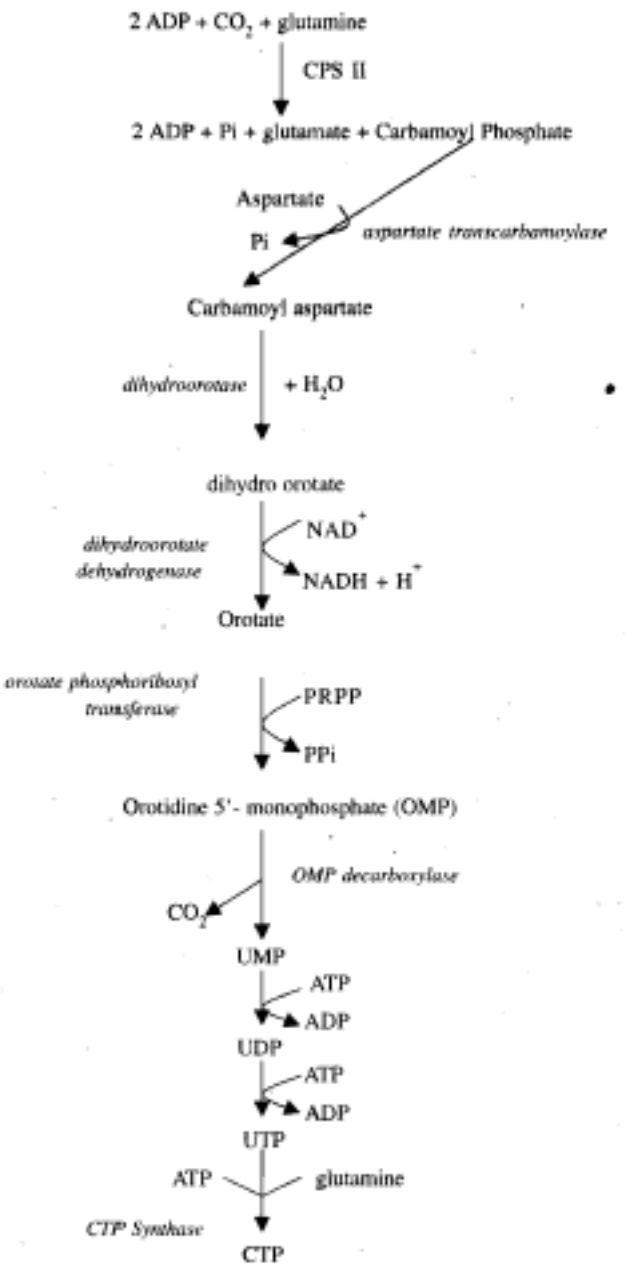

Figure 8.11 : Pyrimydine synthesis

4) *Synthesis of uridine triphosphate U TP and cytidine triphosphate (CTP)*

UMP is first phosphorylated using ATP to form UDP (uridine diphosphate). A further phosphorylation by ATP fornv UTP (uridine triphosphate). The pyrimidine base cytosine isnext aminated i.e. oxygen atom at position 4 is replaced by amino group obtained from glutamine. This converts uracil into cytosine forming CTP (cytidine triphosphate). Thus CTP is produced by amination of UTP catalyzed by

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the enzyme CTP synthase. This step requires energy, which is provided by ATP. The entire pyrimidine synthesis reactions starting from ATP, COO and glutamine to UMP and CTP is presented in Figure 8.11.

5) *Synthesis of deoxyribonucleotides front ribonucleotMes*

The nucleotides synthesized are all ribonucleotides, which can be used as building blocks in RNA synthesis or as nucleotide carriers of other compounds, such as sugars (e.g. UDP-glucose used in glycogen metabolism). The nucleotides required for DNA synthesis are 2' -deoxyribonucleotides (i.e. instead of ribose, there is 2'-deoxyribose where in 2nd position of ribose sugar, oxygen atom is absent), which are produced from ribonucleoside diphosphates. The enzyme ribonucleotide reductase is specific for the reduction of nucleoside diphosphates (ADP, GDP, CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP and dUDP). Since oxygen is removed. it is a reduction reaction.

6) *Synthesis of TMP from dUMP*

The fourth pyrimidine nucleotide commonly present in nucleic acids is thymidine monophosphate (TMP). Figure 8.12 illustrates the biosynthesis of TMP. TMP is present only in I)NA which has deoxyribose. Hence technically it is dTMP, however, it is also generally referred to as TMP. dUMP is converted to dTMP by thymidylate synthetase, which utilizes N5, N10 — methylene tetrahydrofolate as the source of the methyl group when the pyrimidine base uracil is methylated at position 5, it becomes the base thymine (6-methyl uracil). THF not only transfers a C unit but also 2 H atoms resulting in the oxidation of THF to dihydrofolate (DHF). Inhibitors of thymidylate synthetase include thymine analogs (compounds structurally similar to thymine) such as 5-fluorouracil, which serve as antitumor agents. In addition, dihydrofolate reductase, the enzyme that reduces DHF to THF, is inhibited in the presence of drugs such as metrotrexate. By decreasing the supply of THF, these folate analogs inhibit purine synthesis and prevent methylation ofdUMP to dTMP. They lower the cellular concentration of dUMP, which is an essential component of DNA. In this way, DNA synthesis is inhibited and cell growth is arrested or slowed down. Therefore, these drugs are used to decrease the growth rate of cancer cells.

Biosynthesis of thymidine monophosphate

Methylene tetrahydrofolate is the methyl donor. Note that it also acts as a reducing agent, and in the process is converted to dihydrofolate which has to be oxidized to tetrahydrofolate to be reusable in this and other reactions.

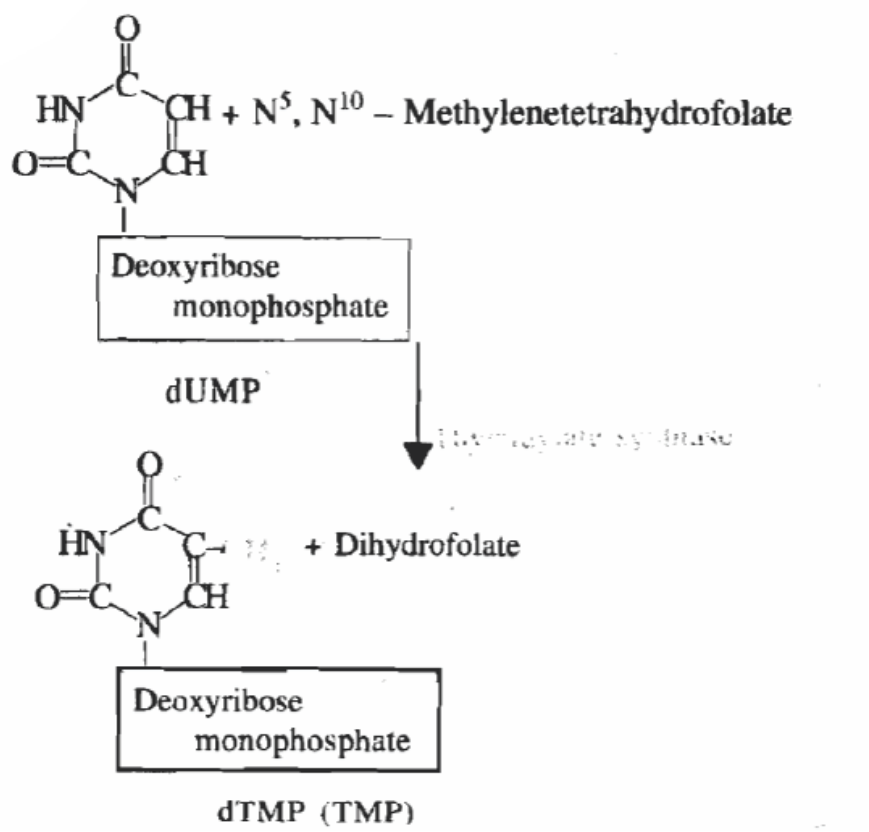

Figure 8.12 : Biosynthesis of TMP

We shall end our study of pyrimedine metabolism by learning about degradation and salvage reactions ofpyrimidine nucleotide.

Degradation of Pyrimidine Nucleotides

The pyrimidine ring can be. opened and degraded to highly soluble structures such as ß alanine and ß amino isobutyrate. They serve as precursors of acetyl CoA and succinyl CoA respectively.

Salvage Reaction of Pyrimidine nucleotide

The pyrimidines, like the purines we saw earlier, can be salvaged and converted into nucleotides by the enzyme pyridine phosphoribosyl transferase wherein again it utilizes PRPP as the source of the ribose-P. In addition, orotate phosphoribosyl transferase (an enzyme ofpyrimidine nucleotide synthesis) salvages (i.e. reuses) orotic acid by converting it to OMP.

8.4.5 Regulation of Deoxyribonucleotide Synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. The regulation of the enzyme is complex. In addition to the single active site, there are 2 sites on the enzyme involved in regulating the activity as given below:

a) Activity site	ATP combines with this site and activates enzyme d ATP inhibits enzyme
b) Substrate specificity sit	ATP, dATP, dTTP or dGTP regulate reduction of specific ribonucleotide

8.5 LET US SUM UP

In this unit we focussed on aminö acid and nucleotide metabolism. We learnt that amino acids play an important role in the synthesis of tissue proteins and have a variety of important biological activities. Transamination, deamination and decarboxylation are some of the important reactions of amino acids. The ammonia formed is convened to urea in the liver and is excreted by the kidneys. The biogenic amines formed through the decarboxylation process have important physiological functions.

The nucleotides are necessary for the synthesis of DNA and RNA. Purine and pyrimidine nucleotides can be synthesized in the body. The end product of purine catabolism in humans is uric acid, the accumulation of which can result in gout. Pyrimidines are degraded to highly soluble structures such as a-alanine and a-amino isobutyrate which serves as precursors of acetyl CoA and succinyl CoA. The nucleotides synthesized are ribonucleotides. The ribonucleotides reductase enzyme reduces the nucleoside diphosphates to their deoxy forms. This enzyme is also responsible for maintaining a balance supply of deoxyribonucleotides for DNA synthesis.

8.6 GLOSSARY

Acute phase proteins proteins : needed during stress, immunoglobulins, leukocytes, lymphocytes, haemoglobin, albumin and enzymes necessary for protein synthesis.

Amino acid Pool : amino acids coming from exogenous and endogenous sources found in circulation.

Analogues : structural similarity.

Hyperuricemia : increased levels of uric acid in circulation.

Melanin : any of a group of naturally occurring dark pigments, especially the pigment found in

skin, hair, fur and feathers.

Protein Energy : also referred to as protein calorie malnutrition; it is a Malnutrition (PEM) potentially fatal body-depletion disorder, which develops disorder in children and adults whose consumption of protein and energy (measured by calories) is insufficient to satisfy the body's nutritional needs.

Urea : the chief nitrogenous end product of protein metabolism and the chief nitrogenous constituent of urine.

8.7 CHECK YOUR PROGRESS

1) What is the metabolic fate of amino acids after the removal of a-amino group?

2) What are the transamination reactions? Give any one example.

3) How is ammonia removed from our body? What is this process called?

4) Indicate the various enzymes and coenzymes involved in the urea cycle.

5) Explain how the nutritional state of an individual affects the metabolic pathway of products formed by the catabolism of C-skeletons.

6)) What specialized products are synthesized from amino acid?

NOTES

UNIT

9

ANTIOXIDANTS

STRUCTURE

9.1 LEARNING OBJECTIVE

After studying this unit, you will be able to;

enumerate how free radicals are formed,

discuss the significance of antioxidants, and

explain the nutrient and non-nutrient antioxidants in health and disease

9.2 INTRODUCTION

Antioxidants as you may be already aware are compounds that scavenge free radicals. Ample evidence exists that the antioxidants protect the body from the load of free radicals which are generated during the normal metabolic processes everyday. This unit gives an overview of physiological mechanisms available in the body to prevent free radical damage. What are free radicals? What are their ill effects? What is the importance of antioxidants in health and disease? These are a few issues discussed in this unit.

9.3 ANTIOXIDANTS AND FREE RADICALS

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What do vitamins A, E, C and beta-carotene, a precursor to Vitamin A, and the trace mineral, Selenium have in common? Well, they are all antioxidants. What are antioxidants?

Any substance that prevents or impedes cell oxidation (destruction) by free radicals, etc. is an antioxidant. Antioxidants are the molecules that work to reduce damage done to cells and DNA by free radicals i.e. charged particles found in the environment and prodåced by processes in the body. Antioxidants are components that combine with free radicals in the body and neutralize their damaging effects. So, antioxidants work together in the body to maintain our health and vigor well into the late decades of life.

We have a fairly clear idea now about what is an antioxidant. Do you know what a free radical is? You may recall reading about antioxidants, free radicals in the Advance Nutrition Course. Well, now once again let us get to know about free radicals.

What is a free radical?

Free radicals are atoms or molecules with an unpaired electron. You will be able to understand this better after reading the discussion on free radicals given herewith:

Oxidation-reduction reactions are central to the supply ofbiological energ. In an oxidation- reduction reaction, electrons from one species are transferred to another. An oxidizing agent is a substance that gains electrons in an oxidation-reduction reaction and in the process gets reduced. A reducing agent is a substance that donates electrons and gets oxidized. Oxidation is always accompanied by reduction, hence there must be both an oxidiiing agent and a reducing agent. Oxidation can take place in several ways:

removal of hydrogen (dehydrogenation)

addition ofoxygen

removal of electrons

Dehydrogenation is the most common form of biological oxidation. Most dehydrogenation occurs by C—H bond cleavage. Since covalent chemical bonds consists of pairs of electrons shared by two atoms, bonds can be cleaved in two ways — both electrons can stay with one atom or one electron can remain with each atom. In most reactions, both electrons stay with one atom. Cleavage of C—H bond almost always produces two ions —

1) if the carbon atom retains both electrons, the carbon containing compound becomes a carbanion (i.e. with a negative charge).

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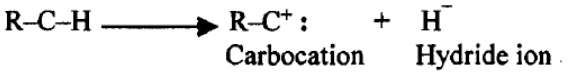
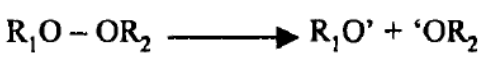
2) if the carbon atom loses both electrons, the carbon containing compound becomes a cationic ion (i.e. with a positive charge) called carbocation.

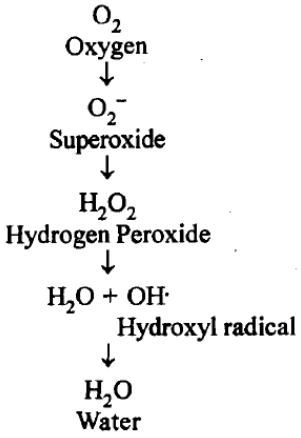


However, if one electron remains with each product, then free radicals are said to be formed.



Thus, you can now understand better that a free radical is a molecule or atom with an unpaired electron.

In Unit 6, you may recall reading that the majority of intracellular oxidation ofsubstrates results in transfer of two electrons to appropriate acceptors such as NAD+ or FAD, which are then oxidized in the electron transport chain. In the last step, 02 is reduced to water, catalyzed by the enzyme cytochrome C oxidase. The electronic structure of 02 favours its reduction by addition of one electron at a time leading to generation of oxygen radicals. Thus the radical has a highly reactive unpaired electron in an outer orbital which can initiate chain reactions by removal ofan electron from another molecule to complete its own orbital. The stepwise transfer of electrons to 02 results in formation of various intermediates called reactive oxygen species, as illustrated herewith.



The oxygen radicals readily react with a variety ofcellular components causing cellular damage. In fact, the most potent oxygen species in biological species is probably the hydroxyl radical (OH^+). Most free radicals are extremely short lived. However, they readily extract electrons from other molecules, converting them to free radicals and thereby initiating a chain reaction. Hydrogen peroxide itself is not a free radical but is converted to the hydroxyl radical in the presence ofFe^{2+} or Cu^+ present in cells.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH—$$

You would be surprised to know that formation of free radicals is a normal oxidation process in foods and are formed during common food treatments such

as toasting, frying, freeze drying, irradiation etc. Free radicals are generally very reactive, unstable structures that continuously react with substances to form stable products.

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So we haye seen that free radicals are highly reactive species that have an unpaired electron e.g. the hydroxyl and superoxide radical. The electron in an atom or molecule orbit the nucleus in shells or layers and the most stable configuration occurs when these electrons are in pairs that orbit in opposite directions. If an atom or molecule within the body loses or gains an electron, the resulting product is highly reactive and can react with and damage DNA, proteins, lipids or carbohydrates. Cellular damage caused by oxygen-derived free radical species has been implicated in the aetiology ofa range of diseases, including cancer, atherosclerosis, cataract and retinopathy. It has been suggested that many of the degenerative changes associated with ageing may be due to the cumulative effects of free radical damage.

Now that we have an understanding about free radicals, surely you can now conceptualize what role these free radicals play. The next section focuses on this aspect.

9.4 ROLE OF OXYGEN FREE RADICALS

Evidence is accumulating that most of the degenerative diseases that afflict humanity have their origin in deleterious free radical reactions. These diseases include atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, degenerative eye disease etc. The process of biological ageing might also have a free radical basis.

Oxygen free radicals can react with DNA to cause breaks in the DNA chain and alteration of bases (mutation). This could initiate carcinogenesis. Free radicals can percxidize polyunsaturated fatty acid (PUFA) residues in low density lipoprotein (LDL). This oxidized LDL is taken up by macrophages and generate foam cells and this ultimately leads to scarring and fibrosis of artery walls seen in atherosclerosis. Unoxidized LDL is considered relatively benign in its effects upon artery wall.

The reactions of free radicals involve the loss or gain of an electron and this creates another free radical, which can initiate a damaging chain reaction unless the free radical is quenched by antioxidant systems and the chain halted. For example, peroxidation ofa PUFA will generate another unstable compound (the lipid peroxyl radical) and this reacts with another fatty acid to produce stable lipid peroxide as shown in Figure 9.1. Susceptibility of PUFA to free radical damage is one ofthe concerns about recommending high levels of PUFA in the diet.

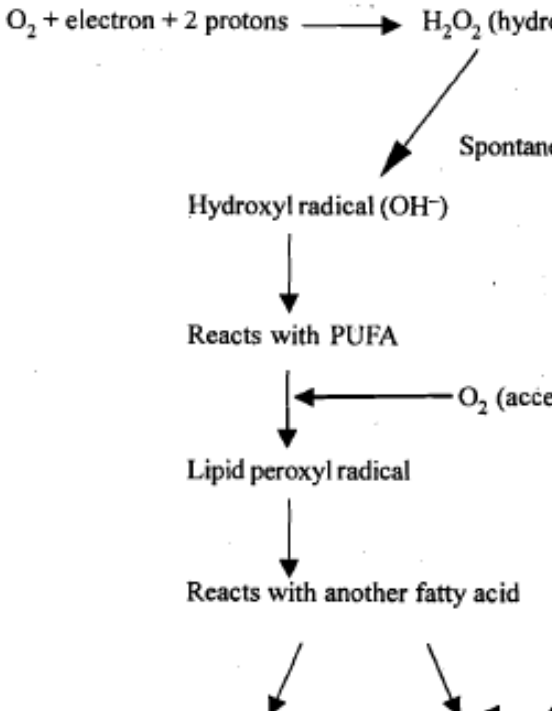


Figure 9.1 : Role of free radical in lipid peroxidation

From our discussion above, Lit is clear that free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed, these highly reactive radicals can start a chain reaction, like dominoes. To prevent free radical damage, the body has a defense system of antioxidants about which we shall learn in section 9.5. But, first let us get to know how are free radicals formed.

9.5 PRODUCTION OF OXYGEN FREE RADICALS

Oxygen free radicals are a normal by-product of the oxidative processes of the cell. Some free radicals arise normally during metabolism. Sometimes the body's immune system's cells purposefully create them to neutralize viruses and bacteria. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals. Some of the processes that generate free radicals include:

Electron transport chain — Free radicals are a by-product of the electron transport chain.

Dissociation of oxygen from haemoglobin generates superoxide radicals.

Certain environmental factors increase the generation of free radicals e.g. cigarette

smoke, exposure to high oxygen tension and ionizing radiation including

sunlight.

Phagocytic white cells generate oxygen free radicals to kill ingested bacteria and destroy other 'foreign bodies'. They can also secrete these reactive species into surrounding tissues (e.g. to kill large parasites) and this can cause significan damage to surrounding tissues. Injured and diseased tissue thus has high levels of free radicals.

9.6 PHYSIOLOGICAL MECHANISMS TO LIMIT FREE RADICAL DAMAGE

We now know that the free radicals are the normal by-products ofthe oxidative processes in cells and thus there are several physiological mechanisms whose specific role is to neutralize these free radicals and limit their tissue damaging effects. Figure 92 highl ights the antioxidant defense system. The primary enzymatic defenses include superoxide dismutase, catalase, peroxidase etc. Non-enzymatic defenses include glutathione, alpha tocopherol, ascorbate, beta-carotene, hydroquinones, flavonoids and phenolic acids

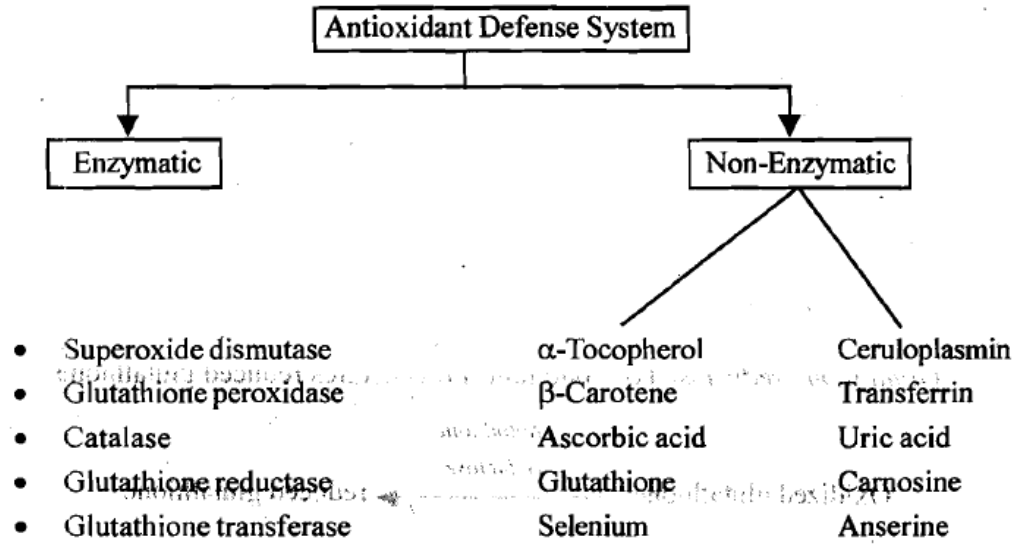


Figure 9.2 : Antioxidant defen'e system

There are a number of metal containing enzymes whose function is to scavenge and dispose of free radicals. Several essential nutrients are components of or cofactors for enzymes that are involved in free radical disposal. Some examples are listed below:

Zinc and Copper are components of the enzyme superoxide dismutase. which disposes of the superoxide radical by converting two superoxide radicals to hydrogen peroxide and oxygen.

Selenium is a component of the enzyme glutathione peroxidase. which neutralizes hydrogen peroxide and converts it to water and oxygen. It also converts peroxidized lipids into stable qnd harmless products thus

breaking the chairireaction of free radical production.

Iron is a component of the enzyme catalase which converts hydrogen peroxide to water and oxygen.

The enzyme glutathione reductase regenerates glutathione, which is oxidized by the glutathione peroxidase reaction mentioned above. This enzyme is a flavoprotein and utilizes a riboflavin derivative as a prosthetic group.

In addition to these enzyme systems, vitamins and other plant pigments (flavonoi s) have antioxidant properties and so have the capacity to scavenge free radicals e.g. vitamin E in ihe lipid phase and vitamin C in the aqueous phase.

Some of the substances in food that are known to have or probably have an antioxidant effect are:

a) the essential minerals- selenium, zinc, copper and iron

b) vitamins C and E

c) p-carotene and several other carotenoids, including lycopene (abundant in tomatoes), lutein (found in green vegetables), a-carotene, zeaxanthin and cryptoxanthin, and

d) other plant pigments. such as polyphenols found in some fruits, tea, olive oil and red-wine and the flavonoids found in grapes, nuts. oranges and strawberries.

Some of the mechanisms involved in the disposal of free radicals are summarized in Box 1.

Box 1: Mechanisms for the disposal of free radicals

Superoxide dismutase (*SOD*) (Zn containing) converts superoxide radicals to H_2O_2

$$\dot{O}_2^- + \dot{O}_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Glutathione peroxidase (Se containing) converts H_2O_2 to water

$$H_2O_2 + \text{reduced glutathione} \xrightarrow[\text{peroxidase}]{\text{Glutathione}} H_2O + \text{oxidized glutathione}$$

Glutathione reductase (B_2 containing) regenerates reduced glutathione

$$\text{Oxidized glutathione} \xrightarrow[\text{reductase}]{\text{Glutathione}} \text{reduced glutathione}$$

The enzyme *catalase* (Fe containing) converts H_2O_2 to water and O_2

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

Vitamin E can quench free radical when it is oxidized.

Free radical + Vitamin E ⟶ Water + oxidized Vitamin E

Lipid peroxyl radical + Vitamin E ⟶ stable hydroperoxide + oxidized Vitamin E

Vitamin E can be *regenerated* by a mechanism that involves Vitamin C

$$\text{Oxidized Vitamin E} \xrightarrow{\text{Vitamin C}} \text{Vitamin E}$$

Some important enzymatic and non-enzymatic physiological antioxidants, their location and properties are summarized in Table 9.1.

Table 9.1 : Important enzymatic and non enzymatic physiological antioxidants

Enzymatic antioxidants	**Location**	**Properties**
Superoxide dismutase (SOD)	Mitochondria and cytosol	Destroys superoxide radicals
Glutathione peroxidase (GSH)	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxide
Catalase (CAT)	Mitochondria and cytosol	Removes hydrogen peroxide
Nonenzymatic antioxidants	**Location / Nature**	**Properties**
Vitamin C	Aqueous phase of cell	Acts as free radical scavenger and recycles vitamin E
Vitamin E	Cell membrane	Major chain-breaking antioxidant in cell membrane
Uric acid	Product of purine metabolism	Scavenger of OH radicals
Glutathione	Nonprotein thiol in cell	Serves multiple roles in cellular antioxidant defense
Lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
Carotenoids	Lipid soluble antioxidants, located in membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
Bilirubin	Product of heme metabolism in blood	Extracellular antioxidant
Ubiquinones	Mitochondria	Reduced forms are efficient antioxidants
Metals ions sequestration: transferrin, ferritin, lactoferrin,		Chelating of metals ions, responsible for Fenton reactions
Nitric oxide		Free radical scavenger, inhibitor of LP

Despite low levels of enzymatic defenses against free radical, human blood plasma possesses highly efficient small molecular weight compounds which act as antioxidants.

NOTES

Some such as glutathione, ubiquinone and uric acid are produced by normal metabolism. Other examples of small molecular weight antioxidants are peptides such as camosine, anserine etc. The role of these small molecular weight antioxidants is highlighted next.

Uric acid has been shown to inhibit lipid peroxidation and scavenge free radicals.

Urate is very efficient scavenger of highly reactive and harmful oxygen species — namely hydroxyl radicals, superoxide anion, singlet oxygen and oxygenated heme intermediates in high iron valence states.

Ubiquinone is the only known fat soluble antioxidant synthesized by animal cells. It is believed to play a role in cellular defense against oxidative damage.

Carnosine and anserine are dipeptides found in skeletal muscles. They have been shown to decrease membrane lipid peroxidation. The antioxidant mechanism has been postulated as being caused by metal chelation and/ or free radical scavenging.

We have studied about the antioxidant defense mechanism to prevent free radical damage above. However, there might be situations that might increase damage by free radicals. What are these situations? Read and find out.

Situations that increase damage by free radicals

The following circumstances may increase the risk of disease due to free radical damage of cel lular components:

increased generation of free radicals beyond the capacity of the mechanib. IS for their Aisposal and repair of the damage that they induce, and

impaired capacity of the disposal mechanisms to handle any free radicals that are generated, for due to dietary deficiency of a key antioxidant nutrient.

9.7 FREE RADICAL IN HUMAN PATHOLOGY AND DISEASE

Overproduction of free radicals, as you may recall reading earlier, have been implicated in the etiology of a host of degenerative diseases including cardiovascular diseases, diabetes, cancer, Alzheimer's disease, retinal degeneration, ischemic dementia and other neurodegenerative disorders and aging. In addition, they also play a role not only in acute conditions, such as trauma, stroke and infection, but also in physical exercise and stress. Let us learn about the role of free radical, in

the context of cardiovascular disease, carcinogenesis and in physiological condition such as aging.

NOTES

Cardiovascular Diseases

Heart diseases we know are one of the main causes of mortality worldwide. Understanding and potentially control ling oxidative events as they affect cardiovascular disease (CVD) therefore, has the potential to provide enormous benefits to our population in health and lifespan.

A high saturated fat diet tends to raise the LDL cholesterol concentration and further cigarette smoking increases free radical production. Ifthe diet does not provide adequate antioxidants then scavenging free radicals becomes a problem. An unquenched free radical can react with LDL and can get converted to atherogenic oxidized form. With a continuated high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque, the symptom of atherosclerosis. Oxidized LDL is atherogenic, and is thought to be important in the formation of atherosclerotic plaques. Furthermore oxidized LDL is cytotoxic and can directly damage endothelial cells. Figure 9.4 presents a schematic representation of how free radicals might contribute to the risk of cardiovascular disease.

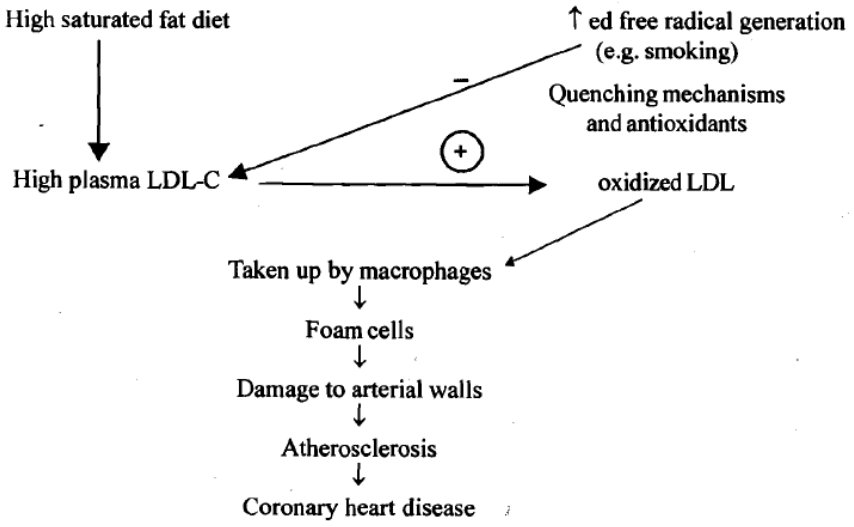


Figure 9.4 : A hypothetical scheme showing how free radicals might contribute to the risk of cardiovascular disease

Carcinogenesis

Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation and transformation, particularly in the past 10 years. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystem could lead to mutation, transformation and ultimately cancer.

Free Radicals and Aging

Strong experimental evidence supports the free radical theory of aging. An increasing number of diseases and disorders, as well as, aging process itself, demonstrate link either directly or indirectly to these reactive and potentially destructive molecules. Not much is known about the mechanism of aging and what determines lifespan. Leading theories attribute these to programs written in DNA and/or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

So what can be done to prevent the onslaught of the free radicals? It is simple. The body relies on the antioxidants, natural or diet-derived to deal with the onslaught. The last section in this unit highlights this aspect.

9.8 NATURAL AND DIET-DERIVED ANTIOXIDANTS

Our daily foods contain a wide variety offree radicals scavenging molecules. Vegetables, fruit, tea, wine are the products rich in natural antioxidant compounds. Fruits and vegetables are rich sources of antioxidants, flavonoids and vitamins. One can get suffcient quantities of these by consuming at least 4-5 servings of fruits and vegetables daily. The recommendation has been based on various epidemiological studies wherein it has been demonstrated that there is a diminished risk of chronic diseases with diets rich in fruits and vegetables. It has been hypothesized that antioxidants, found in fruits and vegetables, may be responsible for this protective effect.

Dietary antioxidants may contribute to the decrease of cardiovascular disease by reduction of free radical formation, as well as, oxidative stress in general, by protection ofLDL oxidation and platelet aggregation and by inhibiting synthesis ofpro-inflammatory cytokines. Epidemiological studies have shown thm a higher intake of these compounds is associated with a lower risk of mortality from cancer and coronary' heart disease.

9.9 LET US SUM UP

In this unit we learnt about antioxidants and free radicals. We studied that free radicals are highly reactive species that are normal by-products of metabolic processes and immune defense mechanism. These free radicals can react with DNA', membrane phospholipids, proteins and other cellular components. Oxidative damage caused by the reaction of free radicals with cellular components has been implicated in the aetiology of several chronic diseases and perhaps even in ageing.

To prevent free radical damage, the body has a defense system of antioxidants. Antioxidants we learnt are substances/molecules that work to reduce damage done to cells and DNA by free radicals, Several cellular enzymes quench free radicals

and these enzymes have riboflavin or a dietary mineral as an essential cofactor. Vitamins E and C and some plant pigments such as the carotenoids have inherent antioxidant and free radical disposal property.

NOTES

9.10 GLOSSARY

Antioxidant : components which combine with free radicals in the body and neutralize their damaging effect.

Free radicals : atoms or molecules with an unpaired electron.

Alzheimer's disease : a specific disease associated with the breakdown of nervous tissue in the brain, giving rise to a dementia in the patient.

Cytokines : powerful chemical substances secreted by cells.

Oxidized LDL : modification ofLDL molecule

9.11 CHECK YOUR PROGRESS

1) What are antioxidants?

2) What are free radicals? Briefly discuss their role in PUFA oxida-**tion.**

3) Enumerate the processes that generate free radicals.

4) Which minerals are associated with the enzymatic antioxidant defense system?

5) Name the vitamins which function as antioxidants.

6) Which vitamin regenerates oxidized tocopherol?

NOTES

UNIT

10

VITAMINS AND MINERALS

STRUCTURE

10.1 LEARNING OBJECTIVE

After studying this unit, you will be able to:

name the storage form of the fat-soluble vitamins,

identify the active form of the vitamin,

describe with chemical reactions the biological role of each vitamin in the body, and

discuss the biological role of different minerals in the body.

10.2 INTRODUCTION

We have already studied the chemical nature of the various vitamins earlier in Unit g. We have also come across vitamins when we have studied the various metabolic pathways (Units 6, 7 and 8). The chemical reactions occurring in our body catalyzed by protein molecules called enzymes. We have read about the characteristics of enzymes in Unit 4. We leamt that many enzymes are active only when other molecules called 'cofactors' are present. In most cases, these cofactors are vitamins. If these cofactors are absent, the enzymes will not act and may lead to the occurrence of disease. Thus vitamins give protection to the body against diseases.

In order to understand the functions and metabolism of vitamins in the body,

you must become very familiar with their structure and propertieS. Hence at this point you should revise Unit 3. You would have read in that section that vitamins may be classified as fat (or lipid) soluble or water soluble. We will continue with this classification in this unit and study about the metabolism of vitamins.

Since vitamins play a vital role in human metabolism, it is but natural that if these micronutrients are deficient/absent in our diets, metabolism will become deranged (disordered). This will lead to a diseased condition accompanied by clinical symptoms. The various vitamin deficiency diseases are discussed in the Course Public Nutrition, in Units 3, 4. The disease is cured (or prevented) only by restoring the intake of the vitamin to the diet.

The next part of this unit focuses on minerals. Minerals are inorganic elements and hence are distinctly different in chemical nature from the other major four nutrients (carbohydrates, proteins, lipids and vitamins), which are organic compounds. Most minerals (except sodium and potassium) form salts and other compounds that are relatively insoluble. Hence they are not readily absorbed and most ingested minerals are excreted in faeces. We shall learn about the metabolism of minerals in this unit.

10.3 VITAMINS

Vitamins, as we all know, are organic compounds required in very small quantities for a variety of biochemical functions and which generally cannot be synthesized in the body and therefore must be supplied in the diet. This definition clearly distinguishes vitamins from inorganic ions which are also essential for life. Further, unlike the macronutrients — carbohydrates, proteins and fats — which are required in large amounts, vitamins are needed in only minute amounts. Apart from some exceptions, all animal species require the major vitamins preformed in the diet due to inability to synthesiæ them from other food constituents. However, in some instances, certain precursors present in the diet are sufficient since these precursors can be converted to the vitamins in the body. One good example of this is the pigment ß- carotene, which you may recall reading, is converted to vitamin A in the body. The vitamins have been named using English alphabets (e.g. A, B, C etc.). In addition, some vitamins are also commonly known by specific names. A few vitamins are not by any alphabet but are referred to only by specific names. Further, we have also studied that they are classified into two categories, namely water-soluble and fat-soluble vitamins. In the subsequent section, we shall study about the different vitamins within each of the two groups.

The vitamins after absorption undergo changes in their chemical structure. They become functional only after undergoing these structural modifications. Hence this is referred to as the 'active form' of the vitamin. Let us get to learn about the metabolism of different water and fat-soluble vitamins. We begin with the fat-soluble vitamins.

10.4 FAT-SOLUBLE VITAMINS METABOLISM

Fat-soluble vitamins are apolar (lacking in ionizable groups), hydrophobic (water repelling or lacking in water affinity) compounds that can only be absorbed efficiently when there is normal fat absorption. This means that bile which helps in digestion and absorption of various dietary lipids is also necessary for absorption of these lipid- soluble vitamins. Hence, apart from low dietary intake, conditions affecting the digestion and absorption of the fat-soluble vitamins such as steatorrhoea (fatty diarrhoea) and disorders of the biliary system can ali lead to deficiency syndromes associated with that particular vitamin. They are transported in blood as constituents of lipoprotein molecules (molecules containing lipid plus protein) or attached to specific binding proteins.

The fat-soluble vitamins are stored in the body if their intake is more than the recommended amounts. This is a desirable feature. It ensures that these vitamins frotn storage become available to the body when dietary intakes are reduced. At the same time, excessive storage of the fat-soluble vitamins leads to signs oftoxicity, which in many instances can be extremely severe.

As you already know, there are four fat-soluble vitamins. These include— A, D, E and K. Now we will discuss each fat-soluble vitamin individually. Revise the structure of the vitamins as given in Unit 3 earlier, before going onto the biological role.

10.4.1 Vitamin A

Vitamin A occurs only in animal tissues. But many plant tissues contain substances (precursors) which can be converted to vitamin A in the body. These are the yellow or rcd coloured carotenoid pigments found in plants. These include carotenes (u, and 0') and the related compounds (cryptoxanthin) known as provitamin A.

You have already learnt earlier in Unit 3, that the active forms of vitamin A are:

retinol having an alcoholic group (CH20H)

retinal (also called retinaldehyde) having an aldehyde group (CHO), and

retinoic acid having a carboxylic group (COOH).

Vitamin A is stored as retinol palmitate (ester of rethol and palmitic acid). This ester is stored in the liver as a lipoglycoprotein i.e. fat-soluble vitamin + carbohydrate + protein. For transport to the tissues, the complex is hydrolyzed and the retinal is bound to a protein called retinal-binding protein (RBP) and secreted into the plasma. Retinoic acid is transported in plasma bound to albumin. Once inside the cell (extrahepatic), retinol is bound by another protein called cellular retinol-binding protein (CRBP). Let us next learn about the role and the mechanism Of action of vitamin A in our body.. Here we shall look at these functions

from the nutritional biochemistry point of view.

NOTES

Functions of Vitamin A

i) One of the functions of vitamin A which has been known for a long time is the role it plays in normal vision. To understand this function, you must revise at this point the physiology of vision discussed in the Course Applied Physiology . in Unit 10 (Physiology of Special Senses).

You may recall reading in this course that retina has two kinds of cells — rods and cones. Rods help us to see in dim light while cones function in bright light. Rods and cones contain light-sensitive protein called opsin which combines with ætinaldehyde as the prosthetic (additional) group to form a complex called rhodopsin (or visual purple) in rods and iodopsin in cones. Figure 10.1 illustrates light activation of rhodopsin. Let us now see the exact mechanism by which the rods ftnction in dim light.

You have seen that vitamin A exists in several isomeric forms. These isomers have the same number of atoms and the same kinds of groups, but differ in the arrangement of groups around the carbon atoms. Thus, retinaldehyde exists in two isomeric forms — all-trans or II-cis. In all-trans isomer, all the double bonds have groups on both sides of the double bond. In II-cis isomer, the double bond between carbons 11 and 12 has groups on one side of the double bond. rhodopsin, the amino acid lysine in the protein opsin forrns a complex with I I-cis retinaldehyde and this helps us to see in dim light. When we look at bright light, a number of complex biochemical changes take place and a nerve impulse is generated. There is isomerization of II-cis retinaldehyde to ll-trans retinaldehyde. This leads to a conformational (structural) change in opsin and different isomeric forms result. When bright light falls on rhodopsin within 10-15 seconds, II-cis retinaldehyde is converted to ll-trans retinaldehyde and the complex photorhodopsin is formed.

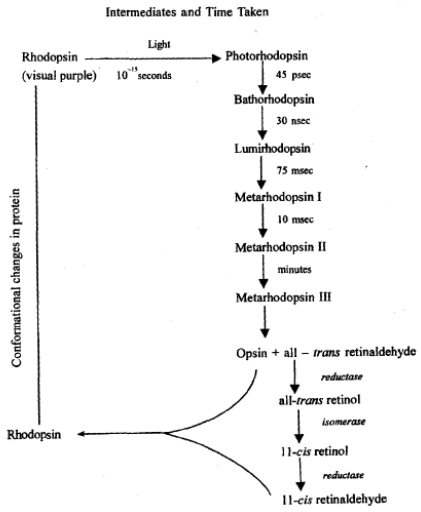


Figure 10.1: Light activation of rhodopsin

bathorhodopsin is formed within pico second of illumination. This is followed by a series of conformational changes and ultimately metarhodopsin Ill is formed. In the final step, this molecule is hydrolyzed to all-trans retinaldehyde and opsin. This is also called bleaching of rhodopsin. So bright light markedly depletes the stores of rhodopsin in the rods. Figure 10.1 explains light activation of rhodopsin just discussed. So what happens when thiS person leaves a well-illuminated room and enters a dimly- lit room. Obviously the person is unable to see. Why? Because rhodopsin in the retinal rods has been hydrolyzed into its two components (opsin and I I-cis retinaldehyde which is isomerized to all-trans retinaldehyde). For vision to be possible, two events have to take place. Firstly, all-trans retinaldehyde in the rods has to be first isomerizes to the specific II-cis isomer and then it has to combine with the protein opsin to form rhodopsin. This accounts for the well-known fact that an individual has difficulty in seeing, on entering a dimly-lit room from a well-lit place. After several minutes, during which time rhodopsin is synthesized, vision improves to the point that one may marvel at one's inability to see a short time previously.

Are you in the habit of entering a cinema hall after the show has started? If so, you would be very familiar with this situation where you feel totally blind on entering, but within a very short time you can see everything! This is called dark adaptation and the time taken to achieve it, is called the dark adaptation time.

For rhodopsin to be reformed, all-trans retinaldehyde is first converted to all-trans retinol which is then isomerized to II-cis retinol. This is then converted to II-cis retinaldehyde which combines with opsin. There is a loss of vitamin A in the photo-chemical (light induced) reactions involving rhodopsin. If the blood is not well supplied with vitamin A (as seen in vitamin A deficiency), the time required for rhodopsin synthesis is lengthened or the total synthesis may not reach optimum quantities. Under such circumstances, dark adaptation time is subnormal. When the condition becomes chronic, it will lead to night blindness. This visual process starts from rhodopsin and comes back to rhodopsin. Hence it is commonly called the visual cycle or rhodopsin cycle, as illustrated in Figure 10.2.

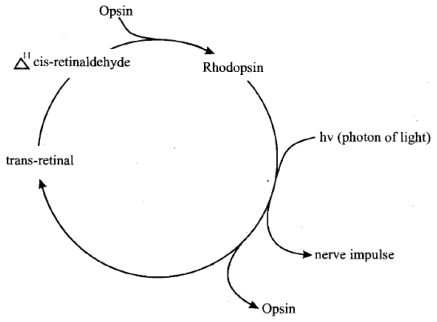


Figure 10.2: Visual cycle (Rhodopsin Cycle)

ii) Vitamin A takes part in the control of cell differentiation (forming different types of cells for different tissues) and turnover (growth). All-trans retinoic acid and 9-cis retinoic acid regulate growth, development and

tissue differentiation. They have different actions in different tissues. In this function, retinoic acid acts like a steroid hormone. It binds to nuclear receptors that bind to specific regions of DNA called response elements. This then causes expression of that gene i.e. synthesis of mRNA (transcription) takes place. Ultimately, this will lead to the synthesis of a specific protein required for growth.

iii) Retinoic acid participates in glycoprotein synthesis. This may partly explain how retinoic acid helps in promoting groMh and differentiation of tissues. It is believed that retinoyl phosphate functions as a carrier of oligosaccharides (carbohydrate containing a few monosaccharide units) across cell membrane. This oligosaccharide is used for synthesis of glycoproteins (carbohydrate + protein), which in turn are necessary for normal growth and also for mucous secretion.

Mucous is the lubricant coating the epithelial cells and it contains a glycoprotein, mucin. This is the reason why in vitamin A deficiency there is a reduction in mucous secretion and keratinization (thickening) of epithelial tissues of eyes, lungs, gastrointestinal and genitourinary tracts. Fissures (cracks) readily develop in such epithelial tissues and make them more susceptible to bacterial invasion and can lead to entry of microorganisms causing various infections particularly in children.

Good vitamin A status in the body prevents illness and hence vitamin A is called the anti-infective vitamin. Additionally, since vitamin A has an important role in differentiation of immune system cells, even mild deficiency leads to an increased susceptibility to infectious diseases. Synthesis of retinol-binding protein is also reduced during infections. This decreases the circulating vitamin and therefore there is a further impairment of immune responses.

iv) Retinol and/or retinoic acid is required for the synthesis of the protein transferrin which is needed for transporting iron in blood. This is the reason why vitamin A deficiency can lead to anaemia

v) Both retinoids and carotenoids have anticancer activity. This again is attributed to the role of retinoids in cell differentiation. vi) A-carotene is an antioxidant, effective at low oxygen concentrations. Compounds like unsaturated tatty acids with double bonds are very susceptible to oxidation (peroxidation) giving rise to what are called 'free radicals', about which you may recall reading in the last unit. These free radicals, as you know, are considered extremely toxic since they damage tissues and cause cancer. ß- carotene plays a role in trapping peroxy free radicals and preventing development of cancerous tissues.

Next, let us look at the mechanism of action of vitamin D vis-å-vis its functions.

NOTES

10.4.2 Vitamin D

You have already studied eäFlier that there are two forms of vitamin D called D2 (ergocalciferol) and D3 (cholecalciferol). They have a steroid structure containing cyclo pentano perhydro phenanthrene ring. Look up Unit 3, sub-section 3.4.2 for the structure. Vitamin and D3 are formed by irradiation of plant sterol, ergosterol and animal sterol 7-dehydrocholesteröl, respectively.

For vitamin D to be physiologically active, it has to be converted into the active forrn. In the liver, cholecalciferol (also called calciol) which has been synthesized in the skin or derived from food is hydroxylated to form the 25-hydroxy derivative called calcidiol. This is then bound to a vitamin D binding globulin which is the main storage form of the vitamin. On release from liver, it enters circulation and goes to the kidney. Here, calcidiol can undergo two reactions-

a) Hydroxylation in position 1 to form 1,25 dihydroxycholecalciferol (also called calcitriol). This is the active form of the vitamin.

b) Hydroxylation in position 24 to form 24,25 cholecalciferol (also called 24- hydroxycalcidiol), which is an inactive metabolite (intermediate). Ergocalciferol undergoes similar hydroxylation to give ercalcitriol (active form). These structures are given in Figure 10.3.

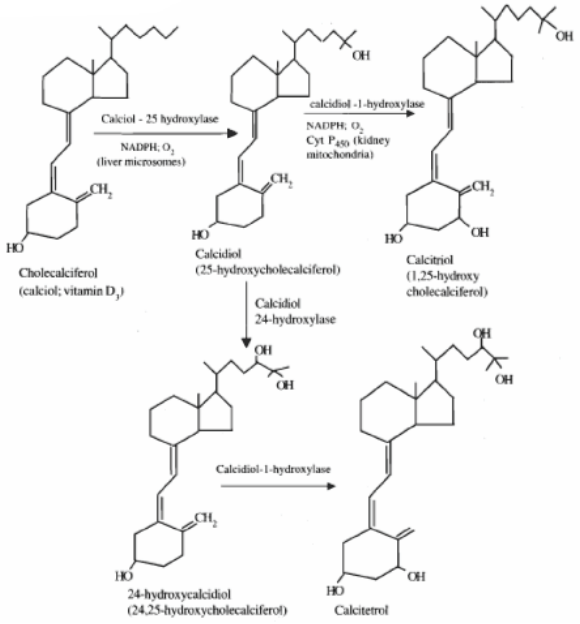


Figure 103: Metabolism of vitamin D

The active form is then transported to the target tissue for exerting its effect, which are discussed herewith under the heading-functions.

Functions of vitamin D

Vitamin D plays an active role in calcium metabolism. Thus it helps, in the control of calcium homeostasis i.e. maintaining normal calcium levels in the body. In fact, vitamin D not only regulates calcium homeostasis, but its own metabolism is in turn regulated by calcium homeostasis. Further, calcium metabolism is interlinked with the metabolism of phosphorus in the body. Hence, metabolism of vitamin D, calcium and phosphorus are all interconnected and interdependent as you will find out while learning about these three compounds

As already mentioned above, the principal function of the active form of vitamin D i.e. 1,25 dihydroxycholecalciferol (calcitriol), is to maintain the plasma calcium concentration at desirable level. It achieves this in three ways. It:

i) increases intestinal absorption of calcium,

ii) reduces excretion ofcalcium by stimulating resorption in the distal tubules (in the kidney), and

iii) mobilizes bone mineral by dissolving calcium deposited in the bone matrix.

How does vitamin D increase intestinal absorption of calcium? For this, the action of vitamin D is like that of a steroid hormone.

When calcitriol from blood enters the intestinal mucosal cell, it binds to a special protein (receptor) in the cell cytosol. This complex is transported to the nucleus, where it binds to a specific DNA. This stimulates the enzyme RNA polymerase II. As the name suggests, this enzyme brings about the synthesis (transcription) of a specific mRNA (messenger RNA). The mRNA is transported to the cytoplasm. Here it attaches itself to a ribosome and brings about the synthesis (translation) of a specific calcium-binding protein called calbindin. One atom of calcium is bound per molecule of protein. When food is digested, this protein enters the intestinal lumen, binds to calcium ions and transports the bound calcium. This then enters the blood stream and raises a lowered blood calcium level. The role of vitamin in calcium homeostasis is shown in Figure 10.4 (a-d). You would realize that calcitriol works along with parathyroid hormone (PTH).

This hormone is secreted by the parathyroid gland when there is a low serum calcium level (Figure 10.1, a). High PTH level promotes the formation of calcitriol (1 ,25 dihydroxycholecalciferol), which as explained above, promotes calcium absorption in the intestine. In bone, calcitriol and PTH act synergistically to promote bone resorption (demineralimtion). Finally, VTH and calcitriol inhibit calcium excretion in the kidney by stimulating calcium resorption in the distal renal tubules. When serum calcium levels are high, reverse reactions take place as shown in Figure 10.4 b. Production of PTH is blocked. Low P TH levels increase the formation of the inactive 24,25 dihydroxycholecalciferol. This leads to an inhibition of bone resorption while calcium excretion is enhanced. Thus we see that vitamin D activity is closely linked with that of PTH. Additionally, when there is a high serum calcium, the hormone calcitonin (CT) is secreted by the thyroid gland.

CT acts in the kidney and increases the excretion of calcium, thereby lowering serum calcium levels. CT also inhibits bone resorption. Figure 10.4 c and d illustrates the pathway of calcium metabolism when vitamin D is low and in excess respecitvely.

a) Low serum calcium

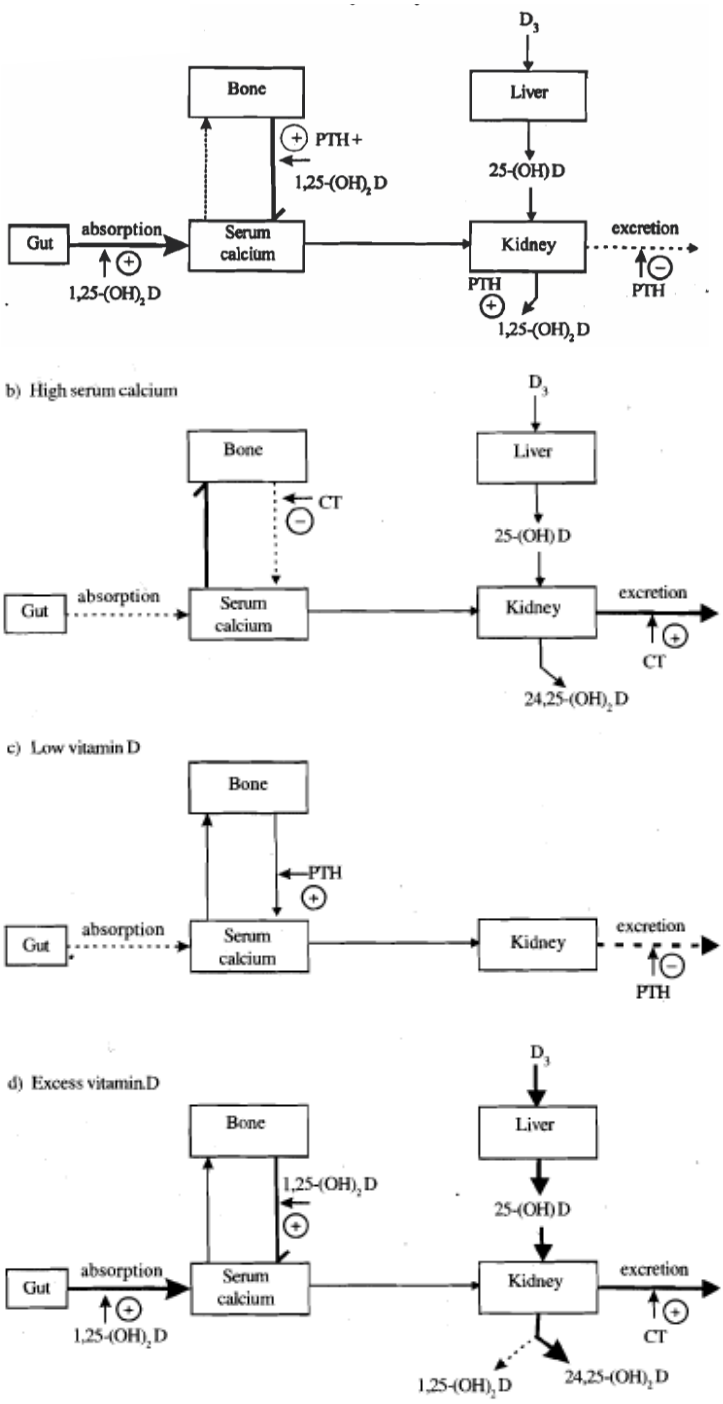


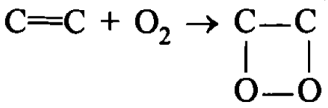
Figure 10.4: Vitamin D and •calcium homeostasis

10.4.3 Vitamin E

Vitamin E is the generic name for two families of compounds called the tocopherols and tocotrienols. Both of these are present in several isomeric forms. You have already learnt about the structure and properties in Unit 3. Look up these structures once again and then move on to the functions discussed herewith.

Functions of Vitamin E

Vitamin E is a natural antioxidant. Since vitamin E is fat-soluble, it accumulates in lipoproteins circulating in blood, cell membranes and fat deposits. Without vitamin E, cell membranes, active enzyme sites and DNA (nucleic acid) are less protected from free radical damage. Well, what are free radicals? As you have already learnt about them in the last unit, you know double bonds are very reactive. Hence, they can easily react with oxygen to form peroxides. Peroxides are formed when the double bond between two carbon atoms is replaced by two oxygen atoms as shown below:



The polyunsaturated fatty acids (PUFA) present in our body can readily undergo peroxidation (autoxidation) by oxygen due to the presence of double bonds. These peroxides undergo further series of reactions to generate free radicals. Examples of free radicals are ROO*, RO*, OH* as you have already learnt in the last Unit. In fact lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. The free radicals are extremely toxic to our tissues. The main function of vitamin E is as an antioxidant. It acts as a chain-breaker and traps free radicals in cell membrane and plasma lipoproteins. It reacts with the lipid peroxide radicals formed when there is oxidation of double bonds present in PUFA. This prevents their establishing a chain reaction. The product formed between tocopherol and the free radical is relatively unreactive. It ultimately forms non-radical compounds. In this way, free radicals are prevented from causing an injury to cells. After taking part in this antioxidant reaction, vitamin E molecules get converted into the tocopheroxyl free radical product in which vitamin E is in an oxidized form. You must be aware that vitamins are present in very minute quantities in our body (this is the reason why they are called micronutrients). Hence vitamin E molecules must be converted back into their original form which is the reduced form. Commonly, the tocopheroxyl radical is reduced back to tocopherol by reaction with vitamin C from plasma as we learnt earlier in Unit 9. In this way, vitamin E is regenerated to continue its antioxidant function.

When vitamin C reacts with vitamin E-free radical complex, the free radical is transferred to vitamin C forming vitamin C-free radical complex called monodehydro ascorbate-free radical (vitamin C is also called ascorbic acid). In this

process, vitamin E is regenerated in its original form. The monodehydro ascorbate-free radical then undergoes enzymic or non-enzymic reaction forming ascorbic acid and dehydroascorbic acid. Neither of these compounds is a free radical and hence non- toxic. In this way, vitamin E acts as a scavenger of free radicals, protecting unsaturated fatty acids (especially membranes) from peroxidation reaction. Figure 10.5 depicts these interactions.

While the presence and functions oftocopherols was known for sometime, information regarding tocotrienolS has been obtained in receht times. Tocotrienols occur only at very low levels in nature. In fact it is now said that the protective effect of tocotrienols as a potent antioxidant is significantly higher than that of tocopherols. Tocotrienols help to reduce cholesterol level and have anti-thrombotic effects and thus are helpful in preventing cardiovascular disease. They also demonstrate anti-cancer effects.

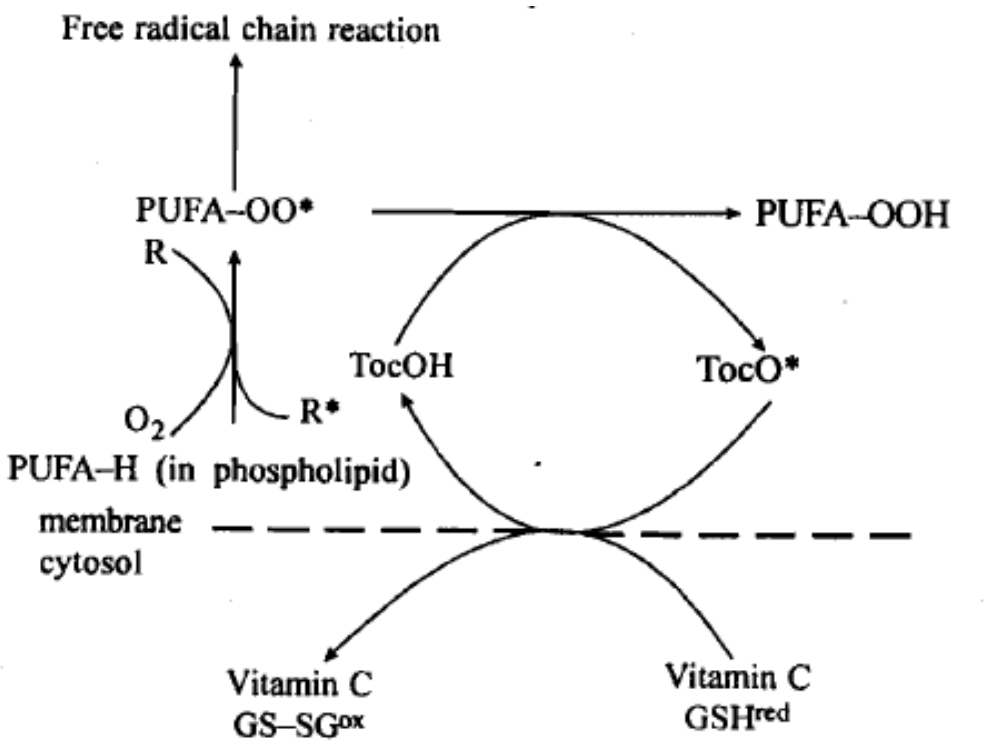


Figure 10.5: Biological role of vitamin E

10.4.4 Vitamin K

If you get a small cut, bleeding occurs. But very soon without even any effort on your part, the bleeding stops. This is because a blood clot is formed. Vitamin K is required for this process. In fact, this is how this vitamin got its name-K for Koagulation vitamin. Different forms of vitamin K have been described.

Functions of Vitamin K

Vitamin K is required for the synthesis of various proteins which are needed for the process of blood clotting. The proteins needed for blood clotting are secreted in an inactive form called precursors. They have to be converted into the active state, following which they help in the blood clotting process. Vitamin K is required for this conversion. Hence low levels of vitamin K result in decreased clotting of blood. The mechanism of this action has been most clearly understood in the case of one of the proteins (clotting factor) called prothrombin. Prothrombin is blood clotting factor II. Prothrombin is synthesized as an inactive precursor called preprothrombin. This, as already mentioned, is a protein molecule and it contains the amino acid glutamic acid units (residues) in its polypeptide chain. Conversion of preprothrombin (inactive) to prothrombin (active) requires carboxylation (introduction of COOH groups) of some of the glutamate residues. This is catalyzed by a carboxylase enzyme which requires vitamin K for its activity. The Y-Carbon (4th carbon) of glutamate residue is carboxylated to form Y-carboxy glutamate, as illustrated in Figure 10.6.

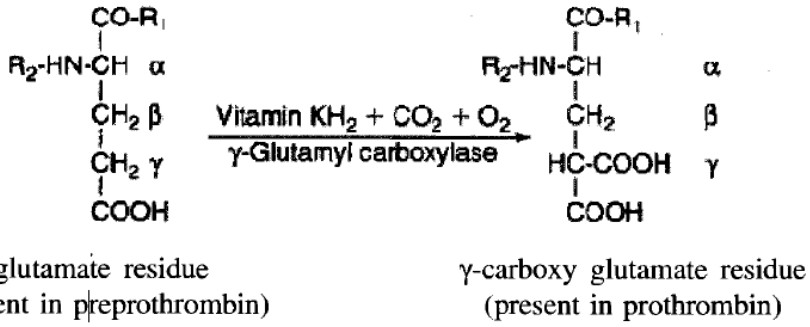


Figure 10.6: Vitamin K dependent carboxylation

The y-carboxy glutanlate residues are good chelators. What do we mean by chelators? Chelators are organic compounds which have the ability to bind to metal ions. In this case, they bind to calcium ions. The prothrombin-calcium complex thus formed is converted to thrombin. Thrombin in turn converts the plasma protein fibrinogen (blood clotting factor I) to Jibrin. Fibrin has a ~hesh-like structure and traps red and white blood cells and other plasma proteins to form the blood clot.

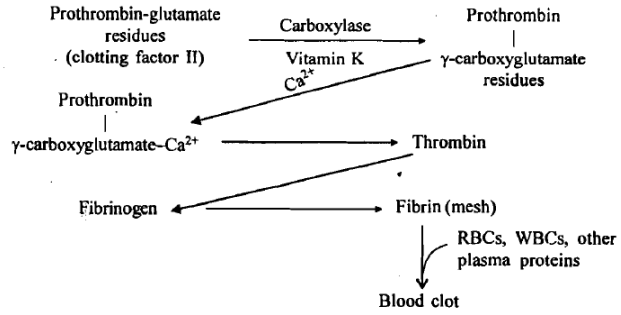


Figure 10.7: Biological role of vitamin K

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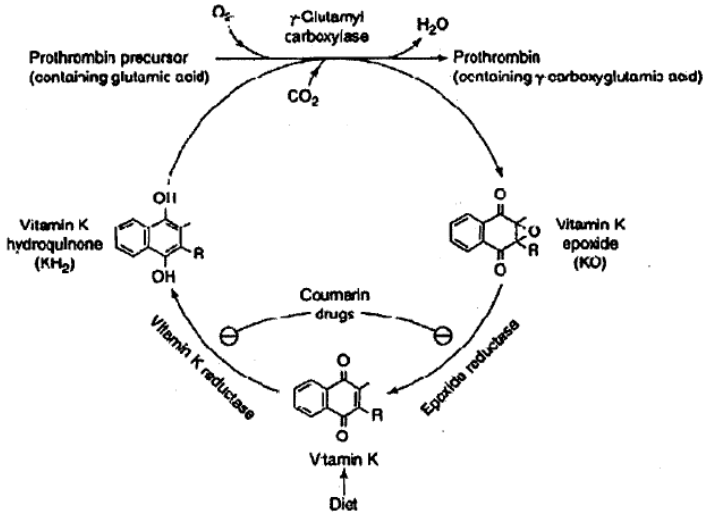
Next, we shall learn about the vitamin K cycle, which you will see is the salvage pathway for vitamin K.

Vitamin K cycle

Figure below depicts the carboxylation reaction and the vitamin K cycle, which is a salvage pathway for vitamin K. Vitamin K epoxide, the product of vitamin K in the glutamyl-carboxylation reaction (discussed above), is recycled to vitamin K hydroquinone by enzymatic reduction. For vitamin K to take part in the carboxylation reaction, it has to be present in the hydroquinone (having 2 OH groups) form. Following carboxylation, vitamin K gets converted to the epoxide structure catalyzed by the enzyme vitamin K epoxidase. In epoxide, 2 carbons share an oxygen atom. Further, the 2 OH groups get converted to double bonded oxygen. For blood clotting to continue, the hydroquinone form must be regenerated. This happens in a 2-step process. A first reduction results in loss of the. epoxide oxygen forming the quinone structure which retains the 2 double bonded oxygen atoms. This reaction is catalyzed by vitamin K epoxide reductase and needs any compound having 2 SH (sulffydryl) groups. The 2 H + atoms (1 each) are added to the 2 carbons which have lost the epoxide oxygen. The compound now will have —S—S— (disufide) group. Vitamin K quinone undergoes the second reduction reaction cataiyzed by vitamin K quinone reductase and vitamin K hydroquinone is regenerated, as can be seen in Figure.This reduction requires the participation again of a sulfhydryl compound to donate H + ions for forming the OH groups. In this way, a cyclic process occurs. Hence if the blood is not well supplied with vitamin K, blood clotting will become a slow process or may not reach completion.



Vitamin K is also needed for the synthesis of two calcium binding proteins in the bone. These are osteocalcin and bone matrix protein. Both contain glutamate residues which have to be carboxylated to form Y-carboxy glutamate residues in the presence of vitamin K. As discussed above, these residues have the property of chelating Ca^{2+} ions. This leads to deposition of calcium in the bone i.e. bone mineralization occurs.

10.5 WATER-SOLUBLE VITAMINS

As the name suggests, these vitamins are soluble in water. Thus they are distinctly different in property from the fat-soluble vitamins we just finished studying. Other major differences include the chemical structure and the biochemical role performed in the body. While the fat-soluble vitamins are chemically derivatives of isoprene units (as informed earlier in Unit 3 and also shown in Figure 10.9 here), there is no one common structure common to all the water-soluble vitamins.

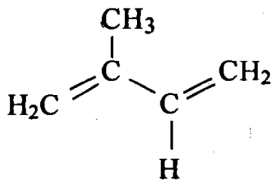


Figure 10.9: Isoprene unit

Fat-soluble vitamins are stored in the body, particularly the liver. Hence accumulation of excess amounts of these vitamins (hypervitaminosis) can occur which can lead to toxic effects. However. the other group of vitamins being water-soluble is readily excreted in the urine and hence are not stored in the body. Accordingly, a state of hypervitaminosis and toxic effects are generally not seen or are rare. But at the same time, deficiency of these vitamins occurs relatively quickly on an inadequate diet. There, the metabolic stores are labile (unstable) and depletion can often occur in a matter of weeks or months.

Another difference observed is the kind of biological role performed in the body. Each fat-soluble vitamin exerts a different type of effect, while a large number of water-soluble vitamins, like the so called B-complex vitamins have one type of activity, functioning as coenzymes in the body. Coenzymes are non-protein molecules which are required for the activity of many enzymes. You should revise the concept of coenzymes given in Unit 4.

The water-soluble vitamins, which we will be discussing, may be classified into 3 groups depending on their function:

energy-releasing water-soluble vitamins : Bp B2, B6, niacin, pantothenic acid and biotin

hematopoietic water-soluble vitamins : folic acid, vitamin B12

other water-soluble vitamins : ascorbic acid (vitamin C).

We begin our study of water-soluble vitamins with energy releasing water-soluble vitamins.

10.5.1 Energy-Releasing Water-Soluble Vitamins

As the name suggests, these vitamins are used in pathways which result in the

production of energy. All the six vitamins, included in this group, namely vitamins - Bp B? niacin, pantothenic acid and biotin, function as coenzymes. After absorption from the intestine, each vitamin undergoes certain modifications and is converted into a form called active form which is able to take part in the reaction. Generally, these active forms are synthesized in the liver. Since these six vitamins have a similar function Of energy generation, deficiencies of these vitamins produce a number of common and overlapping symptoms. Further because Of the central role these vitamins play in energy metabolism, deficiencies show up first in rapidly growing tissues. In many cases, the nervous tissue is also involved due to its high-energy demand. In several cases, the vitamins participate in a number of chemical reactions, thus it is impossible to pinpoint the exact biochemical cause of any given symptom. You will indeed find that these facts are true if you have a look at the diseases resulting from the deficiency of these vitamins. We begin our study of this group of vitamins with thiamin or vitamin B

Thiamin (Vitamin B_1)

Thiamin has a central role in.energy yielding reactions of particularly carbohydrate metabolism. The active form in which thiamin functions in the body, as you may recall reading earlier in Unit 3, is called thiamin pyrophosphate-TPP (also called thiamine diphosphate). The structure is given in Figure 10.10. Here two inorganic phosphate (Pi) groups are introduced into the thiazole ring.

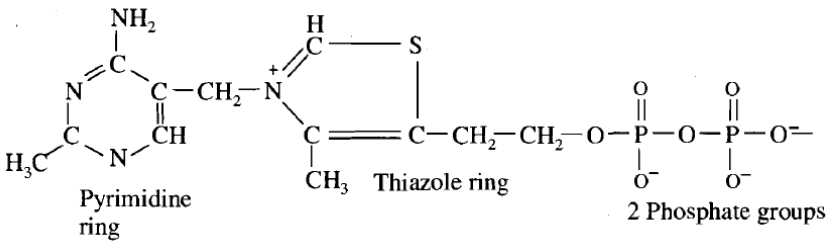


Figure 10.10: Active form of thiamin

Functions of Thiamin

Thiamin diphosphate (TDP) is the coenzyme for three multi-enzyme complexes that catalyze oxidative decarboxylation (oxidation and decarboxylation) reactions. These are:

Pyruvate dehydrogenase in carbohydrate metabolism

a-ketoglutarate dehydrogenase in the citric acid cycle

branched-chain keto-acid dehydrogenase in metabolism of branched-chain amino acids (leucine, isoleucine and valine).

You have already read about the first two reactions in Unit 6 and can refer to these sections for the details of these reactions. You have not read about the third enzyme- complex. It also has similar mechanism. It converts u-keto branched-chain acids (obtained when branched-chain amino acids undergo transamination)

to corresponding coenzyme derivatives. It is now known that enzymes of a multi-step pathway function not individually, but grouped as a complex. Further, within the protein complex is integrated the various required coenzymes. Thus, pyruvate dehydrogenase complex consists of 3 enzymes working sequentially — pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase — along with 5 coenzymes — TDP, lipoic acid, coenzyme A, FAD and NAD+. In all these reactions, finally N ADH is formed, which is oxidized in the mitochondrial electron transport chain with the formation of energy-rich ATP (as discussed in Unit 6).

Additionally, TDP also functions as the coenzyme for transketolase enzyme. You have again read about this in hexose monophosphate (HMP) pathway.

Riboflavin (Vitamin $B_{2)}$

This vitamin which functions as a coenzyme in energy-yielding metabolism has 2 active forms FMN (flavinmononucleotide) and FAD (flavinadenine dinucleotide). Enzymes which use FMN or FAD as coenzymes are called flavoproteins. What is their function? Let's find out.

Functions of Riboflavin

Riboflavin can function as a coenzyme because of its ability to undergo oxidation-reduction reaction, which is given in Figure 10.11. Hence, the overall reaction consists of the addition of 2 hydrogen atoms to the oxidized form resulting in the formation of reduced form of flavin:

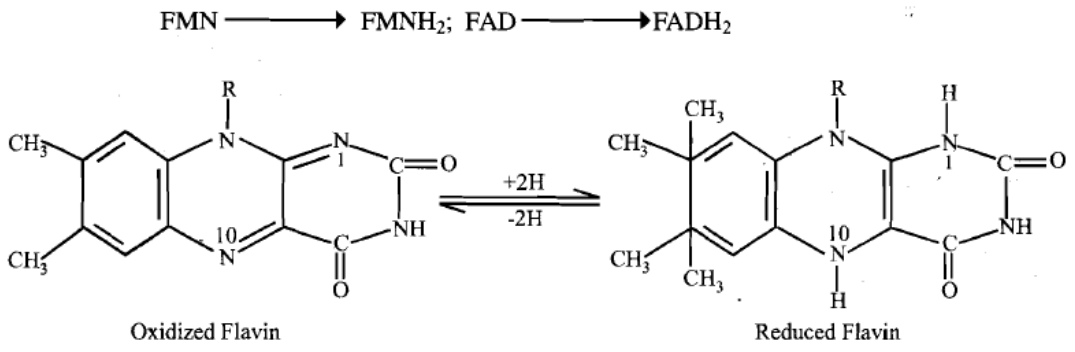


Figure 10.11: Oxidation-reduction property of flavin coenzym

After sometime, all the FAD will exist in the cell as FADH2. Since FAD is a coenzyme, it is present in minute quantities and hence has to be regenerated. Hence, it is oxidized in the mitochondrial respiratory chain. This oxidation, as you already know, is coupled to phosphorylation of ADP, ultimately forming 2 molecules of ATP. Thus participation of FAD in redox (oxidation-reduction) reactions results

in the release of utilizable energy as ATP. However oxidation of FMNH2 does not form ATP since it is directly oxidized by oxygen or some substrate in the cell (not coupled with phosphorylation). Majority of flavoproteins contain FAD as the coenzyme. Thus flavin coenzymes are hydrogen (electron) carriers in oxidation reactions.

Some of the flavoproteins contain metal ions such as iron (Fe3+) and molybdenum (M06+) and are known as metalloflavoproteins. These metals usually participate by being alternatively reduced and oxidized thus making the enzyme able to participate in oxidation-reduction reactions. In our study of metabolic pathways, we have come across several such reactions. Given below are a few examples:

FMN-

L-amino acid oxidase (in kidney) which functions in oxidative deamination of naturally occurring L-amino acids. It removes the amino group as NH3 and oxidizes the remaining portion to the corresponding keto acid. You may recall reading about this reaction in protein metabolism, Unit 8 in sub-section 8.2.2. Look up the reaction once again now.

NADH-dehydrogenase (metalloflavoprotein containing iron) functions in the electron transport chain. It passes H + ions (reducing equivalents) from NADH to ubiquinone (coenzyme Q or Q) (see electron transport chain in carbohydrate metabolism, Unit 6 section 6.11).

FAD

Succinate dehydrogenase functions in citric acid cycle oxidizing succinate to fumarate. Look up the citric acid cycle in Unit 6 section 6.5 for this reaction. The enzyme contains iron.

Acyl CoA dehydrogenase oxidizes coenzyme A ester of fatty acid at 2 and 3 forming A2 trans enoyl CoA.

Xanthine oxidase participates in the last two steps of catabolism of the purine bases adenine and guanine, finally forming uric acid.

Pyridoxine (Vitamin B_6)

It is a generic name for six compounds that have vitamin B6 activity. These are Pyridoxine, pyridoxal, pyridoxamine and their 5'-phosphates. The structures are given in Unit 3, sub-section 3.3.5 and in Unit 4, section 4.9. Look up the section now. It will help you understand the functions as discussed next, better.

Functions of Pyridoxine

Vitamin B6 functions as a coenzyme for many enzymes involved in amino acid metabolism. There are 2 such reactions which require vitamin B6. These are:

Transamination-transfer of 'NH2' groups from amino acids by enzymes called transaminases (or aminotransferases)

Decaboxylation-removal of 'COOH' group of amino acids as C02 catalyzed by the enzymes called decarboxylases.

Transamination- It is a process of combined deamination and amination according to which the amino group of one amino acid may be reversibly transferred to the keto acid of another amino acid, thus effecting amino acid-keto acid interconversion. The general reaction of transamination, is given in Figure 10.12. Do you recall reading about this earlier in Unit 8, sub-section 8.2.1. Thus, transamination represents a process of intermolecular (between molecules) transfer of amino groups without the splitting out of ammonia which is highly toxic to the nervous system (neurotoxin).

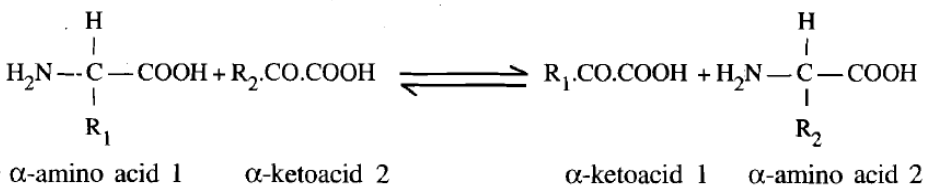


Figure 10.12: General reaction of transamination

The reaction is freely reversible. All amino acids except lysine, threonine, proline and hydroxyproline participate in transamination. Transaminases can function both in amino acid catabolism as well as biosynthesis. Different transaminases are known. Alanine- pyruvate aminotransferase (or alanine transaminase) and glutamate-a-ketoglutarate aminotransferase (or glutamate transaminase) present in most mammalian tissues catalyze the transfer of amino groups to pyruvate (forming alanine) or to a-ketoglutarate (forrning glutamate) as can be seen in Figure 10.13. Serum levels of aminotransferases are elevated in some disease states. Example myocardial infarction, viral hepatitis etc.

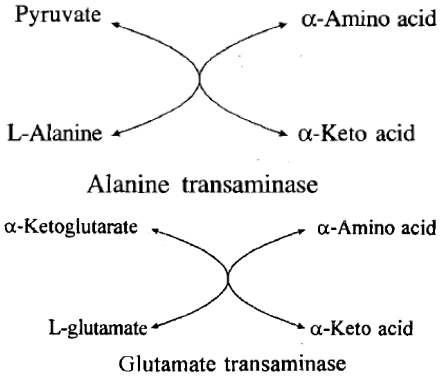


Figure 10.13: Action of transaminases

Approximately 60 specific reactions of amino acids involving pyridoxal phosphate

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have been discovered. Because B6 phosphate is involved in catabolism of amino acids, it is essential for energy production from amino acids and is considered as an energy-releasing vitamin.

Decarboxylation-It is catalyzed by enzymes called decarboxylases which require B6 phosphate as coenzyme. When amino acids undergo decarboxylation, the corresponding amine is formed as shown in Figure 10.14a. We have already studied about this reaction earlier in Unit 8 in sub-section 8.2.7. We learnt that the COOH (carboxylic) group is removed as C02. Thus serine forms ethanolamine (Figure 10.14b) while tyrosine forms tyramine (Figure 10.14c). Decarboxylation of 5-hydroxy tryptophan forms serotonin (10.14d) which functions as a neurotransmitter in the body. Neurotransmitter controls transport of nerve impulse. This may explain the irritability, nervousness and depression seen with mild deficiencies and peripheral neuropathy and convulsions observed in severe deficiencies

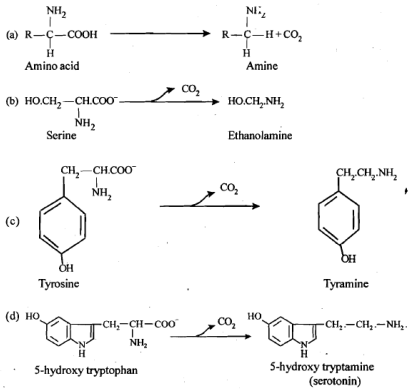

Figure 10.14 : Decarboxylation reaction

Another important enzyme which requires vitamin B6 phosphate is L-glutamate decarboxylase, converting glutamate to y-aminobutyrate (GABA) which is an imporünt intermediate in the body as can be seen in Figure 10.15. The enzyme occurs principally in brain tissue, where it functions as an inhibitory neurotransmitter.

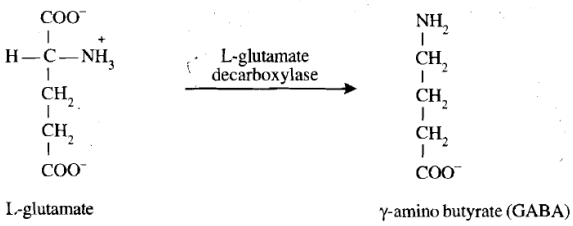

Figure 10.15: L-gluatamate decarboxylase reaction

Pyridoxal phosphate acts as a coenzyme in other physiologically important reactions. It is required for the synthesis of ö-amino levulinic acid, a precursor of heme. Hence B deficiency can result in anaemia.

Pyridoxal phosphate also participates in the synthesis of the sulfur containing amino acid cysteine. This is the reason why cysteine is a non-essential amino acid. This reaction is also important since deficiency of B 6 will result in increased level of homocysteine. High levels of homocysteine (hyperhomocysteinemia) appear to

be a risk factor for cardiovascular disease.

Pyridoxal phosphate is a part of the enzyme glycogen phosphorylase whiéh breaks down glycogen in the body. Hence decreased tolerance to exercise is associated with B6 deficiency.

Pyridoxal phosphate is one of the cofactors required for the conversion of the amino acid tryptophan to the coenzyme NAD^+.

Niacin

It is not a vitamin in the strictest sense of the word, smce some niacin can be synthesized from tryptophan. However conversion of tryptophan to niacin is very small (60 mg of tryptophan forming lmg of niacin) requires other vitamins like Bv B2 and B6 and it is also very inefficient on a marginal diet. Niacin is also called nicotinic acid or sometimes vitamin By Besides nicotinic acid, the diet also provides us nicotinamide which is the amide (CO.NH2) of nicotinic acid (amide is formed when amino group is substituted into carboxylic group). Nicotinamide is converted to nicotinic acid in the body.

Nicotinic acid is converted into 2 active forms by enzymes present in the cytosol of most cells. These are:

NAD+-nicotinamide adenine dinucleotide: and

NADP+ -nicotinamide adenine dinucleotide phosphate.

We have already learnt about these active forms and their structures in Unit 4, section 4.9. We suggest you open Unit 4 right away and revise the structures now, as this will help you understand the functions of -niacin as a coenzyme better.

Functions of Niacin

NAD+ and NADP+ function as coenzymes in several oxidation-reduction reactions. In Unit 4, we learnt that they are coenzymes of many dehydrogenases occurring both in the cytosol, as well as, within the mitochondria. They are therefore key components of many metabolic pathways. Generally, NAD+-linked dehydrogenases catalyze oxidoreduction reactions in oxidative pathways (e.g. citric acid cycle), whereas NADP+-linked dehydrogenases or reductases are often found in pathways concerned with synthesis (e.g. fatty acid biosynthesis).

Figure 10.16 shows the mechanism of oxidation-reduction of nicotinamide coenzymes. As you can see, the nicotinamide portion takes part in this mechanism (R represents the rest of the molecule). One of the hydrogen atoms is removed from the substrate (AH2) as a hydrogen nucleus with two electrons forming the hydride ion W. It is transferred to position 4 of the nicotinamide ring. This results in reorganization of the double bonds in the ring. The second hydrogen removed from the substrate remains free as a hydrogen ion.

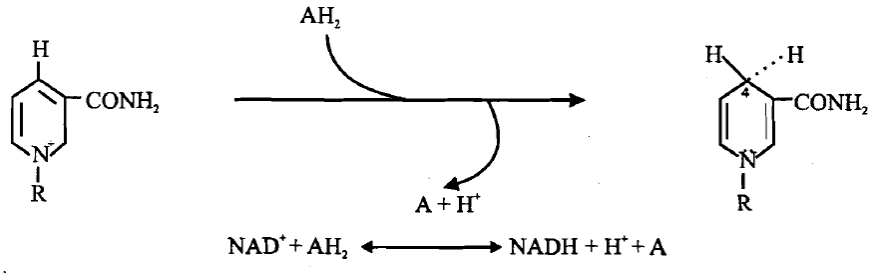

Figure 10.16: Mechanism of oxidation and reduction of nicotinamide coenzyme

You must have observed that the two coenzymes are always written with '+' superscript in the oxidized form. This is because the nitrogen of the nicotinamide moiety (residue) has 4 valencies. Normally nitrogen has 3 valencies. Under exceptional circumstances nitrogen can have 4 valencies when it is called quaternary nitrogen. But in such cases, the nitrogen carries a net positive charge. However on being reduced, the nicotinamide nitrogen has only 3 valencies. Hence NADH and NADPH are written without a '+' superscript.

In the section on metabolic pathways, you have come across several enzymes using NAD+ or NADP+ as the cofactor. Listed below are a few. We have also highlighted them earlier in section 4.9 in Unit 4 on enzymes. We shall not give the details of the metabolic pathway using these cofactors here since we have already discussed them earlier. You will have to go back to the appropriate sections in Units 6, 7 and 8 to get the details of these reactions.

NAD+

— glyceraldehyde-3-phosphate dehydrogenase (look up glycolysis in Unit 6)

— lactate dehydrogenase (look up glycolysis in Unit 6)

— dehydrogenase (look up citric acid cycle in Unit 6)

— L-3-hydroxyacyl CoA dehydrogenase (look up ß-oxidation in Unit 7)

— L-glutamate dehydrogenase (look up oxidative deamination of glutamate in Unit 8).

NADP+

— glucose-6-phosphate dehydrogenase

— 3-ketocyl reductase

— squalene synthetase

— malic enzyme .

In fact you should make your own exhaustive list of enzymes requiring NAD+ or NADP+ as cofactor after studying the various chemical reactions of the different metabolic pathways.

Pantothenic acid Vitamins and Minerals

Pantothenic acid forms coenzymes involved in energy metabolism. It is absorbed readily in the intestine. A phosphate group is attached forming 4-phosphopantothene. This is followed by the addition of the amino acid cysteine. This complex is ultimately converted to coenzyme A, the active form in which pantothenic acid functions in the body. Because of the presence of cysteine, coenzyme A has a free SH group. This is the reactive part of the molecule. It is customary to abbreviate the structure as CoA.SH . Let us now study the functions of this coenzyme.

Functions of Pantothenic Acid

The SH group can combine with carboxyl (COOH) group to form a thioester:

$$\text{R-COOH} + \text{CoA-SH} \longrightarrow \text{R.Co.S.CoA} + \text{H}_2\text{O}$$

(carboxyl group) (thioester)

This process of formation of CoA thioester (or coenzyme A ester) with the substrate is called activation of the substrate. More than 70 enzymes have been described till date that utilize CoA or its derivatives. Thus it is not surprising that coenzyme A is required for the metabolism of fat, protein and carbohydrate. You have already come across many of these reactions, a few of which are listed below:

pyruvate dehydrogenase complex (in pyruvate oxidation in Unit 6)

u-ketoglutarate dehydrogenase complex (in citric acid cycle in Unit 6)

acyl-CoA synthetase (thiokinase) (in ß-oxidation of fatty acid in unit 7)

pantothenic acid is also a component of an interesting heat-stable protein of low molecular weight called acyl carrier protein (ACP). It plays an important role in the biosynthesis of fatty acids

The last vitamin in this list is biotin. Let's look at its function as a cofactor in our body.

Biotin

Biotin acts as a cofactor binding to its specific enzyme protein.

Functions of Biotin

It is intimately associated with carboxylation reactions in which carbon dioxide is added to the substrate. Hence these reactions are called carbon dioxide fixation reactions. The enzymes are called carboxylases. There are 3 such reactions in the

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body. You have already come across all these 3 reactions in metabolic pathways. All biotin catalyzed reactions also need ATP as a source of energy and magnesium ions.

The enzymes which function with biotin are:

pyruvate carboxylase,

propionyl CoA carboxylase,

acetyl CoA carboxylase,

Carbon dioxide takes part in the form of bicarbonate (HCOi) ion as shown herewith:

$$CO_2 + H_2O \longrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

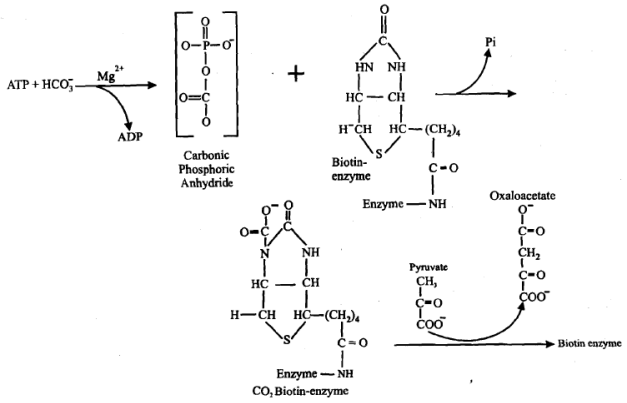

Figure 10.17: Biological role of biotin

In the first step, C02 is added to biotin catalyzed by the enzyme biotin carboxylase forming C02- biotin enzyme. This step is called activation of C02. The C02 is then transferred from biotin to the substrate, pyruvate forming oxaloacetate catalyzed by transcarboxylase and the biotin-enzyme is regenerated. Thus the carboxylase is a multienzyme complex containing three components on one polypeptide chain, comprising a biotin carrier protein, biotin carboxylase and a transcarboxylase. Many such multienzyme complexes are known e.g. ß-oxidase complex and fatty acid synthase comple,

10.5.2 Hematopoietic Water-Soluble Vitamins

Hematopoietic water-soluble vitamins, as mentioned earlier, are those vitamins which are required for the synthesis of red blood cells in the body.

Folic acid

Folacin, as you may already know, is the generic form of folic acid and related substances, having folic acid activity. The simplest form of folic acid, as you can see, has one glutamic acid in the structure. It occurs in diet as polyglutamate derivatives with 2 to 7 glutamic acid residues. These are absorbed into the intestinal mucosal cells. Here, the extra glutamate residues are removed by conjugase, which is a lysosomal enzyme. Folic acid is then reduced by the enzyme folate reductase to

dihydrofolic acid by the addition of 2H+ ions to the pterin ring. Two more ions are added to this ring by dihydrofolic reductase forming tetrahydrofolic acid. Figure 10.18 presents the synthesis of tetrahydrofolic acid. Note that these 2 reactions require NADPH as the cofactor. Glutamate residues are again added to tetrahydro (H4) folate and these polyglutamate derivatives are the active form. Folic acid is also stored as tetrahydrofolate polyglutamate in liver.

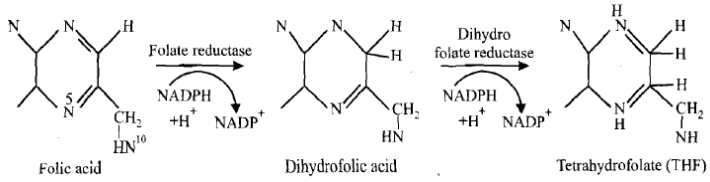

Figure 10.18: Synthesis of tetrahydrofolate

Functions of Folic Acid

Folate functions in what is referred to as 'one-carbon metabolism '. It transfers one-carbon groups (groups containing only one carbon). These include methyl (CH3), methylene (CH2), methenyl (CH), formyl (CHO) and formamino (HC=NH) groups. These groups are obtained from various compounds in the body and get attached to N^5 or N^{10} positions forming one-carbon derivatives which are shown in Figure 10.19. All these forms are metabolically interconvertible. N^5-formyl THF also known as folinic acid is a stable form which is used for therapeutic purposes.

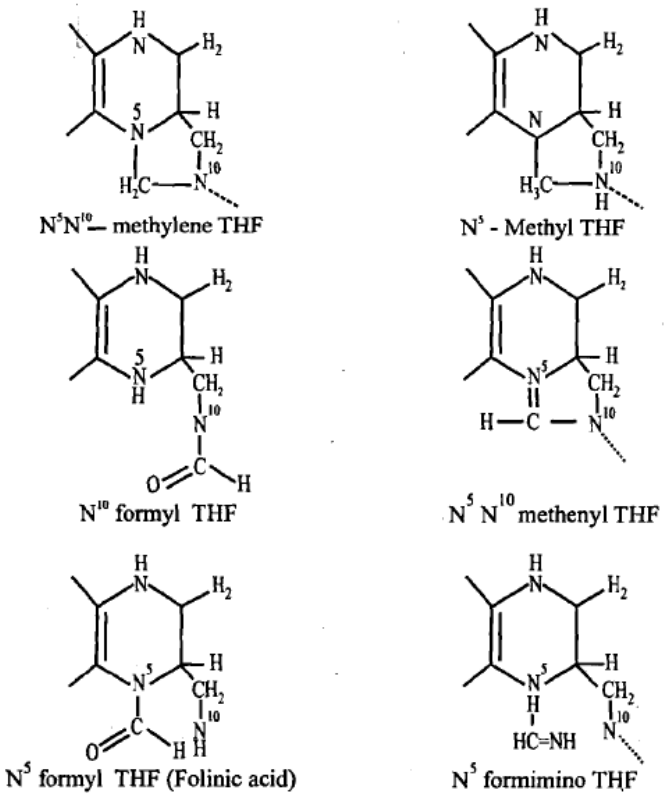

Figure 10.19: One-carbon units attached to tetrahydrofolate

Metabolic reactions using one-carbon derivatives are discussed below.

- Serine-glycine interconversion-

  This is a freely reversible reaction which also requires vitamin B_6 phosphate.

- Synthesis of methionine-

  This reaction requires vitamin B_{12}

- Synthesis of thymidine monophosphate (TMP)-

  TMP is a nucleotide having the pentose sugar, deoxyribose as you have already seen in Unit 2. This reaction is of great physiological significance since TMP is a precursor of DNA synthesis (since it is a constituent of DNA). It is also required for erythrocyte formation. Hence deficiency of folic acid causes anemia. In this reaction, dihydrofolate is formed which is then reduced to active THF by dihydrofolate reductase.

- Catabolism of histidine-

  THF takes part in breakdown of the amino acid histidine to glutamate and is itself converted into the N5-formimino derivative.

- Synthesis of purines-

  N10-formy1 THF is the source of the carbon in position 2 of the purine ring while N5 N10 methenyl THF is the source of the carbon in position 8 of the purine ring (as we have seen in Unit 8. Thus folic acid is required for purine biosynthesis. Purines, as you know, are the constituents of the 2 nucleic acids — DNA and RNA.

Cyanocobalamin (Vitamin B_{12})

Vitamin B12 has been found only in animals and microorganisms and is absent in the plant kingdom. As you have already seen in Unit 3, sub-section 3.3.6, it has a very big structure. The absorption of vitamin B12 requires a highly specific glycoprotein (a compound containing carbohydrate and protein) called the intrinsic factor. After absorption, the vitamin is bound by a plasma protein called

transcobalamin. The vitamin is stored in the liver bound to transcobalamin. We shall look at the functions now.

Functions of Cyanocobalamin

Vitamin B_{12} functions as a coenzyme. For this, the cyanide' (CN-) radical is removed forming cobalamin. This is then substituted with other groups forming the coenzyme. There are two active forms:

- Methylcobalamin As the name suggests, a methyl group is present. It catalyzes the conversion of homocysteine to methionine. This reaction also requires THF, as can be seen in Figure 10.20.

- Deoxyadenosyl cobalamin : Here the cyanide radical is replaced by adenosine (ribose + adenine) moiety (residue). It acts as a coenzyme for methyl malonyl CoA isomerase which converts L-methyl malonyl CoA to succinyl CoA as shown in Figure 10.20. This reaction makes it possible for the body to use propionate as a substrate for gluconeogenesis (as we studied in Unit 6). In vitamin B deficienc this reaction cannot take place and THF cannot be released. Hence all the THF is trapped as methyl THF. This is called the 'folate trap'. It leads to impaired purine and pyrimidine synthesis resulting in impaired DNA synthesis. This, in turn, prevents cell division and formation of the nucleus of new erythrocytes and the presence of immature erythrocytes in blood. Ultimately there is anaemia.

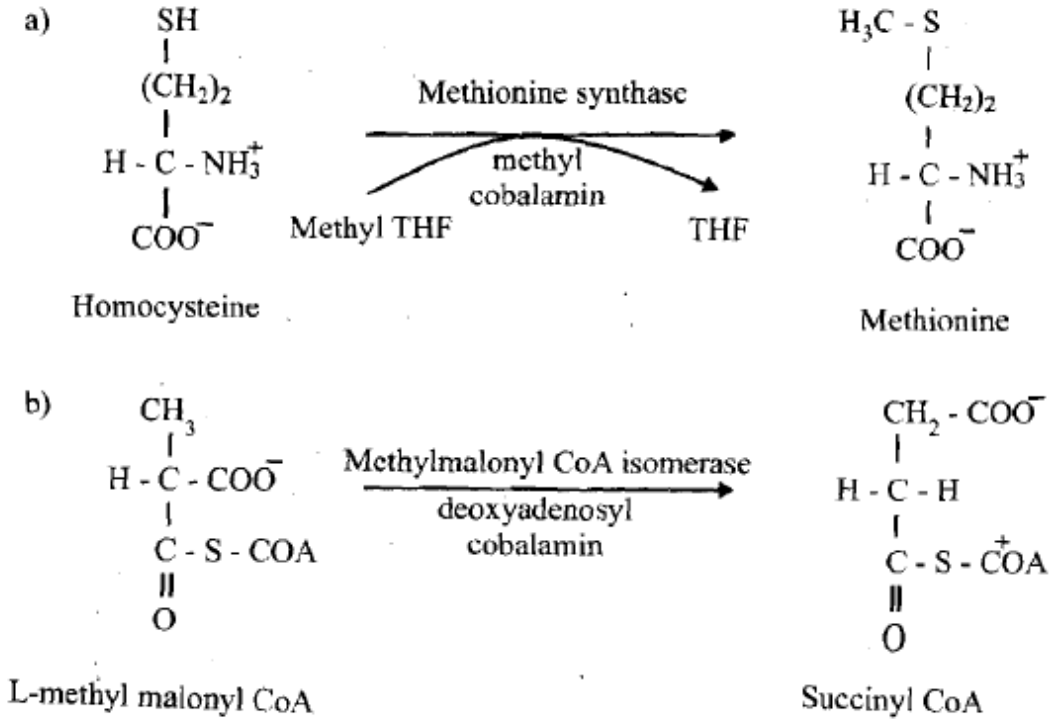

Figure 10.20 : Action of B_2 coenzymes

Next, we shall look other water soluble vitamins, mainly the role of vitamin C

10.5.3 Other Water-Soluble Vitamins

We have looked at the metabolic role of energy releasing and hematopoietic water-soluble vitamins above. One another important water soluble vitamins which has an important metabolic role in our body is vitamin C, which is discussed next. With this, we shall come to an end on our discussion on vitamins. So get started

and get to know about the metabolic role of vitamin C.

Ascorbic Acid (Vitamin C)

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It is necessary to have vitamin C in the diet for primates including human beings and other animals like guinea pig, bats, fishes etc. Most other species can synthesize ascorbic acid from glucose in a multistep pathway. Human beings do not have the enzyme for the last step and hence must have preformed vitamin in the diet. Look up the structure of ascorbic acid, which you have studied in Unit 3 sub-section 3.3.9. You will notice that it possess an enediol group on carbons 2 and 3 (enediol means 2 OH groups between a double-bonded carbon system which you have come across in reducing properties of sugars in Unit 1). Enediol group is a very strong reducing agent. Hence, the biochemical role that ascorbic acid plays is related to it being a good reduciné agent. In many of these processes, ascorbic acid does not participate directly in the reaction, but is required for maintaining the metal cofactor participating in that reaction in a reduced form. This metal cofactor is necessary for the activity of the enzyme catalyzing that reaction. These enzymes are hydroxylases containing copper (Cu+) or iron (Fe2+). During hydroxylation, Cu* (cuprous ion) is oxidized to Cu2+ (cupric ion) and Fe2+ (ferrous ion) to Fe3+ (ferric ion). Reduction back to Cu + or Fe2+ specifically requires ascorbate, which in the process, is oxidized to dehydroascorbic acid. In the body both forms are biologicalty active since dehydroascorbic acid (oxidized form) can be converted back to the reduced form (ascorbic acid) by reducing agents like glutathione (a tripeptide containing the 3 amino acids-glutamate, cysteine and glycine). Thus vitamin C may not function like a typical coenzyme, reacting directly with the substrate.

Important reactions of ascorbic acid are given herewith:

- hydroxylation of basic amino icids, lysine and proline required for the synthesis of the protein collagen. Collagen is a constituent of connective tissues. Thus ascorbic acid is important for maintenance of normal connective tissue and wound healing, since the connective tissue has to be first synthesized for a wound to heal. Vitamin C is also necessary for bone formation, since bone tissue has an organic matrix containing collagen as well as the mineral content. Collagen is a component of the ground (basic) substance surrounding capillary walls and hence vitamin C deficiency is associated with capillary fragility.
- synthesis of the hormones norepinephrine and epinephrine (formerly called noradrenaline and adrenaline) from tyrosine

  Dopamin ⟶ norepinephrine ⟶ epinephrine
- catabolism of tyrosine where p-hydroxy phenyl pyruvate is oxidized (hydroxylated) to homogentisic acid catalyzed by p-hydroxy phenyl pyruvate hydroxylase. Here again vitamin C helps to maintain copper in the reduced state which is required for the maximal activity of thc enzyme. The next step also requires vitamin C where homogentisic acid is oxidized to maleylacetoacetate

by homogentisate dioxygenase, which is a ferrous (iron) containing enzyme. We will find both these chemical reactions in the Unit 12 where we Will discuss the inborn errors of metabolism.

- bile acid formation from cholesterol requires ascorbic acid in the very first step catalyzed by 7a-hydroxylase. You have already learnt about this in Unit 7.
- bile acid formation from cholesterol requires ascorbic acid in the very first step catalyzed by 7a-hydroxylase. You have already learnt about this in Unit 7.
- absorption of iron is significantly enhanced by the presence of vitamin C. As a dietetic student, this must be very clear to you. Dietary iron when present in ferrous (Fe2+) form is more soluble and hence easily absorbed as compared to the ferric (Fe3+) form. Vitamin C being a reducing agent helps to keep iron in the reduced state.
- steroidogenesis (synthesis of corticosteroids) in the adrenal glands. This has several steps involving hydroxylation reactions in which ascorbic acid may be required. In fact the adrenal cortex (where the synthesis takes place) contains large amounts of vitamin C.
- in addition to the above reactions, ascorbic acid may act as a general water-soluble antioxidant in the body. Thus it may act in converting the oxidized form of tocopherol (vitamin E) to the reduced form in the membrane. We have already read about this earlier in sub-section 10.3.3.

Besides the above functions, there is still much controversial debate going on regarding the beneficial effects of high doses (mega doses) of vitamin C in preventing the occurrence of common cold or reducing the duration of its symptoms.

10.6 MINERALS - AN INTRODUCTION

Minerals constitute a wide and complex group occurring widely in the earth's crust. In fact, the list of minerals is continuously increasing with new minerals being discovered.

It had been realized very early in the development of nutritional science that certain minerals were essential for normal health and proper functioning of the body. The importance of minerals has been documented much before nutritional requirement for vitamins became universally accepted.

Minerals are inorganic elements and hence are distinctly different in chemical nature from the other major four nutrients (carbohydrates, proteins, lipids and vitamins), which are organic compounds. Another major difference is the type of function performed. Each mineral in the body has an exclusive function. On the other hand, Nutritional Biochemistry each of the other four nutrients has generally at least one common function in the body:

- carbohydrates — best source of energy
- proteins — structural component

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- fats — high energy fuel
- vitamins — cofactors.

Although in respect to their amounts, the mineral elements are relatively minor components of the tissues, they are essential to many vital processes, such as:

- provide a suitable medium for protoplasmic activity
- many salts are important in acid-base equilibria and the osmotic control of water balance, and
- certain tissues like bones and teeth have a high mineral content which accounts for their hardness and rigidity.

The metabolism of food minerals does not involve the radical changes of molecular form that are found in carbohydrate, protein and lipid metabolism. You have already learnt in detail the extensive metabolic reactions undergone by the above three classes of nutrients in the body. The positive mineral ions such as calcium, magnesium, potassium and sodium taken in our food as salts of organic or inorganic acids or associated with proteins or lipids after absorption are associated with just such negative ions in the body. For example, the calcium ion may partly become associated with plasma protein or protoplasmic -protein or organic (or other inorganic) acids. The phosphate radical can be converted into any of a great number of organic esters in the blood or tissue cells. The positive and negative mineral ions, not used as body structural units, undergo in general no greater chemical alteration than exchange of partners during metabolism and excretion.

Most minerals (except sodium and potassium) form salts and other compounds that are relatively insoluble. Hence they are not readily absorbed and most ingested minerals are excreted in faeces. Mineral absorption often requires specific carrier proteins. These proteins can chelate (combine) with minerals and carry them into the intestinal epithelial cell. In fact, the synthesis of these proteins serves as an important mechanism for control of mineral levels in the body. Even transport in the body and storage in the tissues require specific binding proteins. Excretion of most minerals is accomplished by the kidneys. Many minerals are also secreted into the digestive juices and bile and subsequently lost in the faces.

You may be already familiar with the classification of minerals. A simple classification is also presented herewith for your reference.

Classification of Minerals

One commonly used system of classification is based on the amount of mineral required/present in the body. Accordingly, there are two major classes:

Principal or macrominerals (macroelements)

Trace or micro minerals (microelements)

Minerals may also be classified on the basis of their function(s). In this unit we are only looking at the biological functions. It would be very meaningful to have an

overview of the functions. This is given in Table 10.1. You may have already learnt about these functions in the Advance Nutrition Course, in Units 9 and 10. Here, we shall look at these functions from the biochemical point of view.

Table 10.1: Classification of minerals according to their functio

Function	Minerals
Structural function	Calcium, phosphorus, magnesium
Involved in membrane function: principal cations of extracellular and intracellular fluids, respectively	Sodium, potassium
Function as prosthetic groups in enzymes	Iron, zinc, copper, cobalt, selenium, molybdenum
Regulatory role or role in hormone function	Calcium, chromium, iodine, magnesium, manganese, sodium, potassium
May occur in foods and known to be toxic in excess	Aluminium, arsenic, antimony, fluoride, lead, mercury etc

We will now go on to the specific functions of each mineral. We start with macrominerals.

10.6.1 Macrominerals

As the name suggests, these minerals are required in comparatively larger amounts in the diet, generally greater than 100 mg/day. It follows that they are also present in greater amounts in the body. There are 7 essential principal elements — calcium, magnesium, sodium, potassium, phosphorus, sulfur and chlorine. They constitute 60-80% of all the inorganic material in the body. Let us get to know about them.

Calcium

Calcium is present in the body in larger amounts than any other cation (a positively charged particle), as much as 1200 g in a 70 kg adult. Almost all of it, about 99% is in bones and teeth. The other 1% is in blood, lymph and soft tissues.

Most skeletal calcium is deposited in the form of a crystalline complex called hydroxyapatite Bone also contains considerable amounts of non-crystalline calcium phosphates and carbonates, as well as, small amounts of other salts. These minerals comprise about 50% of the total skeletal muscle, the remaining mass consists of an organic matrix of proteins, glycoproteins and proteoglycans on which the calcium salts are deposited. Glycoproteins and proteoglycans are proteins combined with different types and proportions of carbohydrates. Even though bones and teeth are rigid structures, they continuously undergo remodeling in which calcium and,

phosphorus are removed each day and replaced by new calcium and phosphorus molecules. Let us learn about the functions of calcium in greater details.

Functions of Calcium

- In higher mammals, the most obvious role of calcium is structural or mechanical. Calcium is intimately related with bone development and teeth formation. The organic matter is first formed by the bone cells and then deposition of bone mineral takes place in the form of mainly calcium phosphate. In the tooth, enamel is the most highly calcified part. Without bone development, there can't be growth, so calcium is intimately related with and essential for growth.
- Blood clotting process requires calcium ions for conversion of prothrombin to thrombin. This involves conversion of glutamate residues to Y-carboxy glutamyl residues These residues serve as high affinity binding sites for Ca2+ and hence can chelate calcium ions. Thrombin then acts on fibrinogen to form fibrin. This forms a mesh and entangles the blood cells to ultimately form the blood clot. Besides prothrombin (also called Factor II), several other proteins of the blood clotting system (Factors V II, IX and X and Proteins C and S) are activated by calcium ions. Each contains between four and six Y-carboxy glutamyl residues which chelate calcium ions and so permit the binding of the blood clotting proteins to membranes. We have already learnt about the formation of Y-carboxy glutamate residues in functions of vitamin K in this unit in sub-section 10.3.4. Milk clotting also requires Ca2+. This is important in the digestion of milk in infants.
- Calcium ion is directly related to muscle contraction and nerve impulse transmission.
- Membrane permeability is decreased by calcium and capillary permeability is increased by calcium.
- Calcium is a mediator of hormone action. Hormone carries message to the cell to carry out certain chemical reactions. Hence, hormone-is called the first messenger. On reaching the cell, the communication of this message from the hormone to the intracellular compartment requires intermediary molecules. And in many cases, calcium functions as this molecule. Hence calcium is called the second messenger. You will read more about this in the next unit on hormones.
- Several enzymes and proteins are regulated by calcium. A few examples are given below:
  - adenylyl cyclase
  - Ca2.+-dependent protein kinases
  - phospholipase A2
  - phosphorylase kinase
  - calbindin
  - calsequestrin
  - calmodulin
  - troponin C

You have come across these enzymes in metabolic pathways. It would be a good idea to locate them in the appropriate section. Calbindin is a protein which binds

with calcium and helps in the intestinal absorption of calcium. ~alse~uestriisn a gain a calcium binding protein which helps in calcium storage. Activation of many enzymes by calcium is mediated by calmodulin. Calmodulin has four calcium binding sites and full occupancy of these sites leads to marked conformational (structural) changes which allow calmodulin to activate enzymes. Troponin C is a muscle protein.

Let us now look at the mechanism of action of calcium.

Mechanism of action of calcium

The mechanism of action of calcium is linked to its ability to bind with a large number of cell proteins. Let us see how.

The calcium ion (Ca2+) is able to form coordination bonds with up to 12 oxygen atoms. This makes calcium nearly unique among all cations in its ability to fit neatly into the folds of the peptide chain. By binding with oxygen atoms of glutamic and aspartic acid residues, calcium stiffens the protein molecule-and fixes its tertiary structure. You may recall studying about the tertiary'structure of proteins in Unit 2.

Binding of calcium to a large number of cell proteins results in the activation of their unique functions. These proteins range from those involved with the cell movement and muscle contraction to nerve transmission, secretion and even cell division. In most of these situations, calcium acts as both a signal .transmitter from the outside of the cell to the inside and an activator of the functional proteins involved. In fact, ionized calcium is the most common signal transmitter in all biology. We will learn about the messenger function of calcium later in the next unit.

- Influencing acid-base balance of blood. Phosphorus is present in compounds like disodium hydrogen phosphate (Na2HP04) and sodium dihydrogen phosphate (NaH PO) which act as buffers and maintain pH of blood within the desirable limits.
- Constituent of various physiologically important molecules:

  Nucleic acids, DNA and RNA — These compounds, as you may already know, are important from the stand point of genetics and protein synthesis. You have already learnt about these compounds in Unit 2, under section 2.8 — structure of nucleic acids.

  A variety of coenzymes— NAD+, NADP+, FMN, FAD. You are already aware of a large number of reactions in various metabolic pathways which require these coenzymes for the enzymes to become functional.
- Regulation of metabolic pathways. It is very essential to have mechanisms for regulating all the metabolic pathways. One such mechanism is by covalent modulation. This involves phosphorylation-dephosphorylation of enzymes. While certain enzymes become activated in the phosphorylated form, others are active in the dephosphorylated form. A number of hormones depend upon

phosphorylation for their activation.

So now you realize how important this compound is. Next, let us learn aboutmagnesium.

Magnesium

Occurrence of magnesium is widespread in both plant and animal tissues. The total body concentration of magnesium in a healthy adult is about 20-28g. Somewhat more than half (53%) of the total body magnesium is in bone as magnesium phosphate Mg3 (P04)2 and almost all the rest is in soft tissue. Magnesium is the most abundant divalent mineral cation in cells and is second only in electrolyte quantity to monovalent potassium. Most intracellular magnesium exists in bound form.

Functions of Magnesium

- Being part of the bone tissue, magnesium has an important structural function.
- Magnesium is involved in more than 300 essential metabolic reactions. Magnesium ion (Mg2+) forms complexes with a variety of organic molecules having biologic activities. The binding of functional groups is in descending order: phosphate > carboxylate > hydroxyl, in terms of both relative importance and binding affinities. Mg2+ is essential for many enzymatic reactions and has two general interactions as highlighted herewith-

    a) Mg2+ binds to the substrate, thereby forming a complex with which the enzyme interacts as in the reaction of the kinase enzymes with MgATP, and

    b) Mg2+ binds directly to the enzyme and alters its structure and/ or serves a catalytic role as in the case of enzymes like exonuclease, topoisomerase and RNA and DNA polymerases. Thus magnesium plays an important role in glycolysis, the citric acid cycle, gluconeogenesis, lipid metabolism, amino acid metabolism and nucleic acid metabolism. The transketolase reaction involving thiamin and the transfer of CO to biotin in carboxylation reactions requires Mg2+. Glutathione, a key intracellular antioxidant has Mg2+ requirement for its synthesis.
- Magnesium is important in energy metabolism since ATP the 'free-energy' currency for all cellular processes exists in all cells primarily as MgATP.
- Magnesium, calcium and some other cations react with hydrophilic polyanionic carboxylates and phosphates of the various membrane components to stabilize the membrane and thereby affect fluidity and permeability.
- Cyclic AMP (cAMP) which acts as a second messenger in hormone action is formed from MgATP and the enzyme adenylyl cyclase which is activated by magnesium through its two binding sites. Hence magnesium is required for hormone action.

Before we move any further, let us assess what we have learnt so far, by answering the questions given in check your progress exercise 4.

10.6.2 Microminerals

Microminerals occur in living tissues in minute amounts. In fact early workers who were unable to measure their precise concentrations with the methods then available, frequently referred to them as occurring in 'traces'. For this reason they came to be known as 'trace elements'. Other popular names used include; 'minor elements' or 'oligo-elements' (from the Greek 'oligos' meaning scanty). The microminerals are required in amounts less than 100 mg/day.

The trace elements may be subdivided into 3 groups:

- Essential trace elements — iron, copper, iodine, zinc, manganese, cobalt, molybdenum, selenium, chromium. These have been shown to be dietary essentials vital to the enzymic processes of the living cell.
- Possibly essential trace elements — nickel, tin, vanadium, cadmium, silicon, barium, strontium. They exhibit some metabolic activity, revealed by both in vivo and in vitro studies.
- Non-essential trace elements — aluminium, boron, lead, mercury, fluorine, arsenic.

By far, the greatest numbers are apparently inert in the sense that they have not been shown to perform any vital function or to affect living processes in the concentrations in which they normally occur. However, there is no permanency to membership, particularly in the second and third groups. When the concept of trace elements was elucidated, only iron and iodine were classified as dietary essentials. As physiological and analytical techniques improved, distinctive functions of various mineral elements were identified and these were included in the first group. This is an on-going process. Further it is pertinent to mention here that a reverse thought process has occurred in India with respect to fluorine. This element has long been considered as essential for development of teeth. It still is in the western countries. However, extensive research in India has shown that fluorine occurs in extremely high concentration in our soil and water. Accordingly it may be consumed in large amounts leading to toxicity conditions including excessive mineralization in bones and teeth, leading to irreversible crippling and loss of teeth. Additionally, excessive fluoride can cause extensive damage to epithelial lining of tissues. Hence fluorine definitely belongs to the non-essential group. The 20-30 trace elements of this group that occur in living tissues, a considerable number, notably aluminum, silver, lead, gold, bismuth are believed to be acquired and accumulated as environmental contaminants and their presence merely reflects the contact of the organism with its environment.

We begin our study on each of these microminerals, with iron.

Iron

Iron was a familiar metal in most of the ancient civilization of the Mediterranean coast and hence led to its early medicinal use. In the earliest manuscript of Egypt, rust was prescribed as an ointment to prevent baldness.

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Iron is one of the most abundant elements in the earth's crust. However, the body of an adult weighing 70 kg contains only 4-5 g of iron i.e. 0.006-0.007% of total body weight. Hence it is classified as a trace element. Most of the body iron exists in complex forms bound to protein i.e. as heme compounds .(porphyrin+iron), notably haemoglobin (in blood) and myoglobin (in muscle) or as non-heme protein bound compounds such as ferritin (storage form of iron) and transferrin (transport form of iron). Additionally, it is also present in various enzymes either as heme or as non- heme iron. However this constitutes less than 1% of the total body iron.

We are all familiar with the functions of iron. Let us refresh our knowledge regarding this important mineral.

Functions of Iron

- The major use of iron is for oxygen transport by haemoglobin. The importance of this function cannot be overstressed since oxygen is central to respiration. The process of respiration essentially involves oxidative reactions. Hence oxygen must be supplied to all the tissues. When oxygen enters the lungs, it combines with the iron containing protein, haemoglobin, present in RBCs forming the complex oxyhaemoglobin. On reaching the tissues, the complex dissociates releasing oxygen. Simultaneously, C02 formed as a waste product in catabolic reactions in the tissues, enters RBC and combines with haemoglobin to form haemoglobin Vitamins and Minerals carbamate. On reaching lungs, haemoglobin carbamate dissociates, releasing C02, which is exhaled.
- Oxygen requirement of muscle cells is high because of high level of metabolic activity. To ensure availability, oxygen is stored combined with iron-containing muscle prötein, myoglobin. When strenuous exercise markedly lowers the oxygen content of muscle cells, myoglobin releases oxygen for mitochondrial synthesis of ATP, permitting continued muscular activity. This could well be the reason for fatigue experienced in iron deficiency condition of anaemia. Insufficient iron results in the decreased synthesis of haemoglobin and myoglobin with consequent effects.
- Iron, as a pan of heme, is a constituent of enzymes peroxidase and catalase which catalyze oxidation-reduction reactions. Physiologically, these two enzymes are very important since they bring about degradation of toxic peroxide molecules as presented herewith.

$$H_2O_2 + AH_2 \longrightarrow 2H_2O + A$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

  Accumulation of peroxides can lead to generation of free radicles (ROO*, RO', OH*), about which you learnt in the last unit, which in turn can disrupt membranesand could cause cancer and atherosclerosis.
- Iron, as a part of heme, is present in various cytochromes. Cytochromes

are iron-containing heme proteins. Cytochromes are components of the mitochondrial electron transport chain-b, cp c, a and a3 (cytochrome oxidase). Here they function as carriers of electrons from flavoproteins on the one hand to cytochrome oxidase on the other. Cytochromes are also found in other locations e.g. the endoplasmic reticulum contains cytochromes P 450 and m. In liver microsomes, these two molecules have an important role in detoxificatio (converting toxic compounds into non-toxic intermediates).

- Iron is also present as iron-sulfur combination (FeS) in non-heme enzymes like flavoproteins (metalloflavoproteins) and with cytochrome b. The sulfur and iron are thought to take part in the oxidoreduction mechanism, with the iron atom undergoing oxidoreduction between Fe2+ and Fe3+. Succinic dehydrogenase, an enzyme of the citric acid cycle, contains Fe:S and oxidizes succinate to fumarate. Another metalloflavoprot in enzyme is NADH dehydrogenase containing Fes and FMN. It oxidizes the reduced NADH of the respiratory chain and passes reducing equivalents to ubiquinone or coenzyme Q or also simply called Q. The enzyme aconitase which functions in the citric acid cycle also contains iron-sulfur cluster at its active site. It intimately links the iron Content of cells with energy production through oxidative phosphorylation, both in carbohydrate and lipid metabolism. When there is sufficient iron in mitochondria or in cytosol, aconitase will contain four atoms of iron and four atoms of sulfur. This is the enzymatically active fonn of aconitase. However, in iron-deficiency state, the iron-sulfur complex is modified with only 3 atoms of iron. In this form, aconitase is enzymatically inactive, but becomes the iron regulator protein (IRP). It inhibits synthesis of apoferritin (iron-storage protein), but stimulates the synthesis of 6- aminolevulinic acid synthase (enzyme involved in synthesis of heme) and transferrin (iron-transport protein) receptor. In this way, iron uptake and heme synthesis are regulated at the cellular level to meet the needs of oxidative phosphorylation via the citric acid cycle.

Going through the discussion above, we realize what important biological role iron has in the body. Though required in small amounts, it performs a few major functions. Let us next study about iodine.

Iodine

You are all aware that it is important to use iodized salt i.e. salt to which the microelement iodine is added. In 997, Parliament passed law making sate of iodized salt mandatory. In fact extensive advertisements were carried in print and audio- visual media to educate the common person about the need to use iodized salt. Like iron, iodine too, has had a long and fascinating history in human medicine. The ancient Greeks are reputed to have used burnt sponges successfully in the treatment of human goitre. Iodine is present in abundance in sponges. Use of salts of iodine for the treatment of goitre is documented as early as 1820. The element was discovered in the thyroid gland in 1895.

It is clear that insufficient quantities of iodine in the diet results in the disease

goitre. Goitre, as we all know, is characterized by an enlarged thyroid gland, which becomes visible in the region of the neck. Goitre occurs when soil and water have low levels of iodine and consequently the food (plant and animal sources) and water we consume are not able to meet our daily requirement. Hence goitre will become prevalent over a geographical area and this is referred to as 'endemic goitre'. In our country too, endemic goitre has been an exceedingly serious public health problem. This is associated with cretinism, feeble-mindedness and gcneral physical and mental degeneration. Cretinism is a condition originating in fetal life or early infancy due to severe thyroid deficiency, characterized by stunting of physical and mental development. Since iodine is a micronutrient, the very small amounts required can easily be met by using iodized salt. Surprisingly, recently the government has not made sale of iodized salt mandatory, leaving the choice of selection to the consumers.

The healthy human adult body contains 15-20 mg of iodine, of which about 70-&0% is in the thyroid gland. The thyroid gland which weighs only 15-25 grams possesses a remarkable power for concentrating (accumulating) iodine. The amount of iodine in the gland is closely related to the iodine intake. The content may be reduced to lmg or less in the iodine-deficient enlarged thyroid. The enlargement of thyroid (hyperplasia), then occurs as a compensatory mechanism to utilize as efficiently as possible the decreased amounts of iodine.

What is the role of iodine, which makes it so essential? Let's find out.

Functions of Iodine

Iodine is an integral part of the hormone secreted by the thyroid gland. In fact, there are two compounds secreted by the thyroid gland which are physiologically active- triiodolhyronine (T3) and tetraiodothymnine (T4 or thyroxine). Functions of iodine are essentially the functions of the thyroid hormone, which will now be discussed in detail. All cells of the body with the possible exception of adult brain and testes are target cells for thyroid hormone. Thyroid hormones, bind to specific high-affinity receptors in the target cell nucleus. T3 binds with approximately 10 times the affinity of T4. The role of thyroid hormone include

- Thermo genesis and oxygen consumption — increased heat production and oxygen consumption are characteristics for most tissues responding to thyroid hormone (brain, testes and spleen excluded). Thus thyroid hormone activity is intimately related to the basal metabolic rate (BMR). Much of the energy utilized by a cell is for driving the Na+/K+-ATPase pump. Thyroid hormones enhance the function of this pump by increasing the number of pump units. Since all cells have the pump and virtually all cells respond to thyroid hormones, this increased utilization of ATP and the associated increase of oxygen consumption via oxidative phosphorylation could be the basic mechanism of thyroid hormone function. besity in some patients has been suggested to be

the result of decreased energy and heat production due to diminished ATPase activity.

- Metabolic effects of and T4 — these include alterations in metabolism of carbohydrates, proteins, lipids, electrolytes and water. Thyroid hormone effects on carbohydrate metabolisin involve increased intestinal absorption of glucose balanced by increased glucose utilization. The net effect is one of hyperglycemia and an abnormal glucose tolerance curve.

  Thyroid hormones enhance general protein synthesis and cause a positive nitrogen balance. Thyroid hormones induce or repress proteins by increasing or decreasing gene transcription mechanism. Thyroid hormones act in conjunction with pituitary growth hormone (GH) as the principal anabolic agents during growth and -in maintaining protein stores. T3 enhances transcription of the GH gene so that more GH is produced. Synergistic effects of the two can be demonstrated on protein synthesis in the liver. Very high concentrations of T3 inhibit protein synthesis and cause negative nitrogen balance.

  Cholesterol blood levels are high in hypothyroidism and the high levels can be decreased with thyroid hormone administration. There is an increased lipid utilization with thyroid hormone.

  Retention of water and electrolytes in the hypothyroid state can be reversed by thyroid hormone administration.

  Altering the thyroid hormone state in the human causes well known changes in the central nervous system — in nerve and muscle function, in the gastrointestinal tlact and in the vascular system. The skin is a good indicator of the thyroid state. In hyperthyroidism, the skin is smooth, warm and moist as a result of vasodilation. In contrast, the skin is cold and has a rough texture due to vasoconstriction in the hypothyroid state. The characteristic accumulation of fluid and mucopolysaccharides with the resulting puffiness (pitting edema) of the skin gives rise to the adult hypothyroid state called myxedema.

  The heart reflects the changes in thyroid state, having a slow rate and decreased blood flow in the hypothyroid condition. Hyperthyroid state has an effect on the heart with increased heart rate and cardiac hypertrophy.

- Thyroid hormones are known to be important modulators of developmental processes. The important role of thyroid hormone in human development is apparent in cretinism, a condition brought about by thyroid deficiency during the prenatal period resulting in serious detriment in both mental and physical development in the growing child.

Zinc

Evidence of essentiality of zinc was demonstrated in plants in 1869 and in animals in 1934. Zinc is the most abundant intracellular trace element. It has

been estimated that the newborn contains approximately 60 mg zinc. During growth and maturation. the zinc concentration of the human body increases to approximately 30 mcg/g. The adult total body zinc content ranges from about 1.5 g in women to 2.5 g in men. Zinc is present in all organs, tissues, fluids and secretions of the body. Zinc is primarily an intracellular cation, with well over 95% of total body zinc found within the cells. Zinc is associated with all organelles of the cell, but about 6() to 80% of the cellular zinc is found in the cytosol. Among the major organs and tissues in a normal adult man, skeletal muscle and bone contain approximately 57% and 29% respectively. Skin and liver contain 6% and 5% zinc respectively. While brain contains 1 .50/o of total body zinc, kidneys, heart, hair and blood plasma contain minute quantities (4% of total body zinc). Cornea is the tissue with the highest zinc concentration in the body.

In biological systems, zinc is virtually always in the divalent state (Zn2+). Zinc readily complexes to amino acids, peptides, proteins and nucleotides. Zinc has an affinity for thiol (SH) and hydroxy (OH) groups and for ligands containing electron-rich nitrogen as a donor. Zinc does not exhibit any direct redox chemistry (i.e. loss and gain of electrons). Let us look at the role of zinc.

Functions of Zinc

Zinc is an important trace element required for normal maintenance of human health. It is involved in a multitude of diverse catalytic, structural and regulatory functions.

- Over 30^{O} zinc metalloenzymes have been described to date, including carbonic anhydrase, phosphatases, alcohol dehydrogenase, glutamate dehydrogenase, both DNA and RNA polymerases, fructose bisphosphatase etc.
- A critical function of zinc is its role in the structure and function of biomembranes. Loss of zinc from the membrane results in increased susceptibility to oxidative damage, structural strains and alterations in specific receptor sites and transport systems.
- Zinc plays an important role in cell multiplication and cell growth. Hence reduced cell replication is an early event in zinc deficiency. This has been related in part to the role of zinc in protein and nucleic acid synthesis. Zinc deficiencies in children are usually marked by poor growth and impairrnent of sexual development. Mild zinc deficiency may also affect the quality of growth. In both, children and adults, zinc deficiencies resultin poor wound healing.
- Zinc in many proteins is present in what is called the 'zinc-finger' motif. Zinc fingers enable polypeptides that are too small to fold by themselves to fold stably when stabilized by bound zinc. Several transcription factors have been reported to contain 'zinc-finger' regions.
- Zinc also serves as a stimulator of trans-acting factors responsible for regulating gene expression. This function has been studied most extensively for the expression of the protein metallothionein (MT) which is a copper binding protein'. High intake of zinc induces synthesis of MT in the mucosal cell. This

protein sequesters (holds) copper, making it unavailable for serosal transfer and thus decreases copper absorption.

- Some of the effects of zinc deficiency in humans appear to be mediated through effects on hormonal function. Hormones reported to be affected by zinc status in humans include growth horrnone, the gonadotropins and sex hormones, prolactin, thyroid hormones, corticosteroids and insulin. Zinc plays a role in stabilizing hormone-receptor complexes. We will learn about hormone-receptor complexes in the next unit.
- High levels of oxidation in the tissues lead to the generation of free radicals. These free radicals are implicated in the onset of many degenerative diseases like cancers and diabetes. Adequate zinc intake along with other antioxidants helps in reducing the risk of acquiring these diseases. Further low zinc levels in the body leads to immune deficiencies and susceptibility to a host of infections and non-communicable diseases. Zinc is required for cytokine production by monocytes and T cells.
- Moderate zinc deficiency leads to growth retardation, rough skin and hypogonadism in males. During the 1960s, in the Middle East countries like Iran and Egypt, young boys exhibited severe growth retardation and were shorter in structure. Sex organs and secondary sexual characters were not developed. Additionally, they suffered from anaemia. They had lower zinc levels and were termed 'zinc dwarfs of middle east'. On supplementation with zinc, the symptoms disappeared. This emphasizes the role of zinc in growth and normal development. Zinc deficiency affects women too and may cause dwarfism and amenorrhea.
- Zinc is also present in gustin, a salivary polypeptide that appears to be necessary for normal development of taste buds. Thus zinc deficienc also leads to decreased taste acuity (hypogeusia).

Selenium

Interest in the biological significance of selenium was for many years confined to its toxic effects upon animals. In fact 'alkali disease' in cattle living in soils with high selenium content was known to early settlers in North America. Human beings in these areas can also suffer from selenium poisoning. Condition of excessive selenium is also referred to as 'selenosis'.

The first demonstration of a biochemical function of selenium in animals came in 1973, when it was shown to be a constituent of the enzyme glutathione peroxidase. The importance of selenium in human nutrition was shown in 1979 when Chinese scientists reported that selenium supplementation prevented development of a cardiomyopathy (moderate to severe heart enlargement with varying degrees of heart insuffi iency) known as 'keshan disease' in children living in low selenium areas. It is now well known that selenium is an essential element.

Why? Read the functions of selenium next and find out.

Functions of Selenium

- Most of the selenium in biological systems is present in amino acids as constituents of proteins. Eleven seleno-proteins have been characterized. Cysteine is a sulfur - containing amino acid. However the sulfur atom can be replaced by selenium and the compound is called selenocysteine. While not normally considered an amino acid present in proteins, selenocysteine occurs at the active site of several enzymes. Examples include glutathione peroxidase, thioredoxin reductase and iodothyronine deiodinase.

  You have already learnt the importance and functioning of glutathione peroxidase in pentose phosphate pathway (in Unit 6). This enzyme converts toxic hydrogen peroxide (H202) to non toxic water in the presence of reduced glutathione. Thus selenium functions as a scavenger of peroxides. This reaction is important since accumulation of 1-1202 may decrease the life span of the erythrocytes by causing oxidative damage to the cell membrane, leading to haemolysis. In fact it is now recognized that importance of selenium is on par with vitamin E in maintaining desirable redox potential in the cell.

- The sulfur atom of methionine can also be replaced by selenium forming seleno-methionine which has the same functions as norrnal methionine.

Copper

Copper has been used therapeutically since at least 400 BC when Hippocrates prescribed copper compounds for pulmonary and other diseases. The presence of copper in plant and animal tissues was recognized almost 150 years ago. The first conclusive evidence that copper is an essential dietary component emerged from various studies which indicated that copper in addition to iron was necessary for haemoglobin formation in the rat. The demonstration of the role of copper in haematopoiesis stimulated interest in the biological function of copper at the cellular level.

It was found that certain naturally occurring diseases in grazing sheep and cattle were found to be caused by dietary deficiency of copper or to respond to copper therapy. Copper deficiency was shown to be a causal factor in a disease of sheep and cattle characterized by diarrhoea, anorexia and anaemia. Copper was shown to be vitally concerned in the process of pigmentation, keratinization of wool, bone formation, reproduction and myelination of the spinal cord in addition to that of haematopoiesis.

The healthy human adult body has about 50-120mg of total copper, located mostly in bone, liver, kidney and muscle. This amount is very little when compared with other trace elements such as iron and zinc. Copper present in plasma is transported bound to a protein called ceruloplasmin. It has a pale blue colour because of its high copper content and carries 90% of the copper present in plasma. Each molecule of ceru- loplasmin binds six atoms of copper very tightly. Albumin carries the other

10% of the plasma copper. The functions of copper include:

Functions of Copper

- Copper functions as a part of a number of proteins including many important enzymes. Some of these are: copper-binding proteins, metallothionein, albumin, blood clotting factor V, amine oxidases, ferroxidases, cytochrome C oxidase, superoxide dismutase, tyrosinase, C18, A9 desaturase.

Several important amine oxidases are cuproproteins. Relatively small amounts of these enzymes are found circulating in blood plasma where they inactivate and catabolize physiologically active amines such as histamine, tyramine and polyamines. Histamine stimulates acid secretion in the stomach. In allergic reactions throughout the body, histamine is released in response to exposure to antigens. Lysyl oxidase is a unique amine oxidase and it acts on lysine residues of collagen and elastin and deaminates them to form allysine which is needed for cross-links. Thus this •enzyme functions in the formation of connective tissues including bone, blood vessels, skin, lungs and teeth.

Ferroxidases — Ceruloplasmin is also called ferroxidase I. It contains six (possibly seven) atoms of copper per molecule. It catalyzes the oxidation of ferrous iron and plays a role in the transfer of iron from storage to sites of haemoglobin synthesis. Ceruloplasmin also oxidizes aromatic amines and phenols. Ferroxidase II also catalyzes the oxidation of ferrous ion and accounts for about 5% of the ferroxidase activity in human plasma.

Cytochrome C oxidase — It contains 2 or 3 atoms of copper per molecule. It is present in mitochondria of cells throughout the body and is the terminal link in the electron transport chain. The activity of this enzyme is highest in heart and high in brain, liver and kidney tissues. You can revise this portion in Unit 6.

Superoxide dismutase — It is present in high amounts in the lungs, thyroid and uterus and in small amounts in blood plasma. It functions as a scavenger of superoxide radicals and protects against oxidative damage.

Tyrosinase — It catalyzes the conversion of tyrosine to dopamine and the oxidation of dopamine to dopaquinone which are steps involved in the synthesis of body pigment melanin. Melanin is responsible for the colour in hair, skin and eyes. Deficiency of tyrosinase in skin leads to albinism. Dopamine is also a neurotransmitter (controlling nervous activity).

C 18 , A9 desaturase — This enzyme is responsible for converting stearic acid (C 18 saturated fatty acid) to oleic acid (C18 monounsaturated fatty acid). This may account for the fact that dietary stearic acid does not have the cholesterol-raising property of other saturated fatty acids.

Metallothionein (MT) — MTS are the small nonenzymatic proteins rich in cysteine

that are responsible for binding copper. Each molecule can bind II or 12 copper atoms, as well as, zinc and cadmium. They appear to play a role in metal storage and sequester excess metal ions, preventing toxicity. The concentration is highest in the liver with small amounts in the blood plasma.

Albumin — It is the most prevalent protein in blood plasma and interstitial fluids. Albumin binds and transports copper and also plays a role in binding excess copper that would otherwise be toxic.

Blood clotting factor V — It contains one atom of copper per molecule. This indicates that copper may be required for blood clotting.

- Copper has 2 oxidation states, Cu + and Cu2+. Hence copper is a cofactor for certain enzymes. Copper aécepts and donates electrons and is involved in reactions involving dismutation (destroying highly toxic form of oxygen called superoxide ion), hydroxylation and oxygenation.
- Copper is required for formation and maintenance of myelin, a protective layer covering neurons (nerve cells).
- Other functions — it has been suggested that copper may have a role in thermal regulation, glucose metabolism and immune function.

Thus copper plays an importaht physiological role in oxidation-reduction reactions, connective tissue formation, iron metabolism, central nervous system, melanin (pigment) formation and blood clotting.

Chromium

All plant and animal tissues contain chromium. Dietary chromium occurs in multiple vålence states. Most of the chromium in the food supply is in the trivalent state (Cr3+). Chromium is poorly absorbed in the intestine. Some of the chromium ingested with the food and inhaled as dust finally reaches the tissues. However, thp concentrations are extremely low and highly variable. Chromium is a bone-seeking element and its uptake in bone appears to be rapid. Besides bone, chromium accumulates in spleen, liver and kidney. Generally in human adults, most tissues contain 0.02-0.05 ppm of chromium on wet basis.

Functions of Chromium

- Chromium has been shown to be particularly effective in serving as a crosslinking agent for collagen (a protein present in connective tissue).
- Chromium is a component of a low molecular weight protein called chromodulinwhich potentiates the effects of insulin, possibly by facilitating insulin binding to cell receptor sites. A symptom of chromium deficiency is impaired glucose tolerance, a result of decreased insulin effectiveness.
- A number of beneficial effects of chromium on lipid profiles have been reported. Total cholesterol, LDL-cholesterol and triglyceride levels have decreased, while beneficial HDL-cholesterol and apolipoprotein A levels have increased

- Intense public interest has emerged in using chrolnium as an ergogenic (musclebuilding) aid. Studies have reported an effect of chromium on body composition; young men undergoing resistance training and taking chromium supplementation increased lean body mass and decreased fat mass.

Cobalt

It is also considered as an ultra trace element. Various studies indicate that the retained cobalt is taken up by all tissues, the highest concentrations occumng in the spleen and pancreas. The green leafy vegetables, especially spinach are the richest source of this element and dairy products and cereals are the poorest.

Functions of Cobalt

- Cobalt is a constituent of vitamin B,,. You have already studied the functions of this vitamin. It functions as a coenzyme. The BIZ coenzymes are called cobamides because of the presence of cobalt. You should revise the functions of vitamin B12 done in the earlier section under metabolic pathways.

Manganese

It is classified as an ultra trace element. Ultra trace elements are those elements with estimated dietary requirement of usually less than 1 mg/day. The body of a normal 70 kg man is calculated to contain a total of 12-20 mg manganese. It is distributed throughout the body tissues and fluids .and is not specifically concentrated in any organ or tissue. However, manganese tZnds to be higher in tissues rich in mitochondria and is more concentrated within the mitochondria than in the cytoplasm or the other organelles of the cell. Accordingly, organs rich in mitochondria such as liver, kidney and pancreas have a relatively high manganese concentration. In contrast, plasma manganese in humans is extremely low.

Functions of Manganese

- Manganese as Mn2+ activates a number of plant and animal enzymes including oxidoreductases, lyases, ligases, hydrolases, kinases, transferases and various decarboxylases. While ~nis~ sp'eci fic for glucosyltransferase and xylosyltransferase, other divalent ions may replace Mn2+ as a cofactor in the case of other enzymes. Mitochondria1 superoxide dismutase contains Mn2+. Glucosyltransferase links carbohydrate to protein during the synthesis of glycoproteins. There are only a few manganese metalloenzymes (i.e. containing manganese in the structure). These include arginase (in urea cycle), pyruvate carboxylase (in gluconeogenesis), glutamine synthetase (in glutamine synthesis) and superoxide dismutase (scavenger of peroxide radicals).

10.7 LET US SUM UP

In this unit, we learnt that about the different classes of vitamins, fat-soluble and water-soluble. We had a look at their structure, different forms and various biochemical functions. Then we moved on to the study of minerals — macro minerals, required in much larger quantities than the other class called as micro — minerals. Here we dealt with an exhaustive list of these imperils and got to know about their structure and functions.

10.8 GLOSSARY

Acuity : acuteness of vision or perception.

Basal Metabolic Rate : the rate at which heat is produced by an individual in a resting state.

Cardiac hypertrophy : an enlargement of the heart.

Covalent modulation : the alternation of a protein 's shape and function by covalent bonding of chemical groups to it.

Gluconeogenesis : formation of glucose from non-carbohydrate sources within the liver.

Haematopoietic : pertaining to the formation of blood or blood cells.

Hypogonadism : inadequate functioning of the testes or ovaries as manifested by deficienci s in gametogenesis or the secretion of gonadal hormones.

Hypoguesia : diminished sensitivity to taste.

Keshan disease : selenium deficiency disease that impairs the structure and function of the heart.

Metallonavoproteins : flavoproteins containing metal ions.

Myelination : formation of a myelin sheath around a nerve fibre.

Myelin sheath : insulating layer around some nerves that dramatically speed up conduction of nerve signals.

Myxedema : hypothyriodism or an underactive thyroid gland marked by dry skin and swellings around lips and nose as well as mental deterioration.

Neurotransmitter : a molecule that carries signals between nerve cells.

Purines : a type of nitrogen base; the purine bases in DNA and RNA are adenine and guanine.

Transamination : the process of transferring an amino group from one

compound to another.

Transcription : the organic process whereby the DNA sequence in a gene is copied into mRNA;

Translation : the process whereby genetic information coded in MRNA directs the formation of a specific polypeptide at a ribosome in the cytoplasm.

Vasoconstriction : the narrowing of blood vessels. Vitamins the organic compounds required in very small quantities for a variety of biochemical functions; cannot be synthesized in the body.

10.9 CHECK YOUR PROGRESS

1) How are fat-soluble vitamins transported? What are the factors that lead to deficiency of these vitamins in our body

2) Discuss the role of:

   a) Vitamin E as anti-oxidant.

   b) Vitamin K as anti-coagulant.

   c) Calcitriol in calcium metabolism.

3) List the active forms of vitamin A and vitamin D.

4). Indicate the active forms of the following vitamins along with the reactions catalyæd by them:

   a) Thiarnin

   b) Niacin

   c) Biotin

5) Discuss the role of

   a) Vitamin B6 in transamination reaction.

   b) Pantothenic acid in metabolism of fat, protein and carbohydrate.

UNIT

11

HORMONES

STRUCTURE

11.1 LEARNING OBJECTIVE

After going through this unit, you will be able to:

define and classi$' hormones,

discuss the regulation of the endocrine system,

list and explain the various components involved in the mechanism of hormone action,

compare the mode of signal generation of the two groups of hormones,

discuss the role of second messengers, and

describe the biochemical role of each hormone in the body.

11.2 INTRODUCTION

The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment. Further, the most characteristic property which even human beings possess is their ability to adapt to ever changing scenario, minute to minute, day to day and generation to generation. Thus intercellular (between various cells) communication mechanisms are necessarily required for this adaptation. The nervous system and the endocrine system provide this intercellular, organism-wide communication. In fact there is a remarkable

convergence of these two regulatory mechanisms. They are now even being viewed as an integrated neuroendocrine system.

The physiology of the nervous system and the endocrine system is discussed in Unit 9 and Unit I I of the Applied Physiology Course. Hence it would help you to go through these units. As you go through the Unit on endocrine glands, you would realize that the endocrine system is composed of the endocrine glands, the so called 'ductless glands' whose secretions pass directly into the blood stream. The secretion of-the endocrine gland is called the 'hormone'. Here, in this unit our focus is on hormones. What are hormones? How do we classiö' them? What are the components involved in the mechanism of hormone action? What is the role of hormones in our body? These are a few aspects discussed in this unit. Since our discussion at this point is the detailed study of hormones, brief information about the endocrine system is also given below.

NOTES

11.3 THE ENDOCRINE SYSTEM

The endocrine system, as we mentioned above, is composed of the endocrine glands, the so called 'ductless glands' whose secretions pass directly into the blood stream. The secretion of the endocrine gland is called the 'hormone'. The word hormone is derived from a Greek term that means to arouse to activity. As classicaHy defined, a hormone is a substance that is synthesized in one organ and transported by the circulatory system to act on another tissue.

However this original description is too restrictive because it is now known that hormones can act on adjacent cells (paracrine action) and even on the cell in which they were synthesized (autocrine action) without entering the systemic (general) circulation. Thus today the term hormone refers to any substance in an organism that carries a signal to generate some sort of alteration at the cellular level.

Accordingly, since hormones carry signals or messages, they are also called 'messengers' or specifically 'first messengers Hence It a particular chemical reaction has to take place in a cell, one or the other hormone has to give this message to that cell.

A diverse array of hormones — each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport and metabolism — has evolved to provide homeostatic responses. Hence, the hormones may also be viewed as regulators and the levels of various hormones regulate specific cellular processes. Let us learn about this regulatory mechanism next.

11.4 REGULATION OF ENDOCRINE SYSTEM

What regulates the regulators? The regulation originates in the brain/central nervous system (CNS). The CNS receives inputs from many internal and external

sensors about danger, hunger, dietary intake, blood composition and pressure, for example and orchestrates the production of appropriate hormonal signals by the several endocrine tissues of the body. This results in what is called the hormonal cascade system. Figure 11.1 shows the chain of command in the hormonal signaling hierarchy. A stimulus originates in the external environment or within the organism. The CNS senses the stimulus and this signal may be transmitted as an electrical pulse or as a chemical signal or both. The signal is forwarded to the hypothalamus situated at the base of the brain. The hypothalamus, which is an endocrine gland, secretes the appropriate hormone called the releasing hormone in nanogram (ng) amounts. The releasing hormone is carried by blood to the anterior pituitary (also called adenohypophysis), another endocrine gland. This is the first target of the environmental or interior signal. (In some cases, the posterior pituitary or neurohypophysis is involved.) The anterior pituitary secretes the hormone called trophic hormone or tropin in microgram (mcg) quantities. (Greek tropos means 'turn'). The trophic hormone is carried by blood to its appropriate endocrine gland which gets stimulated. The endocrine gland which is the second target then synthesizes its specific hormone in milligram (mg) amounts. Through blood, this specific hormone travels to specific tissue(s), the ultimate target and brings about the characteristic effects. Thus this signal pathway originates in the brain and culminates in the ultimate target cell. The hypothalamus of the brain is the coordination center of the endocrine system. It receives and integrates messages from the central nervous system.

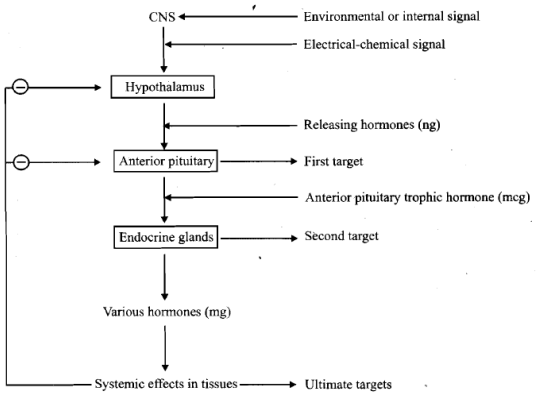

Figure 11.1: General sequence of events in hormonal cascade system

For your better understanding, a specific example is given in Figure 11.2. If there is an infection or haemorrhage or hypoglycemia or the person perceives fear or pain, electrical-chemical signals are generated which are received by the central nervous system. CNS in turn passes on the signals to the hypothalamus. It secretes the corticotropin-releasing hormone (CRH) which goes to the anterior pituitary. The anterior pituitary in turn secretes the adrenocorticotropic honnone (ACTH) which goes to the adrenal cortex (the outer layer of the adrenal gland) and triggers the release of the specific hormone, cortisol. Cortisol, the ultimate hormone, acts in many types of target cells to alter their metabolism. In liver cells (hepatocytes), one of the actions of cortisol is to increase the rate of gluconeogenesis (synthesis of glucose). This ensures that the body gets enough fuel to overcome the adverse situation.

From Figures 11.1 and 11.2, it is clear that in the hormonal signaling hierarchy, at each level, a small signal elicits a large response. The initial electrical signal to the hypothalamus results in the release of a few nanograms of CRLwhich elicits the release of a few micrograms of corticotropin. Corticotropin on the adrenal cortex to cause the release of milligrams of cortisol, for an overall amplification of at least a millionfold. This is called the hormonal cascade system. fie hormonal cascade system amplifies a specific signal. Thus the cascade mechanism means that smalBmounts of an extracellular compound can affect large number of intracellular enzymes without crossing the • plasma membrane or binding to each target protein.

At each level of a hormone cascade, there is the possibility of feedback inhibition of earlier steps in the cascade, an elevated level of the ulpate hormone or one of the intermediate hormones inhibits release of the earlier hormones in the cascade from the hypothalamus or pituitary. This ensures that a product is made (or released) only until the necessary concentration is reached

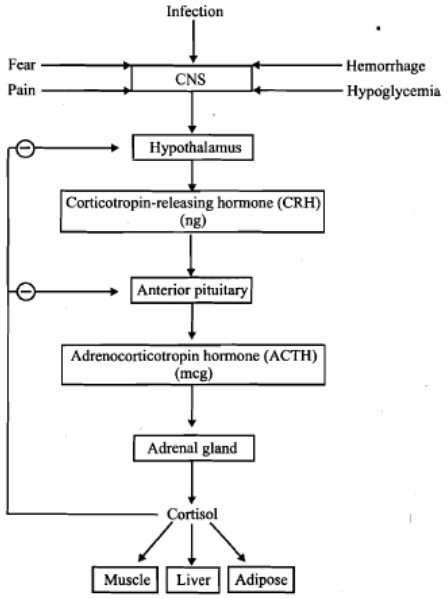

Figure 11.2: Hormone cascade system for cortisol

Not all hormone-producing cells are a part of such long cascades. Insulin release by the pancreas for example, is largely regulated by the level of glucose in the blood supplied to the pancreas.

11.5 MECHANISM OF HORMONE ACTION

Even the brief insight into the endocrine system along with its regulation, presented in the section above, must have impressed upon you the complexity of this endocrine system. Accordingly, you would realize that the mechanism of action of the hormones is not a very simple one. It is for this reason that detailed information on this aspect has only been obtained in the last few decades, despite

the fact that the functions of many of the major hormones had been described much earlier. However, it is now well known that the mechanism of hormone action involves many sequential steps and requires the participation of various components, each having a well-defined role. These components are discussed next.

11.5.1 The Target Cell Concept

There are about 200 types of differentiated cells in humans. Only a few produce hormones, but virtually all the cells in the human body are target of one or more of the over 50 known hormones. It was thought that hormones affected a single cell type or only a few kinds of cells and that a hormone elicited a unique biochemical or physiologic action. However, a hormone can affect several different cell types. Further, more than one hormone can affect a given cell type and hormones can exert many different effects in one cell or in different cells.

11.5.2 Hormone Receptors

Hormones are present at very low concentrations in the extracellular fluid, which is generally in the range of 10-15 to 10-9 mol/L. This concentration is much lower than that of the many structurally similar molecules like sterols, amino acids, peptides, proteins etc. and other molecules that circulate at concentrations in the range of 10-5 to 10-3 mol/L. Hence target cells must distinguish not only between different hormones present in small amounts but also between a given hormone and the 106 to excess of other similar molecules. This high degree of discrimination is provided by cell-associated recognition molecules called receptors. Thus a target cell has the ability to selectively bind a given hormone to its specific receptor. Hormones initiate their biologic effects by binding to specific receptors. Accordingly, hormone- induced actions are terminated when the hormone dissociates from the receptor. The characteristics of receptors are discussed next.

Characteristics of receptors

All receptors have at least two functional domains-

- a recognition domain that binds the hormone, and

  a region that generates a signal that couples hormone recognition to some intracellular function.

The coupling (signal transduction) occurs in two ways depending upon the chemical nature of the hormone-

- polypeptide and protein hormones and the catecholamines (like epinephrine and norepinephrine) bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, which is often by changing the activity of an enzyme.

  steroid, retinoid and thyroid hormones react with intracellular receptors

and this hormone-receptor complex directly provides the signal, generally to specific genes whose rate of transcription is thereby affected. In fact, it is now known that these hormone receptors have several functional domains. These sites are-

i) which binds the hormone

i) which binds to specific DNA region

iii) which is involved in the interaction with other coregulator proteins that result in the activation (or repression) of gene transcription, and

iv) which may specify binding to one or more proteins that influence the intracellular trafficking of the receptor

Thus both recognition and coupling domains occur on receptors. The dual functions of binding and coupling~ultimatelyd efine a receptor. It is the coupling of hormone binding to signal transduction, which is also called receptor-effector coupling that provides the first step in amplification of the hormonal response. This dual purpose also distinguishes the target cell receptor from the plasma carrier proteins that also bind hormone but do not generate a signal.

11.5.3 Classification of Hormone

Hormones can be classified in several ways according to chemical composition, solubility properties, location of receptors and the nature of signal used to mediate hormonal action within the cell. One of the most common systems of classification is based an the location of receptors and the mechanism of action of the hormone. According to this system, the hormones are grouped into 2 major classes -. Group 1 and Group 2.. .

Group I-Hormones that bind to intracellular receptors
• Thyroid hormones (T3 and T4) • Glucocorticoids • Mineralocorticoids • Retinoic acid • Calcitriol (1,25 dihydroxy-D3) • Androgens • Oestrogens
Group II- Hormones that bind to cell surface receptors
A - The second messenger is cAMP • Glucagon • Calcitonin • Parathyroid hormone

- α_2-Adrenergic catecholamines
- β-Adrenergic catecholamines
- Thyroid-stimulating hormone (TSH)
- Antidiuretic hormone (ADH)
- Adrenocorticotropic hormone (ACTH)
- Human chorionic gonadotropin (HCG)

B - The second messenger is cGMP

- Atrial natriuretic factor

C - The second messenger is calcium or phosphatidyl inositol or both

- Acetylcholine
- α_1-Adrenergic catecholamines
- Oxytocin
- Antidiuretic hormone (ADH)
- Cholecystokinin

D - The second messenger is a kinase or phosphatase cascade

- Insulin
- Growth hormone
- Prolactin

Each group of hormones that are listed in Table 11.2 has distinct properties. The hormones in Group I are lipophilic or fat-soluble. After secretion, these hormones associate with plasma transport or carrier proteins. This overcomes the problem of their insolubility in the aqueous medium of the plasma. At the same time, it prolongs the plasma half-life of the hormone since they cannot be eliminated easily when bound to proteins. The relative percentages of bound and free hormone are determined by the binding affinity and binding capacity of the transport proteins. The free hormone, which is the biologically active form, readily traverses (crosses) the lipophilic (lipid containing) plasma membranes of all cells. The receptors for Group I hormones are present either in cytosol or nucleus of target cells. It is here that the hormone- receptor complex is formed. It is thought that this complex, the ligand-receptor complex, is the intracellular messenger which conveys the message brought from the CNS to the specific cell.

The hormones in Group II are hydrophilic or water-soluble. They cannot cross the lipophilic plasma membrane. Hence they bind to the plasma membrane of the target cell. Their receptors are located in the plasma membrane. Such hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called second messengers. The hormone itself is called the first messenger. These second messengers are generated as a consequence of the ligand-receptor interaction. For example, when epinephrine binds to the plasma membrane of certain cells, the concentration of cyclic AMP (cAMP) increases. Hence cAMP mediates the effects of many hormones. Hormones listed in Group II A of Table 11.2 use cAMP as the second messenger. The general

characteristics of group I and group II hormönes are given in Table 11.2. As can be seen in Table 11.2, GMP (cGMP) is used as second messenger by atrial natriuretic factor (Group II B). Many hormones use ionic calcium (Ca2+) or phosphatidyl inositol (or both) as the second messenger. These hormones have been classified in Group II C (refer to Table 11.2). You have read about phosphatidyl inositol in chemistry of lipids (Unit 2). The intracellular messenger for Group II D hormones is a protein kinase-phosphatase cascade. You have come across such a cascade in glycogen metabolism

Table 11.2: General characteristics of group I and group I1 hormones

Characteristic	Group I hormones	Group II hormones
Type of hormone	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport Proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	Second messenger-cAMP, cGMP, Ca, phosphoinositols, kinase cascades

Having learnt about the hormones, next, we will see how the hormone action brings about the signal transduction, which is the next component in the mechanism of hormone action.

11.5.4 Signal Transduction

The homeostatic adaptations an organism makes to a constantly changing environment are mainly achieved by altering the activity and amount of proteins. This in turn is brought about by the action of hormones. A hormone-receptor interaction results in generation of an intracellular siY1al that can:

i) regulate the activity of a select set of genes, which will alter the amount of certain proteins in the target cell or

ii) affect the activity of specific proteins including enzymes and transporter or channel proteins. (Cell membranes have channels or passages for movement of molecules).

The signal can influence the location of proteins in the cell and can affect general processes such as protein synthesis, cell growth and replication. These changes may be brought about by influencing gene expression. The signal transduction that occurs as a result of hormone action ultimately affects homeostatic mechanisms

in the body. Figure I I .3 shows hormonal response to any stimulus. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. In the case of an organism as a whole, this generally involves the nervous system, as well as, various senses including sight, hearing, pain, smell, touch. At the cellular level, recognition involves physicochemical factors such as pH, oxygen tension, temperature, nutrient supply, osmolarity and even production of undesirable metabolites. Accordingly one or more of hormones would be secreted which will bring about the desired adaptive responses.

So from our discussion above it is clear that the action of every hormone is mediated through the generation of a signal. Let us get to know more about the signal generation component next. But, before doing so we shall try to recapitulate what we have studied so far. Answer the questions given in the check your progress exercise 2 and check your progress so far.

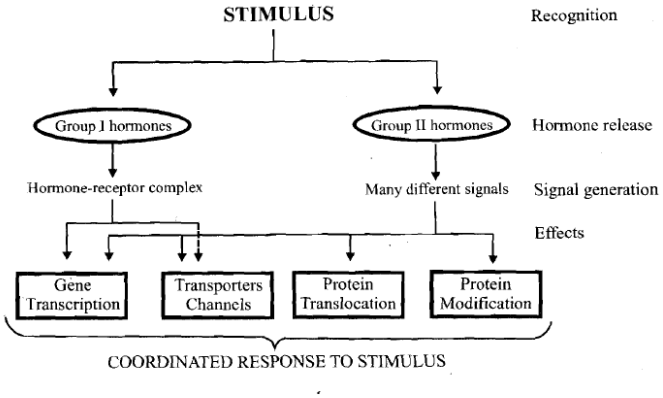

Figure 11.3: Response of group I and group II hormones to a stimulus

11.5.5 Signal Generation

Even though action of every hormone is mediated through the generation of a signal, there are great differences in the kind of signal generated and the manner in which it occurs. The difference is determined by the nature of the hormones involved i.e. group I hormones or the group II hormones. Let us get to know more about this aspect next.

Group I Hormones

The ligand-receptor complex is the signal for group I hormones. These hormones which are lipophilic, easily diffuse through the plasma membrane of all cells and encounter their specific, high-affinity intracellular receptors in target cells. As already mentioned, these receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone-receptor complex undergoes, first, an activation reaction. This can occur by the following two mechanisms :

- In the case of glucocorticoid hormones, the receptors are present in the cytoplasm of the target cells as illustrated in Figure 11.4. These receptors are

bound to a protein called heat shock protein 90 (hsp 90). When the hormone binds to the receptor, there is dissociation of hsp 90 from the receptor. This is an essential step before the glucocorticoid receptor can be translocated into the nucleus of the cell. In fact the receptor itself contains nuclear localization sequences which help in this process of translocation from the cytoplasm to nucleus. The activated receptor then moves into the nucleus. It binds with high affinity to DNA at a specific sequence called the hormone response element (HRE) When the particular hormone is a glucocorticoid, this region is also specifically called glucocorticoid response element (GRE) as shown in Figure 11.4.Once the activated hormone-receptor is bound to HRE, one or more proteins which act as coactivators also bind to this region resulting in accelerated gene transcription.

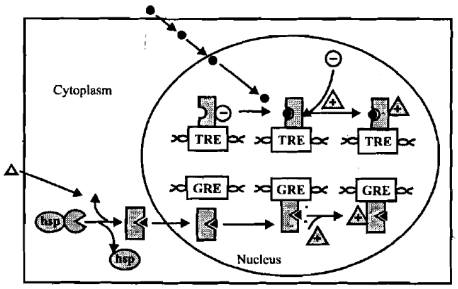

Figure 11.4: Regulation of gene expression by Group I hormones

Thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go directly into the nucleus. In this case, the respective receptor is already bound to the HRE which can be also called thyroid response element (TRE) if we are talking of thyroid hormone. Figure 11.4 above highlights this element. However this DNA-bound receptor fails to activate transcription because it is complexed with a corepressor. In fact the receptor-corepressor complex is an active repressor of gene transcription. But when the hormone binds to this complex, there is dissociation of the corepressor.

Further, the hormone affects less than 1% of the genes, mRNA or proteins in a target cell. However, the effect produced can be profound. Apart from exerting their effect on modulating gene transcription, hormones can act at any step in the information pathway as shown in Figure 11.5

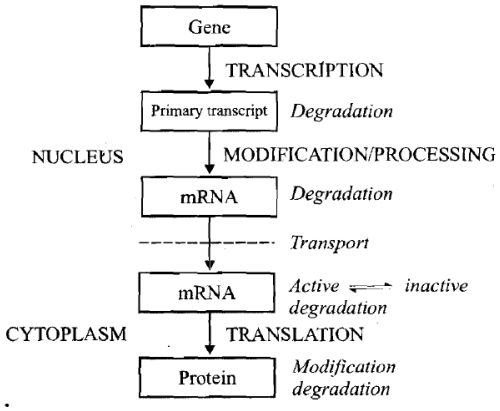

Figure 11.5: The information pathway

Group II *Hormones*

Group II hormones being water soluble cannot cross the plasma membrane. Hence they have their receptors in the plasma membrane and thus have to use intracellular messenger molecules to communicate their message to the cell. These molecules called second messengers are the intracellular signals they generate. Many of these second messengers affect gene transcription, as well as, many other processes in the cell. Receptors of Group II hormones work in collaboration with a specific protein called G Protein. Hence these receptors are also called G protein-coupled receptors or GPCR. The G protein is called the transducer. After the hormone (first messenger) binds to its specific receptor on the surface of the target cell, the signal is passed through the membrane located G protein transducer to a membrane-bound effector. The action of the effector molecule generates an intracellular second messenger, which is usually a small molecule or ion like cAMP, cGMP, Ca2+ and phosphatidyl inositides. The diffusible second messenger carries the signal to its ultimate destination, which may be in the nucleus, an intracellular compartment or the cell cytosol resulting in specific cell response. This general mechanism of signal transduction is shown in Figure 11.6.

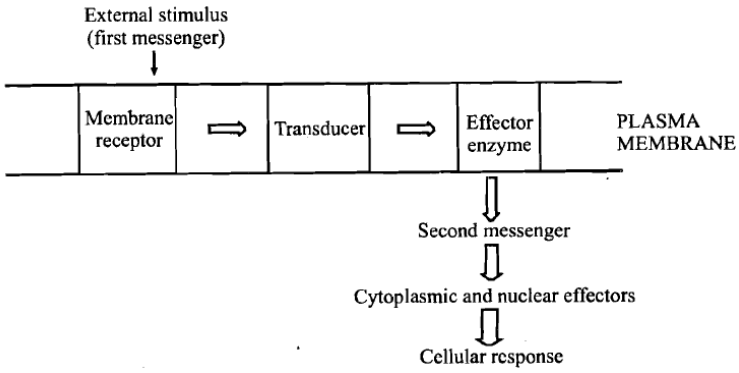

Figure 11.6: General mechanism of signal transduction

As discussed earlier, an important feature of signaling pathways is amplification. In this case, a single hormone-receptor complex can react with a number of transducer molecules, each of which can activate several molecules of effector protein. This will form several molecules of second messenger which can activate many kinase molecules, which in turn will catalyze the phosphorylation of many target proteins. This series of amplification events results in a cascade effect. The cascade mechanism means that small amounts of an extracellular compound can affect large numbers of intracellular enzymes without crossing the plasma membrane or binding to each target protein.

In the section above we learnt that Group II hormones work in collaboration with a specific protein called G Protein and an intracellular second messenger, which is usually a small molecule or ion like cAMP, cGMP, Ca2+ and phosphatidylinositides. These are the other important components of the mechanism of hormone action. Let us get to know a bit more about these components, next.

11.5.6 G Protein-Coupled Receptors (GPCR)

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Receptors of-Group II hormones have seven domains (areas) spanning the plasma membrane. This is shown in Figure 11.7. The seven domains are depicted as seven interconnected cylinders which extend through the lipid bilayer of the membrane (plasma membrane consists of a protein layer in between two lipid layers). The G protein consists of 3 polypeptides (subunits), each having a different amino acid composition, i.e. it is a heterotrimeric protein. The 3 polypeptides are denoted as α, ß and ɣ in Figure 11.7. The a subunit of G protein is bound to guanosine diphosphate (GDP). The G protein is inactive in this form. Further the G protein as shown in Figure 11.7 is anchored to the plasma membrane but not linked to the receptor.

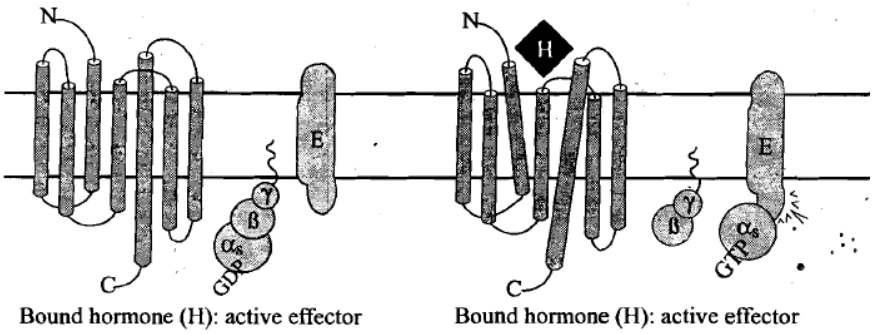

Figure 11.7: G-Protein coupled receptors (GPCR)

When a Group II hormone binds to the receptor, there is presumably a conformational change of the receptor.This has been depicted as tilted membrane-spanning domains in Figure11.7. Following the conformational change, a GTP molecule replaces the GDP molecule attached to the α subunit.The ß and ɣ subunits then dissociate from the α subunit as shown in Figure 11.7. The ß and ɣ subunits however are always associated (ßɣ). These changes result in activation of the G protein. The a subunit along with the attached molecule of GTP then binds to the effector molecule (E) which is also present on the plasma membrane.The effector can be the enzyme adenylyl cyclase, Ca^{2+}, Na^{+}, K^{+} or Cl^{-} channels, the enzyme phospholipase C or the enzyme cGMP phosphodiesterase.

The a subunit has intrinsic (built-in) GTPase activity. This means it can act as the enzyme GTPase and hydrolyze GTP into GDP and Pi. Once this happens, the protein gets inactivated. The trimeric complex (αßɣ) is reformed and is ready for another cycle. Cholera toxin catalyzes the ADP-ribosylation (combining with ADP) of a subunit. This modification disrupts the intrinsic GTP-ase activity and hence the a subunit cannot reassociate with ßɣ and is therefore irreversibly activated.

There is a large family of G proteins. The α subunits of different G proteins are distinct, but the and ɣ subunits are similar and often interchangeable. Humans

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have 24α proteins, 5ß proteins and 6ɣ proteins.

Next, let us get to know more about the second messengers.

11.5.7 Second Messengers

To understand the second messengers better, let us look at Table 11.1, presented earlier. As you can see, in Table 11.1, Group II hormones are further subdivided on the basis of the intracellular signals (second messengers) they generate. For example, Group II A hormones function through cyclic AMP, Group II B hormones function through cyclic GMP and so on. Cyclic AMP, GMP, calcium, phosphatidyl inositols etc. are all second messengers. Let us get to know more about these messengers next, starting with cyclic AMP.

A) *cyclic AMP (cAMP)*

Figure I I .8 illustrates the formation of cyclic AMP. As can be seen, it is formed from ATP by the enzyme adenylyl cyclase (effector molecule). Several components comprise a system for the generation, degradation and action of cAMP. These are discussed next.

- Adenylyl cyclase

Not all hormones on binding to G proteins activate this enzyme. In the case of some 'hormones, binding to G proteins results in inhibition of adenylyl cyclase. Such G proteins are referred to as Gt proteins. Accordingly G proteins which cause activation (stimulation) of adenylyl cyclase are referred to as Gs proteins. Hence Group II A hormones may be subdivided on the basis of this characteristic as given in Table 11.3.

Table 113: Subclassification of Group II A Hormones

Hormones that stimulate Adenylyl cyclase (Hs)	Hormones that inhibit Adenylyl cyclase (Hi)
• Glucagon	• Acetylcholine
• Calcitonin	• α_2-Adrenergic catecholamines
• Parathyroid hormone	
• β-Adrenergic catecholamines	
• Thyroid-stimulating hormone (TSH)	
• Antidiuretic hormone (ADH)	
• Adrenocorticotropic hormone (ACTH)	
• Human chorionic gonadotropin (HCG)	

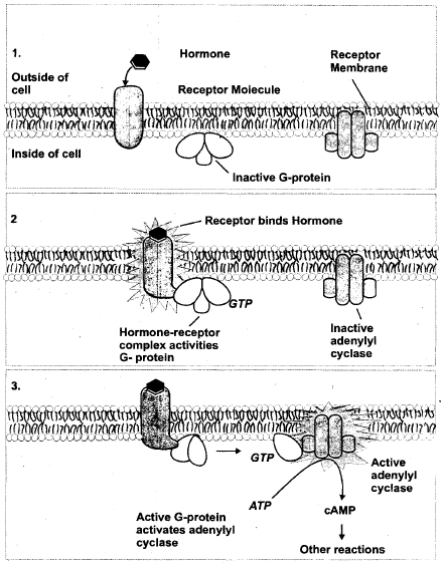

Figure 11.8: Formation of cyclic AMP

Thus two parallel systems have been identified, a stimulatory (s) and an inhibitory (i) system. Further each system consists of a receptor Rs or Ri, and a regulatory complex protein Gs and q. As discussed earlier, Gs and Gi are each trimers composed of a, and y subunits. However the a subunit in Gs differs from that in Gi. Hence they are designated as and al respectively. Both as and ai bind to guanine nucleotides. GTP activates as and and GDP inactivates as and ai. There is a large family of G proteins.

The a subunits and the ßy complex have actions independent of those on adenylyl cyclase. Some forms of ai stimulate K + channels and inhibit Ca2+ channels and some as molecules have the opposite effects. Some members of the G family activate the phospholipase C group of enzymes. The ßy complexes have been associated with channel stimulation and phospholipase C activation./G proteins are involved in many important biologic processes in addition to hormone action. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

Ligand binding to a cell-surface receptor almost invariably results in the activation of protein kinases. These enzymes catalyze the transfer of a phosphoryl group of ATP to various protein substrates. Some proteins are activated by phosphorylation, whereas, others are inactivated. The cAMP produced by the action of adenylyl cyclase, binds to a protein kinase called protein kinase A (PKA) that is a heterotetrameric molecule. It is a serine-threonine protein kinase, catalyzes phosphorylation of the hydroxyl group of specific serine-threonine residues in target enzymes. This phosphorylation can be reversed by the action of protein phosphatases, which catalyze hydrolytic removal of the phosphoryl groups.

Protein kinase A consists of two regulatory subunits (R) and two catalytic subunits (C) in a configuration of R2C,. On binding with cAMP, the following change takes

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place:

$$4\ cAMP + R_2C_2 \longrightarrow R_2 \cdot (4\ cAMP) + 2\ C$$

The enzyme protein kinase A has no enzymatic activity when present in the configuration R2C2. However binding of cAMP by R dissociates R from C as shown in the equation above. This frees the C subunit and becomes active. The active C subunit catalyzes the transfer of the Y (last) phosphate of ATP to serine or threonine amino acid residues in a variety of proteins resulting in the formation of phosphoproteins.

1) Phosphoproteins

The effects of camp in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues of protein molecules as discussed above. The control of any of the effects of cAMP, includmg such diverse processes as carbohydrate and fat metabolism, ion transport, enzyme induction, gene regulation, cell growth and replication etc. could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phospllorylation. These substrates help define a target tissue and are involved in defining the extent of a particular response within a given cell.

2) Phosphodiesterases

The ability to turn off a signal-transduction pathway is an essential element of all signaling processes. Actions caused by hormones that increase cAMP concentration can be terminated by the action of phosphodiesterases. They catalyze the hydrolysis of cAMP to 5'-AMP. The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once tlle hormonal stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes.

Having studied about cyclic AMP, we move to the other second messenger, cyclic GMP.

B) cyclic GMP (cGMP)

It is also an intracellular signal. It is made from GTP by the enzyme guanylyl cyclase. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis (excretion of Na+ in urine), diuresis (increased excretion of urine), vasodilation and inhibition of secretion of the hormone, aldosterone. These peptides bind to and activate the membrane-bound guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold and brings about the above mentioned effects. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. This then results ip relaxation of smooth muscle and vasodilation. Termination of this

effect is brought about by the action of the enzyme cGMP phosphodiesterase which hydrolyzes cGMP to 5'- GMP.

Next, we shall learn about the role of calcium and phospatidylinositols as second messengers in the mechanism of hormone action.

C) Calcium or Phosphatidylinositols

Calcium

Several hormones act through calcium as second messenger. Ionized calcium is also an important regulator of a variety of cellular processes, including muscle contraction, blood clotting, enzyme activity and membrane excitability. The extracellular Ca2+ concentration is about 5mmoVL and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca2+) is very low: 0.05-10 gmoVL. Inspite of this large concentration gradient and a favourable electrical gradient, Ca2+ is restrained from entering the cell. A considerable amount of energ is expended to ensure that the intracellular Ca2+ is controlled, as prolonged elevation of Ca2+ in the cell is very toxic. The class IIC hormones, about which you have already studied in Table 11.1, by binding to receptors that are themselves Ca2+ channels, enhance membrane permeability to Ca2+ and thereby increase Ca2+ influx. Hormones also indirectly promote Ga2+ influx by modulating the membrane potential at the plasma membrane. Ca2+ can also be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

Once calcium enters the cell, it brings about its effect through the calcium-dependent regulatory protein calmodulin. Calmodulin has four Ca2+ binding sites. When all these sites are attached to, there is a marked conformational change of the molecule. This allows calmodulin to activate enzymes and ion channels. The interaction of Ca2+ with calmodulin is conceptually similar to the binding of cAMP to protein kinase A and the subsequent activation of protein kinase A. Calmodulin is one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. Some of the enzymes regulated directly or indirectly by Ca2+, probably through calmodulin is given in Table 11.4.

Table 11.4: Enzymes regulated directly or indirectly by Ca2+ through calmodulin

- Adenylyl cyclase
- ca2+- dependent protein kinases
- ca2+- Mg ATPase
- Cyclic nucleotide phosphodiesterase

- Phosphorylase kinase

Next, let us look at the role of phosphatidylinositols. '

Phosphatidylinositols

Some signal must provide communication between the hormone receptor on the plasria membrane and the intracellular Ca2+ reservoirs. This is provided by two molecules, both derived from a plasma membrane phospholipid, phosphatidylinositol 4,5-bisphosphate. When hormones like antidiuretic hormone, acetylcholine and at- type catecholamines bind to their respective receptors on the cell surface, the signal is transduced through the G protein. The active GTP-bound form of the G protein activates the effector enzyme phospholipase C. This enzyme catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol triphosphate and 1,2-diacylglycerol. Both these intermediates are second messengers that, transmit the original signal to the interior of the cell.

Inositol triphosphate diffuses through the cytosol and binds to a calcium channel in the membrane of the endoplasmic reticulum. This binding causes the calcium channel to open for a short time, releasing Ca^{2+} from intracellular storage sites in the endoplasmic reticulum into the cytosol. Accumulation of high levels of Ca^{2+} in the cytosol results in the activation of Ca^{2+}-calmodulin-dependent kinases and many other Ca^{2+}-calmodulin-dependent enzymes.
These then modify substrates and thereby alter physiologic responses.

1,2-diacylglycerol is capable of activating the enzyme, protein kinase C, whöse activity also depends upon Ca^{2+}. Protein kinase C can phosphorylate specific proteins to form the corresponding phosphoproteins which then bring about cellular responses. Signaling via the inositol-phospholipid pathway just discussed, is turned off in several ways. First, when GTP is hydrolyzed, the G protein returns to its inactive form and no longer stimulates phospholipase C. The activities of the two second messenger molecules, inositol triphosphate and 1,2-diacylglycerol are also transient. They are rapidly converted to inactive intermediates. The calcium signal is also short-lived since Ca^{2+} is pumped back into the lumen of the endoplasmic reticulum when the channel closes.

Finally; let us study about the last second messenger i.e. protein kinase cascade.

D) Protein kinase cascade

Many growth factors operate by a signaling pathway that includes a multifunctional transmembrane protein called tyro'ine •kinase. The receptor, •transducer and effector functions are all found in this protein. As the name suggests, the kinase preferentially phosphorylates tyrosine residues: Phosphorylation of tyrosine residues is not common (< 0.03% of total amino acid phosphorylation) in mammalian cells. Binding of the ligand to an extracellular domain of the receptor activates tyrosine-kinase catalytic activity in the intracellular domain,

by dimerization (combination of two units) of the receptor. When the two •receptor molecules associate, each tyrosine-kinase domain catalyæs the phosphorylation of specific tyrosine residues of its partner, a process called autophosphorylation. This initiates a complex series of events. The phosphorylated receptor next phosphorylates various receptor protein substrates, again on tyrosine residues. There are at least four such substrates. The phosphorylated substrates then bind to specific domains of a variety of proteins that are directly involved in mediating the different effects of the hormone. This entire sequence sets off a cascade of events in the cell. It is now well known that the hormone insulin w rks through such a receptor.

To end the tyrosine kinase cascade effect, phosphoryl groups are removed from both the receptors and their protein targets by the action of protein tyrosine phosphatases. These enzymes play an important role in regulating the tyrosine-kinase signaling pathway.

You may have found the discussion above on mechanism of hormone action and signal transduction a bit tough. We tell you that only a brief outline (for easy understanding) has been given in the above discussion on mechanism of hormone action and signal transduction. Extensive research conducted in this area has shown that the whole process is extremely complex involving the interplay of a host of receptor proteins, intermediates, regulators and coregulators. We hope you must have understood the mechanism involved. If not, we suggest you go through this section again, step by step, and recapitulate. This information for would see is the basis for your understanding the biochemical role of different hormones discussed next.

11.6 BIOCHEMICAL ROLE OF HORMONES

Before we get to know about the role of hormones, let us quickly review where the various hormones are synthesized. Hormones are synthesized in a variety of cellular arrangements. You may already know that some are synthesized in specialized organs designed solely for this specific purpose — like thyroid (triiodothyronine), adrenal (glucocorticoid and mineralocorticoid) and the pituitary (TSH, growth hormone, ACTH etc). Some organs are designed to perform two distinct but closely related functions: like the ovaries produce mature oocytes, as well as, the reproductive hormones estradiol and progesterone. Hormones are also produced in specialized cells within other organs — like small intestine (glucagon-like peptide), kidney (angiotensin II). Synthesis of some hormones requires the parenchymal cells of more than one organ — like the skin, liver, kidney are required for the production of 1,25 dihydroxy D3 or calcitriol.

Now then, what is the role of hormones in our body? Let's find out.

The functions of hormones vary widely. A hormone may bring about its effect in a specific cell type, tissue or organ. Or a hormone can cause changes in more than

one cell type, tissue or organ. A hormone can also have one effect in one tissue and a totally opposite effect in another tissue. Most hormones exert their influence on more than one reaction/metabolic pathway in the body. Again a certain type of effect can be caused by more than one hormone. Some authors also tend to categorize the biological role of hormones as being predominantly either physiological or biochemical. Probably these effects are in many cases overlapping. Hence, all this adds to the complexity of hormones as a class of biologically important molecules in the body. Nevertheless, due to painstaking research in the last few decades, the functions of the various hormones have been spelt out. There are two ways of elucidating the functions of hormones. First is a direct assessment where data has been obtained regarding its activity. Second is by looking at what happens in a state of absence/deficie cy of the hormone and attributing the effects to lack of those actions of the hormone.

We will now look at the biochemical role of hormones. Included for your study are some of the major hormones from different endocrine glands. However, you must know, there are many more hormones which have been identified and whose functions have been enumerated. This can call for a separate study altogether. Here our focus is on a few major hormones. We shall take them one by one, starting with the hormones produced by the pancreas.

11.6.1 Pancreas

The two major hormones secreted by the pancreas, as you may already be aware, include insulin and glucagon. Let us get to know about their role.

A) Insulin

Insulin is secreted by the a-cells of the islets of Langerhans and was first isolated from the pancreas in 1922 by Banting and Best. Almost overnight this changed the outlook for the severely diabetic patient from one of rapid decline and perhaps death to that of a nearly normal person. Historically, insulin has been associated with 'blood sugar' and accordingly insulin has profound effects on carbohydrate metabolism. In fact it is now known that insulin affects fat and protein metabolism as much as it does carbohydrate metabolism.

Insulin is associated with energy abundance. When there is great abundance of energy-giving foods in the diet, especially excess amounts of carbohydrates, insulin is secreted in large quantity. Hence insulin plays an important role in storing the excess energy substances. Thus carbohydrates are stored as glycogen mainly in the liver and muscles and as fats in the adipose tissues. In the case of proteins, insulin has a direct effect in promoting amino acid uptake by cells and conversion of these amino acids into protein. So let us see the effect of insulin on the metabolism of these molecules.

- Effects on carbohydrate metabolism

Immediately after a high-carbohydrate meal, the glucose that is absorbed into the

blood causes rapid secretion of insulin, The insulin in turn causes rapid uptake, storage and utilization of glucose by almost all the tissues of the body, but especially by the muscles, adipose tissue and liver. Glucose enters all cells by facilitated diffusion. In muscles, adipose and a variety of other tissues, insulin facilitates glucose entry into the cells by increasing the number of glucose transporter in the cell membrane. The glucose transporter molecule is called GLUT 4. In the liver, glucose induces hexokinase and this increases the phosphorylation of glucose. As a result, the intracellular concentration of free (unphosphorylated) glucose remains low, facilitating the entry of glucose into the cell.

Insulin influences the intracellular utilization of glucose in a number of ways as shown in Figure 11.9. In a normal person, about half the glucose ingested is converted to energy through the glycolytic pathway and about half is stored as glycogen or fat. Glycolysis (breakdown of glucose into pyruvate and lactate) decreases in the absence of insulin, and the anabolic processes of glycogenesis (synthesis of glycogen) and lipogenesis (synthesis of fat) are affected. Only 5% of an ingested glucose load is converted to fat in an insulin-deficient diabetic.

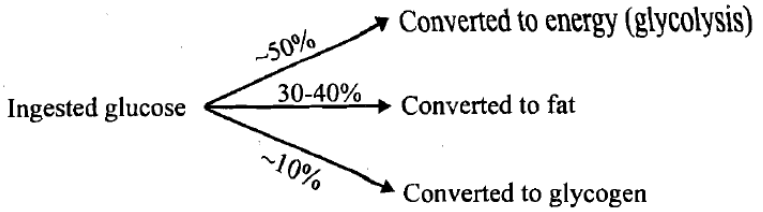


Figure 11.9: Utilization of glucose

Insulin increases hepatic glycolysis by increasing the activity and amount of several regulatory enzymes including glucokinase, phosphofructokinase-I (PFK-I) and pyruvate kinase. Enhanced glycolysis increases glucose utilization and thus indirectly decreases glucose release into the plasma. Insulin also decreases the activity of the enzyme glucose-6-phosphatase, an enzyme found in liver but not in muscle. Since glucose-6-phosphate cannot cross the plasma membrane of the liver cell (only glucose can), this aetion of insulin results in the retention Of glucose within the liver cell. In liver and muscle, insulin stimulates the conversion of glucose to glucose-6-phosphate, which then undergoes isomerization to. glucose-I -phosphate and is incorporated into glycogen by the enzyme glycogen synthase. You may recall reading about this in Unit 6, under Carbohydrate Metabolism. The activity of this enzyme is stimulated by insulin. This effect is an indirect one. Insulin activates a phosphodiesterase causing hydrolysis and conversion of cyclic-AMP (cAMP) to 5'-AMP. Hence intracellular cAMP levels are decreased leading to low activity of cAMP-dependent protein kinase which normally phosphorylates glycogen symnase. Accordingly glycogen synthase remains in the dephosphorylated active form, promoting glycogen synthesis. Additionally insulin also activates a phosphatase that dephosphorylates glycogen synthase and maintains it in the active form. Low intracellular levels of cAMP also do not promote phosphorylation of phosphorylase, keeping it in an inactive form and decreasing glucose liberation from glycogen. Hence the net effect of insulin on glycogen metabolism is highly

anabolic. The glycogen. can increase to a total of about 5-6% of the liver mass, which is equivalent to almost 100 g of stored glycogen in the whole liver.

The actions of insulin on glucose transport, glycolysis and glycogenesis occur within seconds or minutes, since they mainly involve the activation or inactivation of enzymes by covalent modulation through phosphorylation or dephosphorylation. A more long- term effect on plasma glucose involves the inhibition of gluconeogenesis by insulin. Many of the gluconeogenic enzymes are activated by glucocorticoid hormones, and to a smaller extent by a- and ß-adrenergic agents, angiotensin II and vasopressin. Insulin inhibits these same steps.
The key gluconeogenic enzyme,, is phosphoenolpyruvate carboxykinase (PEPCK). Insulin decreases the amount of this enzyme by selectively inhibiting transcription of the gene that codes for the mRNA for PEPCK.

Insulin stimulates lipogenesis from glucose. The net action of all these effects of insulin is to decrease the blood glucose level. In this action, insulin stands alone against a group of other hormones that counteract this effect.

Next, let us study about the role of insulin in lipid metabolism.

- Effects on lipid metabolism

As already mentioned above, lipogenesis is promoted by insulin. This is by:

- providing acetyl-CoA and NADPH required in fatty acid synthesis
- maintaining a normal level of the enzyme acetyl-CoA carboxylase, and
- pröviding the glycerol moiety required for triacylglycerol synthesis.

Thus effect of insulin on fat is anabolic. Insulin is also a potent inhibitor of lipolysis in liver and adipose tissue and thus has an indirect anabolic effect. Since insulin decreases tissue cAMP levels, protein kinase activity is decreased resulting in dephosphorylated form of the enzyme lipase. This is the inactive form and hence cannot cause hydrolysis of fat. In addition, insulin also exhibits direct action of its anti- lipolytic activity. It activates a phosphates as a result of which the lipase is maintained in a dephosphorylated inactive form. The net effect is decreased levels of free fatty acids, a situation that promotes glucose utilization (free fatty acids have a glucose- sparing action). In patients with insulin deficiency, lipase activity increases, resulting in enhanced lipolysis and increased concentration of free fatty acids in plasma and liver.

Insulin apparently affects the formation or clearance of VLDL and LDL, since levels of these metabolites, and consequently the level of cholesterol, are often elevated in poorly controlled diabetes. Accelerated atherosclerosis, a serious problem in many diabetics, is attributed to this metabolic defect. So now you can appreciate what important role insulin has in lipid metabolism.

Next, we shall look at the role of insulin in protein metabolism.

- Effects on protein metabolism

Insulin generally has an anabolic effect on protein anabolism since it stimulates protein synthesis and retards protein degradation. It stimulates the uptake of neutral amino acids into muscle. Among the amino acids, most strongly transported are valine, leucine, isoleucine, tyrosine and phenylalanine. The effects of insulin on general protein synthesis in skeletal and cardiac muscle and in liver are thought to be exerted at the level of mRNA translation. As already discussed, insulin depresses the rate of gluconeogenesis. Because amino acids are quantitatively the most important substrates for gluconeogenesis, the suppression of gluconeogenesis conserves the amino acids in the protein stores of the body. Accordingly, protein wasting is one of the most serious of all the effects of severe diabetes mellitus.

Finally, let us look at the effect of insulin on growth and its synergistic effect with growth hormone.

- Effect on growth and synergistic effect with growth hormone

Because insulin is required for synthesis of proteins, it is equally as essential for growth of an individual as is growth hormone. Animal experiments have shown that a depancreatized, hypophysectomized (removal of pancreas and pituitary) rat without therapy hardly grows at all. Administration of either insulin or growth hormone one at a time also causes almost no growth. However a combination of the two hormones results in dramatic growth. Thus it appears that the two hormones — insulin and growth hormone — function synergistically to promote growth, each performing a special function that is separate from that of the other. Probably, a small part of this necessity for both hormones results from the fact that each promotes cellular uptake of different types of amino acids, all of which are required if growth is to be achieved.

Having studied the role of insulin in our body, can you now suggest what would happen if there was a deficiency of insulin in our body. Read the next section and tally your responses with the effects given herewith.

- Insulin deficiency

The central role of insulin in carbohydrate, lipid and protein metabolism can be best understood by examining the consequences of its deficiency in humans. The moét prominent feature of diabetes mellitus is hyperglycemia (high blood sugar levels). This is due to .

decreased entry of glucose into cells

decreased urtilization of glucose by various tissues, and

increased production of glucose (gluconeogenesis) by the liver.

Figure 11.10 depicts the pathophysiology of insulin deficiency. Thus polyuria, polydipsia and weight loss in spite of adequate caloric intake are the major symptoms of insulin deficiency. High levels of blood sugar lead to excretion of sugar in urine (glycosuria). The urine volume is increased due to osmotic diuresis leading to polyuria and polydipsia. Due to non-utilization of glucose as a source of energy, alternative sources of energy like fats are used leading to weight loss.

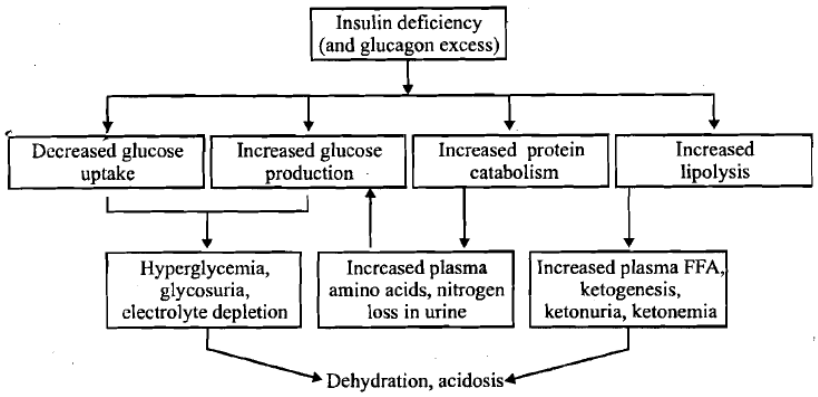


Figure 11.10: Pathophysiology of insulin deficienc

Next, let us get to know about glucagon, the other hormone produced by the pancreas.

B) Glucagon

You may already be aware that glucagon is the hormone secreted by the p-cells of the islets of Langerhans of the pancreas. Glucagon has several functions, but, diametrically opposed to those of insulin. Hence, one of the most important functions of glucagon would be to increase the blood glucose concentration. Secretion of glucagon is inhibited by glucose, emphasizing the fact that the actions of glucagon oppose those of insulin.

While insulin as we have just seen promotes energy storage by stimulating glycogenesis, lipogenesis and protein synthesis, glucagon causes the rapid mobilization of potential energy sources by various mechanisms. Let us study the effects of glucagon next.

Effects on glucose metabolism

The major effect of glucagon on glucose metabolism is:

breakdown of liver glycogen (glycogenolysis), and

increased synthesis of glucose from non-carbohydrate sources (gluconeogenesis) in the liver.

Thus liver is the primary target of glucagon action. However, both of these effects of glucagon greatly enhance the availability of glucose to the other organs of the body. Let us now see how these effects are brought about.

As a result of glycogenolysis in the liver, the blood glucose concentration increases within minutes. Glucagon, being a Group II hormone, binds to specific receptors in the hepatic cell plasma membrane which results in the activation of the enzyme adenylyl cyclase. As discussed earlier, adenylyl cyclase forms cAMP, which activates a protein kinase and causes phosphorylation of several proteins including the enzyme glycogen phosphorylase. Being active in the phosphorylated

form, phosphorylase enhances the rate of gwcogen degradation, releasing glucose to blood. Simultaneously glycogen synthase is also phosphorylated by the kinase. However, the synthase is inactive in this form and glycogen synthesis is inhibited. Thus while glucagon on one hand adds glucose to blood, on the other hand it prevents glucose from leaving blood for glycogenesis. The overall effect is a large increase in blood sugar concentration. In fact, this whole sequence of events has a cascading effect. Hence, only a few micrograms of glucagon can cause the blood glucose level to double or more within a few minutes. It should be noted here that glucagon has no effect on glycogenolysis in muscle tissue. You may recall reading about this earlier also in Unit 6, sub-section 6.7.5.

The increased level of cAMP following release of glucagon, stimulates the conversion of amino acids to glucose by inducing a number of enzymes involved in the gluconeogenic pathway. This includes the key regulatory enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK). Glucagon through cAMP increases the rate of transcription of mRNA from the PEPCK gene, and this stimulates the synthesis of more PEPCK. Additionally, glucagon also causes induction of fructose- 1,6-bisphosphatase and glucose-6-phosphatase. In fact glucagon is considered to be the most potent gluconeogenic hormone.

Next, we shall read about the effect of glucagon on lipid metabolism.

- Edect on lipid metabolism

Glucagon is a potent lipolytic agent. It increases the cAMP levels in the adipose cell. This causes activation of a kinase that phosphorylates the hormone-sensitive enzyme lipase. ?he lipase hydrolyzes the stored fat into its components. glycerol and fat. Thus glucagoil secretion results in mobilization of fat. The fatty acids released are transported by blood to be metabolized for energy by other body tissues. High levels of fatty acids in blood have a sparing action on the utilization of glucose, thus conserving low blood glucose for those tissues that are totally or in great part dependent on glucose as fuel. ~owever,'excessiveb reakdown of fatty acid in turn promotes ketogenesis (synthesis of ketone bodies-acetone, acetoacetate and 3-hydroxy butyrate). Invariably, it leads to the undesirable condition of ketosis.

With this, we end our study of the biochemical role of hormones of the pancreas. Next, we shall study about the role of hormones produced by the thyroid.

11.6.2 Thyroid

The thyroid gland produces two hormones — triiodothyronine (T 3) and tetraiodothyronine or thyroxine (T 4). Additionally, the thyroid has now been recognized to be involved in the secretion of the third hormone, calcitonin in human beings. Let us get to know about these hormones.

A) T3 and T 4

Hormones, T 3 and T 4 , are unique in that they require the element iodine for

biological activity. Hence the biological role of these two thyroid hormones has already been discussed under the functions of iodine in the section on trace minerals in Unit 10, earlier. We suggest you look up the unit once again now to study their functions. We will move on to calcitonin.

B) Calcitonin

In human beings, calcitonin is secreted not by the parathyroid glands but by the thyroid gland. It has weak effects on blood calcium but opposite those of parathormone (PTH). Calcitonin reduces blood Ca2+ concentration by decreasing resorption of bone mineral. Hence'it functions to increase deposition- of Ca2+ and phosphate in the bone. What about the parathyroid? What are the hormones produced in this gland? Read and find out

11.6.3 Parathyroid

The parathyroid gland of the adult human being consists mainly of cells called chief cells which secrete the parathyroid hormone or parathormone (PTH). You may recall reading about the parathyroid in the Applied Physiology Course, Unit 11. Let us study about the role of the parathormone hormone here once again.

Parathormone

This hormone is intimately associated with the metabolism of calcium and phosphorus in the body. Much of the effect of PTH on its target organs is mediated by the second messenger, cAMP. This ultimately results in the activation of the intracellular enzymes or proteins that finally mediate the biologic actions of the hormone. These actions are concerned with calcium and phosphate metabolism, which are discussed next.

- Effect on calcium homeostasis

We have already seen that calcium ion regulates a number of important physiologic and biochemical processes. To ensure that these processes operate normally, the plasma Ca2+ concentration is maintained within very narrow limits. The physiologic maintenance of calcium balance depends on the long-term effects of PTH acting on intestinal absorption.

This effect is brought about in an indirect manner by stimulating the synthesis of calcitriol (vitamin D hormone) in the intestine. Calcitriol then promotes the absorption of Ca2+ from the intestinal lumen. (The details of this have already been discussed in the section on vitamin D, in Unit 10). However, if there is a prolonged deficiency ofcalcium in the diet resulting in inadequate intestinal absorption of Ca2+, then P TH acts directly on bone and kidney. P TH increases the rate of dissolution of bone, both organic and inorganic phases and the concentration of Ca2+ in the extracellular fluid (ECF) is increased. P TH also reduces the renal clearance or excretion of calcium. This again results in an increased concentration

of Ca2+ in ECF. The most rapid changes occur through the action on the kidney. But because bone contains the maximum amount of Ca2+, the largest effect is from the bone.

- Effect on phosphate homeostasis

Calcium in the body is usually associated with phosphate. The hydroxyapatite crystal in bone consists mainly of calcium phosphate. Hence phosphate is released along with calcium from bone following the action of PTH on dissolution of bbne mineral. But at the same time, PTH increases renal phosphate clearance by diminishing proximal tubular reabsorption of phosphate ions. So the net effect of P TH on bone and kidney is to increase the ECF calcium concentration and decrease the ECF phosphate concentration.

This ensures that calcium and phosphate concentrations are maintained in an inverse proportion. This is essential to prevent the development of a supersaturated concentration of calcium and phosphate in the plasma and precipitation of the two mineral ions. PTH also increases the rate of tubular reabsorption of sodium, potassium and amino acids.

Having looked at the role of parathormone, next let us study about the hormones produced by the adrenal medulla and the cortex.

11.6.4 Adrenal Medulla

The adrenal medulla produces the catecholamine hormones dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline). The major product of the adrenal medulla is epinephrine which constitutes 80% of the catecholamines in the adrenal medulla. Tyrosine is the immediate precursor of catecholamines.

The effect of the catecholamine hormones depends on the kind of receptors they bind to. They act through 2 major classes of receptors called a-adrenergic and ß-adrenergic. Each is divided into 2 subclasses— cq and 02 and ßl and

Hormones classification is based on the relative order of binding of various agonists (molecules that mimic the action of the horrnones) and antagonists (molecules that oppose the action of the hormones). Different catecholamine hormones have different binding affinities for the four types of the adrenergic receptors. In fact no hormone has exclusive affinity for any single receptor.

A hormone could bind to more than one type of receptor. Further the degree of affinity for each receptor would be variable. Hence the effect a particular hormone can exert would depend upon the sum total of the effects of the individual receptor and the affinity with which the hormone binds to the receptors.

NOTES

Table 11.5: Actions elicited through adrenergic receptors

α_1	α_2	β_1	β_2
Increased glycogenolysis Smooth muscle contraction • Blood vessels • Genitourinary tract	Smooth muscle relaxation • Genitourinary tract Smooth muscle contraction • Some vascular beds Inhibition of • Lipolysis • Renin release • Platelet aggregation • Insulin secretion	Stimulation of lipolysis Myocardial contraction • Increased rate • Increased force	Increased hepatic Gluconeogenesis Glycogenolysis Increased muscle Glycogenolysis Increased release of Insulin Glucagon Renin Smooth muscle relaxation Bronchi Blood vessels Genitourinary tract Gastrointestinal tract

Table 11.5 indicates that some of the functions of the a receptors are excitatory while others are inhibitory. Similarly ß-receptors also have both, excitatory and inhibitory effects. Hence a and receptors are not identified by excitation or inhibition but only with the affinity the hormone has for the receptors in a specific target organ. It is also generally believed that there is a less distinct division of a receptors into and 0.2.

Catecholamines belong to Group II class of hormones. Hence their receptors are located on the cell surface. However the second messenger is different. 02 and ß adrenergic receptors function through cAMP while the adrenergic receptor utilizes Ca2+ or phosphatidyl inositols as indicated in Table 11.1 earlier.

What is the role of the hormones epinephrine and norepinephrine? Let's find out next.

Epinephrine and Norepinephrine

Both these hormones have somewhat different effects in exciting the a and ß receptors. Norepinephrine binds to and activates mainly the a receptors, while the receptors are activated to a lesser extent. On the other hand, epinephrine binds to and activates both and receptors to an equal extent. Therefore, the relative effects of nor epinephrine and epinephrine on different target organs will be determined

by the type of receptors in the organs. If they are all ß receptors, then epinephrine will have greater effect on- that target organ.

Stimulation of adrenal medulla causes release of both the hormones into circulation. The circulating norepinephrine causes constriction of essentially all the blood vessels of the body. Some of the effects include increased activity of the heart, inhibition of the gastrointestinal tract, dilation of the pupils of the eye etc.

Epinephrine causes almost the same effects but with some differences since it activates a and ß receptors to the same extent. It has greater effect on cardiac stimulation than norepinephrine. It also causes only weak constriction of the blood vessels in the muscles. A further difference relates to their effects on tissue metabolism. Epinephrine has 5 to 10 times as great a metabolic effect as norepinephrine. It can increase the metabolic rate of the whole body to as much as 100% above normal.

Epinephrine increases glycogen breakdown in liver and muscle tissue. ß-adrenergic stimulation activates glycogen phosphorylase and inhibits glycogen synthase through cAMP-dependent mechanism. You are already familiar with the effect of epinephrine on glycogenolysis in liver and muscle and release of glucose into the blood (carbohydrate metabolism) and a revision of that portion would be merited. Catecholamines also stimulate hepatic gluconeogenesis through a-adrenergic mechanisms. They increase delivery of gluconeogenic precursors to the liver — lipolytic (glycerol) and glycogenolytic (lactate and pyruvate). In addition, there is also increased hepatic uptake of amino acids. The net effect is increased availability of glucose in circulation.

Both epinephrine and norepinephrine activate hormone-sensitive lipase in adipose tissue, liver, heart and skeletal muscle. Any kind of physical or mental stress (or alarm) results in the secretion of these hormones into blood. Accordingly these hormones are also referred to as fight or flight hormones since they help the person through their actions to • fight the stress condition. In the case of animals, they decide to either stand and/or fight the alarm condition or to run away from the situation. Hence the alternative name given is flight.

11.6.5 Adrenal cortex

The hormones secreted by the adrenal cortex are called corticosteroids. They all have a steroid structure and in fact are synthesized from cholesterol. There are three types of adrenocortical hormones — glucocorticoids, mineralocorticoids and androgens. Qunatitatively the first two hormones are more important. Only small amounts of androgens are secreted. We will be discussing the two major classes of the hormones, next.

A) Glucocorticoids

The glucocorticoids are 21-carbon steroids with many actions. One of their most

important functions is to increase the blood glucose concentration and hence get their name. Cortisol, also known as hydrocortisone, is the predominant glucocorticoid in humans. Corticosterone is less abundant in humans, but is the main glucocorticosteroid in rodents. Glucocorticoid hormones affect basal metabolism, host defense mechanisms, blood pressure and response to stress. Let us learn about these effects and the effects of this hormone on carbohydrate, protein and lipid metabolism, next.

- Effect on carbohydrate metabolism

One of the most important functions of glucocorticoids is stimulation of gluconeogenesis in the liver, which could be as much as 6- to 10-fold. They increase all the enzymes required to convert amino acids into glucose in the liver cells. This is brought about by activating DNA transcription in the nucleus, with the formation of messenger RNAs that in turn lead to the synthesis of all the enzymes required for gluconeogenesis. Additionally, the hormones cause mobilization of amino acids from the extrahepatic tissues, mainly from the muscles. Hence, there is an increased delivery of amino acids (the gluconeogenic substrate) to the liver from the peripheral tissues, thereby promoting gluconeogenesis. Thus glucocorticoids counteract the effects of insulin at numerous steps in glucose homeostasis. Glucocorticoids also increase hepatic glycogen deposition by promoting activation of the enzyme glycogen synthase. In this regard, they lesemble insulin.

Glucocorticoids also cause a moderate decrease in the rate of glucose utilization in the body. Though the exact mechanism is not known, glucocorticoids may depress the reoxidation of NADH to NAD+ resulting generally in decreased glycolysis. Both the increased rate of gluconeogenesis and the moderate reduction in the rate of glucose utilization by the cells result in increased blood glucose concentration. This increase could even be 50% or more above the normal.

- Effect on protein metabolism

One of the principal effects of cortisol is reduction of protein stores in essentially all body cells except those of the liver. This is brought about by decreased protein synthesis, as well as, by increased catabolism of protein already present in the cell. There is decreased amino acid transport into extrahepatic tissues, as well as, decreased synthesis of RNA especially in muscle and lymphoid tissue. Great excess of glucocorticoids can result in extreme wasting of muscle tissue. The oppoSte effect is seen in the liver where there is increased synthesis of protein.

- Effect on fat metabolism

Glucocorticoids promote mobilization of fat from the adipose tissue. This increases the concentration of free fatty acids in the plasma, consequently increasing their utilization as energy sources. Cortisol may also have a direct effect on promoting oxidation of fatty acids in the cells. This helps in conserving glucose in the condition of low blood glucose concentration.

Exces:s of cortisol secretion or adlninistration can cause obesity, with excess

deposition of fat in the chest and head regions of the body giving a buffalo-like torso and a rounded 'moon face'.

- Effect in stress and inflammation

All types of stress, physical and neurogenic, cause an immediate and marked increase in secretion of cortisol. When large amounts of cortisol are secreted or administered, it has anti-inflammatory effects. It can, not only prevent inflammation from occurring, but once the inflammation has started, it can also cause rapid resolution of inflammation. It affects several stages in the inflammation process to bring about these effects. Cortisol also blocks the inflammatory response to allergic reactions.

- Effect on blood cells and on host defense mechanisms

Cortisol decreases the number of eosinophils and lymphocytes in the blood. Administration of large doses of cortisol also causes significant atrophy of all the lymphoid tissue throughout the body, which in turn decreases the output of both T cells and antibodies from the lymphoid tissue. The level of immunity is greatly decreased. At the same time, the ability of glucocorticoids in suppressing immunity makes them among the most useful of all drugs to prevent immunological rejection of transplanted organs like kidneys, heart etc.

Excess of glucocorticoid leads to bone dissolution by decreasing bone formation and increasing bone resorption. This can ultimately lead to osteoporosis. Hence using corticosteroids as therapeutic agents should be done only under expert medical supervision.

- Permissive effects

Small amounts of glucocorticoids must be present for a number of metabolic reactions to occur, although the glucocorticoids do not produce the reactions by themselves. This effect is called permissive action. Permissive effects include the requirement for glucocorticoids to be present for glucagon and catecholamines to exert their calorigenic effects, for catecholamines to exert their lipolytic effects and for catecholamines to produce pressor responses and bronchodilation. Having studied about the glucocorticoids, next let us get to know more about other hormone produced by the adrenal cortex i.e. the mineralocorticoids.

B) Mineralocorticoids

Mineralocorticoids, like glucocorticoids are also 21-carbon steroids. Aldosterone is the most potent hormone and accounts for about 90% of all mineralocorticoid activity. Deoxycorticosterone is secreted in about the same amount as aldosterone, but has only 3% of the mineralocorticoid activity of aldosterone. Even cortisol which is the major glucocorticoid secreted by the adrenal cortex has significant amount of mineralocorticoid activity. Let us get to know about the role of these hormones in our body.

- Effects on transport of ions

The major actions of aldosterone and other steroids with mineralocorticoid activity are on ion transport. They increase the reabsorption of Na+ from the urine, sweat, saliva and gastric juice. Na+ diffuse out of the urine (or sweat, saliva and gastric juice) into the surrounding epithelial cells and are actively transported from these cells into the interstitial fluid. The amount ofNa+ removed from these fluids is proportionate to the rate of active transport ofNa+. Thus mineralocorticoids cause retention ofNa+ in the extra cellular fluid (ECF). They also function in the transport of K + and to a lesser extent, the transport of H+. In fact the increased amounts ofNa+ are exchanged for K + and H+ in the renal tubules, resulting in excretion of K+ causing K+ diuresis. Therefore, the net effect of excess aldosterone in the plasma is to increase the total quantity of Na+ in the ECF while decreasing the K + concentration. In the kidneys, they act primarily on the epithelium of the cortical collecting ducts.

The transport of these ions involves an active transport mechanism through the Na+/ K + pump. Energy for the functioning of this pump is provided by hydrolysis of ATP. Aldosterone increases the activity of several mitochondrial enzymes, and this could result in the generation of the ATP required to drive Na+/K+ pump. The proteins synthesized also include the Na+-K+ ATPase molecules. The NADH/ NAD+ ratio increases and when the NADH is oxidized in the mitochondrial respiratory chain, ATP will be formed. One of the mitochondrial enzymes which increases in concentration is citrate synthase. This is brought about by the action of aldosterone in directly inducing the transcription of the enzyme gene. Aldosterone also increases the number of membrane Na+ channels, and this presumably increases intracellular Na+. Additionally, aldosterone binds to the cell membrane arid by a rapid nongenomic (not involving the gene) action increases the activity of membrane Na+-K+ exchangers. This produces an increased intracellular Na+ and the second messenger involved is inositol triphosphate.

- Effect on extracellular fluid volume

When Na+ is reabsorbed by the kidney tubules, there is simultaneous osmotic absorption of almcst equivalent amounts of water. Therefore, the extracellular volume increases almost as much as the retained sodium. Hence the net effect is that there is not much change in the ECF concentration of sodium. Finally, let us study about the role of hormones produced by the pituitary gland.

11.6.6 Hypophysis (The Pituitary Gland)

The newer name for the pituitary gland is hypophysis. Physiologically (you may recall reading in the Applied Physiology Course, Unit 11) it is divisible into two distinct portions — the anterior pituitary also known as the adenohypophysis and the posterior pituitary also known as the neurohypophysis.

The anterior pituitary secretes six important hormones and several less important hormones. Two of the six important hormones, namely the thyroid stimulating hormone and the growth honnone, are already discussed above. The posterior pituitary secretes two hormones—vasopressin and oxytocin. Let us get to know

about the role of these hormones, starting with the hormones of the anterior pituitary i.e. the adenohypophysis.

NOTES

A) Adenohypophysis (Xnterior Pituitary)

Thyroid stimulating hormone (TSH)

TSH is also referred to as thyrotropin or thyrotropic hormone. The target gland of TSH is specifically the thyroid gland. TSH regulates the functions of the thyroid gland. TSH has several acute effects on thyroid function. These occur in minutes and involve increases of all phases of the biosynthesis of the two hormones of the thyroid gland — triiodothyronine (TN) and tetraiodothyronine or thyroxine (T 4). These include iodide concentration, organification, coupling and thyroglobulin hydrolysis. You have already come across these two hormones in the section on iodine in Unit 10. TSH also has several chronic effects on the thyroid gland. These require several days and include increases in the synthesis of proteins, phospholipids and nucleic acids in the thyroid gland as well as increase in size and number of thyroid cells. All this leads to increased production of T3 and T 4 that will in turn exert their effects in the body. Thus long-term metabolic effects of TSH are due to the production and action of the thyroid hormones.

Growth hormone (GH)

GH exerts its effects on all or almost on all tissues of the body. It is required for postnatal growth and is also called somatotropic hormone or somatotropin. Thus it is required for normal carbohydrate, lipid, protein and mineral metabolism as discussed herewith.

- Effect on growth

GH causes growth of almost all tissues of the body that are capable of growing. The effects of GH on growth are mediated by growth factors called somatomedins. They are secreted by liver and other tissues in response to stimulation by GH. The first of these factors isolated was also called 'sulfation factor' because it stimulated the incorporation of sulfate ·into cartilage. However, since it also stimulated collagen formation, its name was changed to somatomedin. In human beings, the growth related effects are primarily mediated by somatomedin C or insulin-like growth factor I (IGF-I) and to a much lesser extent by insulin-like growth factor II (IGF-II). Effect on protein metabolism

GH. increases the transport of amino acids into muscle cells resulting in an increased protein synthesis. There is increased positive nitrogen balance. At the same time, there is a decrease in the breakdown of cell protein resulting in a decrease in plasma and urinary levels of amino acids and urea. The net effect is a generalized increase in protein content of the body.

Thus GH is a protein anabolic hormone. Increase in protein synthesis is accompanied by increased transcription of DNA to form RNA and increased translation of RNA

to form proteins. In these respects GH actions resemble some of the actions of insulin.

- Effect on carbohydrate metabolism

GH generally antagonizes the effects of insulin. GH decreases peripheral utilization of glucose and simultaneously increases hepatic production by stepping up gluconeogenesis. Both these effects lead to increased blood glucose levels. GH increases liver glycogen, probably from activation of gluconeogenesis from amino acids. Impairement of glycolysis may occur at several steps. There is mobilization of fatty acids from triacylglycerol stores in adipose tissue which may also contribute to the inhibition of glycolysis in muscle. Hence prolonged administration of GH may cause diabetes mellitus.

- Effect on lipid metabolism

As already mentioned above, GH promotes the release of free fatty acids and glycerol from adipose tissue, increasing the level of circulating free fatty acids. This results in increased oxidation of fatty acids in the liver. This could also lead to increased ketogenesis, particularly in diabetes. The increased mobilization of fat has a protein-sparing action that promotes protein deposition and growth.

- Effect on mineral metabolism

GH, probably through IGF-I promotes a positive calcium, magnesium and phosphate balance and causes the retention ofNa+, K + and Cl-. A positive calcium, magnesium and phosphate balance is a result of action of GH in bone where it promotes growth of long bones at the epiphyseal plates in growing children. GH also increases formation of cartilage. Having learnt about the role of the hormones produced by the anterior pituitaor, we move on to the hormones produced by the neurohypophysis i.e. the posterior pituitary. What role do they play? Let's find out.

B) Neurohypophysis (Posterior Pituitary)

Within this, we shall study the role of oxytocin and vasopressin.

Oxytocin

Oxytocin, as you may already know, acts primarily on the uterus and breasts. Let us get to know what effect it has on these organs.

- Effect on the uterus

Oxytocin causes contraction of the smooth muscle of the uterus. In late pregnancy, the uterus becomes very sensitive to oxytocin which is accompanied by a marked increase in the number of oxytocin receptors and the mRNA of the oxytocin receptors. Secretion of oxytocin is increased during labour leading to high plasma levels. Oxytocin may also act on the non-pregnant uterus to facilitate sperm transport.

- Effect on the breas ts

Oxytocin plays a very important role in lactation. Jt causes milk to be expressed from the alveoli into the ducts so that the baby can obtain it by suckling. This mechanism is called milk letdown or milk ejection.

Finally, let us review the biochemical role of yasopressin, the other hormone produced by the posterior pituitary.

Vasopressin

This hormone is also called antidiuretic hormone (ADH). Its principal physiologic effect is the retention of water by the kidney. Extremely minute quantities of ADH, even as little as 2 nanograms when injected into a person can cause antidiuresis, that is, decreased excretion of water by the kidneys and hence the name ADH. In the presence of ADH, the permeability of the collecting tubules and ducts to water increases greatly and allows most of the water to be reabsorbed as the tubular fluid passes through these ducts. This results in conserving water in the body and producing very concentrated urine. In the absence of ADH, the urine is not concentrated and may be excreted in amounts exceeding 2L/day. ADH receptor is linked to adenylyl cyclase system which causes synthesis of cAMP. The cAMP is thought to mediate the effects of ADH in the renal tubule.

Higher concentrations of ADH have an effect of constricting the arterioles everywhere in the body and therefore of increasing the arterial pressure. Hence this hormone is also called vasopressin.

With our discussion on vasopressin, we end our study on the biochemical role of few of the hormones in our body.

11.7 LET US SUM UP

This unit focused on the study of hormones. Initially the unit presented an overview on the endocrine system highlighting the hormones produced by the endocrine glands. 'The classificatio of hormones and the various components involved in the mechanism of hormonal action was discussed subsequently.

Finally, the biochemical role of some important hormones, namely, the insulin, glucagon secreted from the pancreas, the T3 and the T 4 secreted from the thyroid, parathormone of the parathyroid gland, epinephrine / norepinephrine and glucocorticoid, minerocorticoid, produced by the adrenal medulla and adrenal cortex and thyrotropin, growth hormone, oxytocin and vasopressin from the pituitary, were discussed.

11.8 GLOSSARY

Homeostatic responses : body's internal self-correcting mechanism that helps

it maintain systemic balance following disruption.

Hormone : any substance in an organism that carries a signal to generate some sort of alteration at cellular level.

Receptor : cell-associated recognition molecules.

Signal Transduction : the biochemical events that conduct the signal of a hormone or growth factor from the cell exterior, through the cell membrane, and into the cytoplasm. This involves a number of molecules, including receptors, proteins, and messengers.

Transcription : the synthesis of an RNA copy from a sequence of DNA (a gene); the first step in gene expression.

11.9 CHECK YOUR POGRESS

1) Define the following

a) Endocrine system

b) Hormone

2) What is a receptor? What is its role in maintenance of hormonal action?

3) Give the simple classification of hormones based on the mechanism of action.

4) Explains the role play by Horomones in the Develpoment of the body ?

5) What do you mean by G protein Coupled Receptor?

UNIT

12

INBORN ERRORS OF METABOLISM

STRUCTURE

12.1 LEARNING OBJECTIVE

After studying this unit, you should be able to:

explain general concepts underlying inborn errors of metabolism,
ideqti$' the specific biochemical defect in each disease
list the clinical symptoms occurring in each disease,
compare the incidence/prevalence of different diseases,
name the mode of inheritance, and
formulate therapeutic diets wherever useful.

12.2 INTRODUCTION

As you have com leted the study of Units I-I l, you have virtually finished studying this Course of Nutritional Biochemistry. You saw that this course consisted basically of two arts — chemistry of hysiologically im ortant molecules and the metabolic athways undergone by these molecules in our body. You may wonder then what is Unit 12 all about. If Units I to II are considered as 'Basic Knowledge', then Unit 12 may be looked u on as 'A lications'. erha s, more a tly it may be described as "Misa lications". Yes, as you would have guessed rightly, this unit

will give you a brief insight into what would ha en if normal metabolic ste s (about which you learnt earlier) do not take lace correctly.

When we fall ill, we ask ourselves why something went wrong in our body. But after learning about the myriad of metabolic reactions occurring in our body and the fine- tuning of homeostatic regulatory mechanisms which are in place, you must have realized how efficient nature really is in giving us good health

In this unit we will consider specific inborn errors of metabolism. It is impossible to discuss all the known diseases. The diseases which will be discussed have been chosen for different reasons like:

they are of some historical importance,

they occur commonly in our country,

they can be managed at least to some extent by nutritional intervention,

mass screening programs have been conducted worldwide for early detection, and

surprisingly, no clinical symptoms are encountered.

12.3 INBORN ERRORS OF METABOLISM - GENERAL CONCEPTS

Sir Archibald Garrod coined the term 'Inborn Errors of Metabolism' in 1902. He said that there are a number of metabolic abnormalities that are congenital, present throughout life and hereditary. He suggested that metabolic blocks resulting from an abnormality of certain specific enzymes caused the hereditary diseases.

This can be because the enzyme molecule is completely absent and hence unable to do its work. Or more likely, the protein molecule is present, but there is a genetically determined mutation at the reactive site. It may also be possible that the enzyme is present and structurally normal too, but unable to function properly because of alterations within the cell. A deficiency of cofactors or the presence of inhibitors could produce such an effect.

At the time when Garrod described inborn errors of metabolism, it was presumed that these errors were due to the deficiency of enzymes. However, now it has been realized that these could be due to deficient working of carrier transport molecules, receptor molecules, certain hormones and of course, enzymes. For example, blood is a vehicle for transporting various molecules from intestine, the site of absorption, to the liver. From there, these molecules are transported to all the tissues which need them, or if not needed at that time, to tissues which store them.

It is now known that all molecules transported in blood are carried bound to different kinds of proteins called carrier transport molecules. Thus no molecule is really 'free' in blood. You have also read that only after a hormone binds to a

protein molecule present in a cell can it exert its effect on the cell. This protein molecule is called a receptor molecule. Thus it helps the hormone to recognize the cell where it has to act.

NOTES

Now to get back to our discussion on general concepts of inborn errors of metabolism, you will notice that one common factor among inborn errors of metabolism is that these defects are concerned with protein molecules. You have already learnt that synthesis of proteins is genetically controlled. Hence, these diseases are manifested when genetic mutations occur and also become inherited. What do we mean by genetic mutation? A mutation occurs when a DNA gene is damaged or changed in such a way as to alter the genetic (inherited) message carried by that gene. What is a 'gene '?

Under metabolism of nucleotides that gene is a segment of the DNA (nucleic acid) chain that contains the instructions for a complete protein. Hence, the gene is the fundamental unit of genetic information. These genes contain instructions that affect not only structure, size, colour and other physical attributes but also intelligence, susceptibility to disease, life span and even some aspects of behaviour.

All these characteristics are transferred from one generation to another. A lot of information is now available as to how various characteristics (e.g. colour of hair and eyes, height etc) are transferred from parents to the offspring. This is the science of genetics. It was Mendel, a monk, who carried out experiments on garden pea and formulated the principles of genetics. These are called Mendelian Laws of Inheritance (Mendelian Genetics). To get a basic idea of this concept, we suggest you look up the NCERT Biology book for Class XI and XII or any other reference related to the topic.

Mendelian Laws of Inheritance holds good for animal species including human beings. In fact not only physical characteristics, but even diseases are inherited from parents, again according to these laws of inheritance. Thus it could be an autosomal (chromosomal) recessive characteristic or a dominant characteristic or could be a sex-linked (i.e. through X or Y sex chromosomes) inherited characteristic. As these names suggest, inheritance is in less number of off springs in recessive inheritance. When it is dominant, many more offspring will get the disease. In sex-linked diseases/ characteristics, the disease is inherited only from the father or the mother, as the case may be.

In most of the diseases, failure of a metabolic step leads to the excretion of intermediate products which cannot be carried further along the metabolic path. Very often accumulation of these intermediates leads to clinical symptoms. The identification of the intermediate metabolic products in urine aids in diagnosis of the disease. In many diseases, the clinical symptoms are variable and non-specific. Hence biochemical estimations in biological fluids such as blood, urine etc. is essential to confirm the existence of the particular disease

Clinicql symptoms could appear as early as in the first week of birth. Hence,

timely diagnosis is of prime importance. Further, at the present time, no effective treatment is available for most of the diseases. Thus the inevitable end is death of the affected person, very often within 1-2 years of birth. Hence genetic counseling and prevention of such births is the only course open. Accordingly, prenatal diagnosis of many of the diseases is now regularly resorted to. For this, the amniotic fluid is analyzed and assayed for the enzyme in question, or for the metabolite that accumulates or for the defective product synthesized.

The estimated burden of inborn errors of metabolism (IEM) is 3-4 per 1000 live births. About 20% of acute illnesses in newborns in the developed countries are due to IEM. In India, there is a paucity of information regarding incidence/ prevalence of IEM. This is because we have a poor system of record keeping.

Further, the actual figures would be much higher than what is available, since deaths due to IEM would go unreported due non-diagnosis of the disease. This would be particularly true in our vi Ilages where the primary health care services are non-existent or poorly availed of by the people. According to an ICMR multicentric study, about 5% of genetic causes of mental retardation are due to IEM. Other studies have quoted a figure of 0.5-2.5%.

Next, moving on to the treatment of IEM. Treatment, wherever possible, is Still only symptomatic i.e. alleviating the pain etc. or preventing the disease from progressing to more severe consequences. Nutritional management has been useful in many instances. However the ideal treatment would be adoption of devices which would enable the cell to start synthesizing the right kind of protein. This evidently would involve gene manipulation, which sounded far-fetched just a few years back. But enormous progress made recently in the field of molecular genetics has made gene therapy in humans a potential therapeutic approach in the near future. This is the advent of recombinant DNA technology or what is commonly referred to as 'genetic engineering '.

What is genetic engineering? Look at Figure 12.1 which illustrates the genetic engineering process. This involves isolation and manipulation of DNA, wherein the defective portion of the DNA molecule (wrong base sequences) is removed. The right sequences are inserted at this point to produce the correct desired DNA molecule. Molecules containing both human and bacterial DNA sequences joined together have been produced. These are called chimeric DNA.

The hybrid DNA molecule is then inserted into small circular duplex DNA molecules called plasmids which are present in bacteria. These are called vectors. When the plasmids replicate or duplicate, identical DNA molecules are produced in large numbers. This method of DNA amplification is called cloning.

The correct DNA molecule is cleaved off from the plasmid. If this is inserted into cells where the DNA was originally defective, the cell will now also replicate the correct gene, which in turn can direct the synthesis of the right protein molecule.

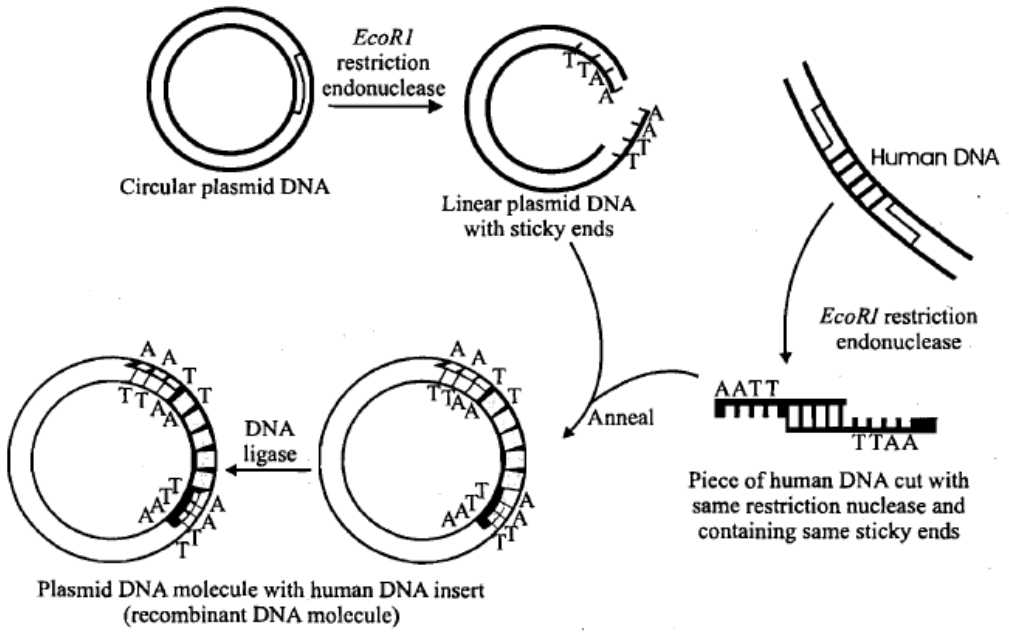


Figure 12.1: Genetic Engineering

Extensive research is needed before gene therapy can become a reality. The first step in this quest was to obtain complete information about the human gene. It is now suspected that virtually every disease, to some extent, has a genetic component. And hence a 'Gene Hunt' was launched. You should read Box I for more information on this very interesting subject.

We just discussed how DNA can be cloned. You must have read about 'Clomngi in the newspapers. Does DOLLY ring a bell? Yes, Dolly was the first clone. It was the first mammal, a sheep, which was cloned from cells of an adult animal in 1986 by scientists of Roslin Institute in Scotland. Then Polly was created, a lamb with a human gene in its genome. Italian researchers have made a strain of-pigs that carry' human genes in their hearts, livers and kidneys. This gives rise to the possibility that genetic disorders may someday be treated with drugs supplied by sheep and other animals or provide organs for transplantation. Subsequently many animals are reported to have been cloned. These include pigs, goats, cats, rabbits, mice, mule and horse. So there is a large cloned animal farm. Wait, hold your breath — a cloned human child is expected to be born soon!

Before going through this unit, you must revise the earlier units to reinforce your knowledge regarding Metabolic Pathways. It will also be interesting for you to read Mendelian Laws of Genetics. You should also try to formulate therapeutic diets for diseases where such dietary regimen is beneficial. As you are specializing in dietetics, this will not only be a challenge to you, but at the same time, it will also be very interesting and possible for you.

With this knowledge, let us move on to learn about the inborn errors of metabolism.

As you read on, you will find that these inborn errors are specific to defects in the metabolism of carbohydrates, proteins, fats etc. .

Box I : The Gene Hunt

A Human Genome Advisory committee was formed in 1989. Its goal was to map (decipher) the human genome (all the genes present in the human body) and spell out for the world the entire message hidden in its chemical code. The committee consisted of biologists, industry scientists and engineers, computer experts and eth icists. This Human Genome Organization had 42 scientists representing 17 nations. Hence it was called 'The UN of gene mapping'.

The human genome has now been mapped to a large extent. In addition to the above group headed by Francis Colins, a second group (privately funded), Celera Genomics founded by biologist, Craig Venter has been working on gene mapping. Thus two almost identical findings of the human genome have been published. The key findings are:

- There are approximately 30,000 genes in human beings (instead of 10,000 as was believed earlier).
- This is the same range as in mice, twice that of roundworms, three times as many as fruitflies and only five times more than bacteri
- All human races are 99.99% alike. So racial differences are genetically insignificant. This could mean we all descended from the same original mother who was from Africa.
- Most genetic mutations occur in the male of the species. So men are the agents of change. They are also more likely to be responsible for genetic diseases.
- It is quite humbling because not only are the numbers similar, the genes themselves, barring.a few, are alike in mice and men. Hence this is a pointer that 'science cannot tell man from mouse'.

Very soon people might have access to computer readout of their own genome with an interpretation of their genetic strengths and weaknesses. Hence James Watson (who elucidated the structure of DNA) has said— 'we used to think that our fate was in our stars. Now we know, in large measure, our fate is in our genes'.

Being able to read the entire genetic message and perhaps alter it, is also alarming. Such knowledge could create many moral and ethical problems. Some people feel genetic testing may constitute an invasion of privacy. It could lead to discrimination against the 'genetically unfit'. Another question raised is should someone destined to be stricken with a deadly genetic disease be told about ones fate, especially if no- cure is available? This would result in creating the so-called 'worried-well' people who are well now but have found out what might ail them in the future. Insurance companies may demand this information from clients before offering a policy. Hence, a word of caution is necessary. Will human genome mapping usher in an era of ' genetic apartheid'?

12.4 DISORDERS OF PROTEIN METABOLISM

We will first have a look at disorders of aromatic amino acids followed by other amino acids (non-aromatic). You have already learnt about the chemical structures of amino acids in Unit 2. Amino acids like phenylalanine, tyrosine, tryptophan have a phenyl (benzene) ring in their structure and hence are called aromatic amino acids. The rest of the amino acids which do not have a phenyl ring are called non- aromatic (or aliphatic) amino acids.

The diseases we will be considering here are Alcaptonuria, Phenylketonuria (PKU), Tyrosinemia— Types I, II and Neonatal Tyrosinemia and Albinism.

All the disorders included in this study have an autosomal recessive pattern of inheritance. Additionally, ocular albinism also has X-Iinked pattern of inheritance. Further occurrence of these diseases is rare. Wherever reported incidence is available, it has been given in Table 12.1.

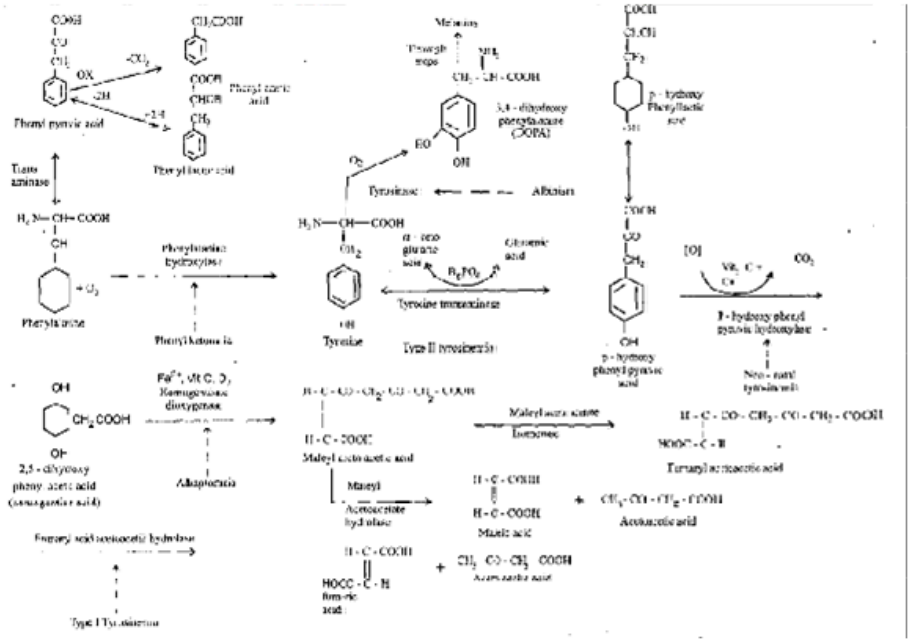
Along with the discussion presented here for each disorder, other characteristics have been listed in Tables 12.1 and 12.2. Read these tables carefully. This will make it easy for you to recapitulate, as well as, follow the information presented in the text. The chemical reactions involved are given in Figure 12.2.' In fact, Figure 12.2 gives all the chemical reactions along with the names of enzymes involved in the catabolism of the aromatic amino acids phenylalanine and tyrosine. For an easy understanding, defective step leading to diseases are coloured red, steps unaffected are coloured green and alternate pathways used to reduce accumulated metabolites (intermediates) are coloured blue. So let's get started.

12.4.1 Alcaptonuria

Alcaptonuria is a very rare hereditary disorder of tyrosine (an aromatic amino acid) metabolism. The disease is characterized by the excretion of urine, which upon standing, gradually becomes darker in colour and may finally turn black. There is a defect in the enzyme homogentisate oxidase (also called homogentisate dioxygenase). In a normal person, tyrosine is metabolized through steps to homogentisic acid and further to maleylacetoacetic acid as can be seen in Figure 12.2. Absence or deficiency of the enzyme homogentisate oxidase (homogentisate dibxygenase) causes a block in the metabolic pathway at homogentisic acid. Furtherhletabolism of homogentisic acid is prevented. Hence this intermediate compound (homogentisic acid) accumulates in blood. In order to decrease the level in blood, homogentisic acid is therefore excreted in urine. The homogentisic acid in urine, on exposure to atmospheric oxygen (air), is oxidized to a coloured pigment. Urine also has strong reducing properties and gives a violet colour with ferric chloride. These characteristics can be used for diagnosis of this disorder.

This condition — Alcaptonuria — is compatible with long life since there are np clinical manifestations. Late in the disease, there is a generalized pigmentation of

connective tissues called ochronosis. This is due to the oxidation of homogentisate by polyphenol oxidase, forming benzoquinone acetate, which polmerizes and binds to connective tissue macromolecules. This is also linked to high incidence of cardiovascular disease. Accumulation of homogentisic acid in cartilage causes arthritis in older patients. Alcaptonuria enjoys the historic distinction of being the human disease that led to elucidation of the concept of inborn errors of metabolism by Garrod.



12.4.2 Phenylketonuria (Phenylpyruvic Oligophrenia)

Phenylketonuria was discovered in 1933 and is commonly referred to as PKU. The disease is caused due to deficiency of the enzyme phenylalanine hydroxylase which is responsible for the breakdown of the essential amino acid 'phenylalanine' in the body. In most cases, less than 2% activity of normal phenylalanine hydroxylase is seen in PKU.

In normal people, the enzyme phenylalanine hydroxylase converts phenylalanine to tyrosine which is then utilized by the body. In PKU, since phenylalanine cannot be hydroxylated to tyrosine, it accumulates. In an attempt to lower the concentration, it goes through alternate pathways and is transaminated to phenylpyruvate, which in turn is reduced or oxidized to form phenyllactate and phenylacetate respectively as can be seen in Figure 12.2. Much of the phenylacetate is conjugated (combined) in the liver with glutamine and excreted in the urine as phenylacetylglutamine.

The accumulated metabolites cause damage to the CNS (Central Nervous System) resulting in acute neurological symptoms. Hence such inborn errors of metabolism with neurological abnormalities are also called neuro-metabolic disorders. Accordingly half of phenylketonurics exhibit mild to marked

microcephaly (small head). Bioterin, a protein molecule synthesized in the body, is an obligatory (binding) cofactor. Hence any metabolic condition which interferes with its production will lead to PKU symptoms.

Relief of symptoms in PKU can be achieved by nutritional management which involves use of phenylalanine restricted diets. Several low phenylalanine diets are available in the west. However the cost of importing is the limitation.

These are mainly protein hydrolysates (natural protein subjected to controlled hydrolysis to remove phenylalanine and tyrosine molecules) derived from casein or ox serum protein. Special corn starch products have also been used successfully. Based on the phenylalanine content of various foods eaten in India, you can calculate the dietary exchanges and formulate a suitable diet.

The following information given in Box 2 will help you in formulating a dietary regimen for PKU.

Box 2 : Guidelines for dietary management in PKU

- The brain of a foetus with classic PKU develops normally in intrauterine
- stage.
- The critical period of human brain growth and development extends over the first 6 months of neonatal life requiring that dietary therapy be instituted right
- after birth.
- Myelination may not be completed until 5 or 6 years of age and hence dietary restriction must be rigidly followed.
- The proportion of dietary phenylalanine that is utilized for protein synthesis varies with age-50-60% during early growth and only about 10% for normal adult.
- Blood phenylalanine levels must be maintained between 3-15 mg/dl.
- For a phenylalanine restricted diet, 50-80% of the natural protein must be replaced by a protein preparation that contains little or no phenylalanine.
- Most natural proteins contain about 50 mg phenylalanine/g protein.
- The composition of the preparation should meet all nutritional requirements for all the nutrients.
- Tyrosine must be supplemented in the diet.
- Usually one-third to one-tenth of normal phenylalanine content is recommended.
- Infections in the infant should be avoided to prevent tissue catabolism and increased phenylalanine levels in blood.
- Higher dietary phenylalanine intakes may be allowed after 6-10 years of age along with frequent clinical and biochemical supervision.
- Strict dietary restrictions should be adhered to by phenylketonuric women during pregnancy to prevent damage to the fetus.

Screening of newborn infants for PKU is compulsory in the US.

12.4.3 Tyrosinemias

NOTES

Tyrosinemias, as the name suggests, are due to defects in tyrosine catabolism. Depending on which enzyme is absent/defective, different kinds of symptoms appear. Accordingly, three distinct defects hav;e been identified. These are:

Tyrosinemia Type I

Tyrosinemia Type II

Neonatal Tyrosinemia

Let us get to know them one by one.

Tyrosinemia Ope I (Trosinosis) (Hepatorenal Orosinemia)

Tyrosinemia Ope I was originally called Tyrosinosis. Failure to properly break down tyrosine leads to an abnormal accumulation of tyrosine and its metabolites in the liver, resulting in severe liver disease. There is a progressive liver and kidney failure in this disease and hence is also called hepatorenal tyrosinemia.

What is the cause for this disorder? This occurs due to the deficiency of the enzyme fumaryl acetoacetate hydrolase (the terminal enzyme in the tyrosine pathway), which as shown in Figure 12.2, converts fumaryl acetoacetic acid to fumaric and acetoacetic acids. Deficiency of this enzyme allows the accumulation of fumaryl acetoacetate and its conversion to succinyl acetone.

Fumaryl acetoacetic acid is formed from maleylacetoacetic acid which, in turn, is the breakdown product formed from tyrosine through various steps. Maleyl acetoacetic acid can also be acted upon by the enzyme maleyl acetoacetate hydrolase to forty maleic acid and acetoacetic acid. Very often in Tyrosinemia.Type I, this enzyme is also defective leading to accumulation of maleylacetoacetate too. In this disease, there is a formation of an extremely toxic compound called succinyl acetone. This causes impairment in the movement of various molecules in and out of cells in the body. Many other enzymes in the liver do not function properly. There is also decreased synthesis of heme, which as you know, is a component of haemoglobin. Accumulation of fumaryl acetoacetate and maleyl acetoacetate leads to changes in chemical composition of DNA causing formation of tumor. Various symptoms observed in the acute form are listed in Table 12.2.

Plasma Wrosine levels are elevated (6-12 mg/dl) as are those of methionine. In the acute form, without treatment, death from liver failure ensues in 6-8 months. There is also a chronic form in which similar but milder symptoms lead to mild mental retardation and death occurs by age 10. Diet low in tyrosine and phenylalanine and in some cases, low in methionine will provide relief. When the diet is low in methionine, cysteine supplementation must be recommended. Inclusion of hematin (hydroxide of heme with the iron in the ferric state) compensates reduced heme biosynthesis.

Plasma phenylalanine levels should be maintained between 40-80 mol/L and plasma tyrosine levels between 50-150 mol/L. In this disease, prenatal diagnosis is possible, which involves measuring fumaryl acetoacetate hydrolase activity in cultured amniotic fluid cells

Tyrosinemia Type II (Richner-Hanhart Syndrome) (Oculocuta-neous Type)

This condition is caused by deficiency of hepatic tyrosine aminotransferase (also commonly called tyrosine transaminase). It catalyzes the first step in the catabolism of tyrosine forming the corresponding keto acid, p-hydroxy phenyl pyruvic acid as shown in Figure 12.2. Here too, plasma tyrosine levels are elevated (4-5 mg/dl) and tyrosine crystals deposit in cells causing inflammation. This is the more common type. There is moderate mental retardation. Tyrosine and phenylalanine restricted diet is essential.

Neonatal Wrosinemia

In neonatal tyrosinemia, the defective enzyme is p-hydroxy phenyl pyruvic hydroxylase which normally converts p-hydroxy phenyl pyruvic acid to homogentisic acid. The condition is asymptomatic and is more common in premature infants.

12.4.4 Albinism

Albinism is another congenital defect of tyrosine metabolism in which there is a decrease or absence of melanin formation (hypomelanosis). Melanin, you may already know, is a pigment which gives colour to skin, hair and eyes. Melanins are the polymers of tyrosine catabolites.

The defect is due to the deficiency of the enzyme tyrosinase (tyrosine hydroxylase), a copper-containing oxidase, which converts tyrosine to 3,4-dihydroxyphenylalanine (DOPA), as can be seen in Figure 12.2.

Normally DOPA through a series of steps gets converted to melanin. The defect may involve the entire melanocyte system or only one locus of melanocyte. In addition to the actual synthesis of melanin, there are also processes involving the formation and transport of the melanin containing bodies (melanosomes). Defects could be present in any of the above mechanisms.

There are various Wpes of hereditary albinism in which pigment is lacking on!y from certain parts of the body such as eyes, patches of skin and areas of hair. Ten forms of human oculocutaneous (defect in eye and skin) have been identified. All ten forms can be differentiated on the basis of their clinical, biochemical, ultrastructural (cell structure) and genetic characteristics. Most severe form is tyrosinase-neggtive (ty- neg) where there is a total absence of pigments. In tyrosinase-positive (ty-pos) patients, as they grow older, some pigmentation develops and visual activity increases. There is a universal, generalized or localized albinism.

Patients with universal albinism or generalized albinism have white or very pale yellow hair which is silky in texture. The pupils of the eye appear to be red and the iris is pink or bluish from reflected light. Patients are very sensitive to light (photophobia). Hence prevention of exposure to sunlight and proper protection of eyes by dark glasses is recommended. Patients are also susceptible to skin cancer since the light coloured skin permits UV rays of the sun to penetrate inside. Albinism is transmitted by autosomal recessive inheritance. Ocular albinism in addition to autosomal recessive inheritance also shows X-Iinked pattern of inheritance.

Table 12.1: Disorders of aromatic amino acid metabolism

Name of disease	Defective enzyme	Amino acid involved	Amino acid/metabolite accumulated	Reported incidence	Beneficial diet therapy
1) Alcaptonuria	Homogentisate oxidase	Phenylalanine Tyrosine	Homogentisate	2-5 per million live births	Phenlyalanine and tyrosine restriction Ascorbic acid supplementation
2) Phenylketonuria (PKU)	Phenlyalanine hydroxylase	Phenylalanine	Phenylalanine, phenylpyruvate, phenyllactate, phenylacetate	1:10,000 white newborns 1:132,000 black newborns	Phenylalanine restricted diet Tyrosine supplementation
3) Tyrosinemia Type I (Tyrosinosis)	Fumaryl acetoacetate hydrolase and maleyl acetoacetate hydrolase	Tyrosine Phenylalanine ± Methionine	Tyrosine, phenylalanine, succinyl acetone, fumaryl acetoacetate, maleyl acetoacetate, δ-amino levulenate, ± methionine	–	Diet low in tyrosine, phenylalanine, ± methionine High carbohydrate feeds (65-75% of calories), hematin supplementation
4) Tyrosinemia Type II (Richner-Hanhart Syndrome)	Tyrosine transaminase	Tyrosine	Tyrosine, p-hydroxy phenylpyruvate, p-hydroxy phenyllactate, p-hydroxy phenylacetate, N-acetyl tyrosine, tyramine	–	Phenylalanine and tyrosine restriction required less severe than Type I Early but transient dietary protein restriction
5) Neonatal tyrosinemia	p-hydroxy phenyl pyruvate hydroxylase	Tyrosine	Tyrosine, phenylalanine, p-hydroxy phenyl acetate, N-acetyl tyrosine, tyramine	–	–
6) Albinism	Tyrosine hydroxylase (tyrosinase)		–	1:20,000	–

Table 12.2: Clinical symptoms of disorders of aromatic amino acid metabolism

Name of disease	Clinical symptoms
1) Alcaptonuria	In later life, ochronosis and deforming arthritis.
2) Phenylketonuria (PKU)	Mental retardation, unusual irritability, epileptic seizures, tremors, hand posturing, rhythmic rocking back and forth, severe temper tantrums, eczema, decreased pigmentation leading to blue eyes, blond hair and fair skin, 'mousey odour'.

3) Tyrosinemia Type I	Diarrhoea, vomiting, 'cabbage-like' odour, failure to thrive, renal tubular dysfunction, vitamin D-resistant rickets,

Name of disease	Clinical symptoms
1) Alcaptonuria	In later life, ochronosis and deforming arthritis.
2) Phenylketonuria (PKU)	Mental retardation, unusual irritability, epileptic seizures, tremors, hand posturing, rhythmic rocking back and forth, severe temper tantrums, eczema, decreased pigmentation leading to blue eyes, blond hair and fair skin, 'mousey odour'.
	retardation.
5) Neonatal tyrosinemia	Generally asymptomatic, but long term effects may include mild mental retardation.
6) Albinism	Tyrosine hydroxylase positive (ty-pos)- presence of some visible pigment and white-yellow to light tan hair. Tyrosine hydroxylase negative (ty-neg)- absence of total visual pigment.

12.4.5 Arginemia (Hyperargininemia)

Arginemia is a genetic defect caused due to the defective enzyme arginase. In this disease, one step in the synthesis of urea from ammonia is affected. You have already studied urea cycle. Look up Unit 8 for this reaction. As you know, urea synthesis involves five steps. In fact there are diseases associated with each of the five enzymes involved in urea synthesis. We will have a look at just one of them

So we have seen that the enzyme defective in case of this disorder is arginase, hich in normal persons catalyzes the conversion of arginine to urea and ornithine. Hence there is also accumulation of arginine. The excess arginine competes with lysine and cystine for reabsorption in the renal tubules, which are then excreted in rine (lysine-cystinuria). Aim of dietary treatment should be to maintain blood NH levels below 50 mmoI/L. A low-protein diet lowers plasma ammonia levels and _abolishes lysine-cystinuria. 12.3.6 Homocystinuria (Homocysteinemia)

omocystinuria is a rare metabolic condition characterized by an excess of the compound homocystine in the urine. In this disease, metabolism of the sulfur containing essential amino acid, methionine is affected.

In most cases, homocystinuria is caused by reduced activity of an enzyme known as ystathionine beta-synthase (CBS). Hence as shown in Figure 12.3, the homocysteine ormed from methionine cannot be further metabolized to cystathionine. Accordingly, omocysteine accumulates. Two molecules of homocysteine combine to form the isutfide, homocystine which is excreted in urine. Various types of homocystinurias re known. The most common (Type I) is discussed here. Upto 300 mg of homocystine s excreted daily in urine. Normally homocysteine is further catabolized via ystathionine to yield cysteine. Hence cysteine becomes an essential amino acid in his condition.

Diet has to be complemented with special preparations low in or free f

methionine. Additionally, choline should be present as a methyl donor along with •ysteine. Such commercially produced formulae are not available and have to be emported at a high cost. Plasma levels of methionine must be maintained between .03-0.1 mmol/L and cystine levels between 0.037-0.085 mmol/L.

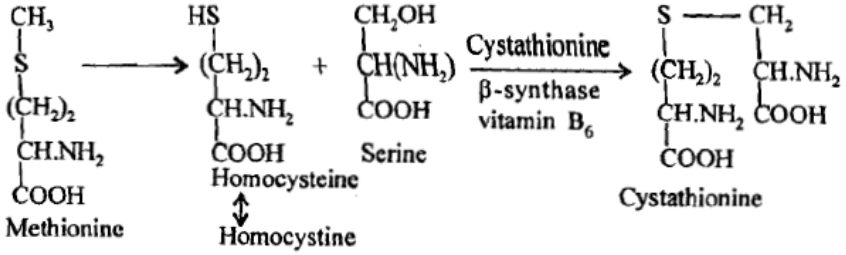


Figure12.3 : Metabolism Of methionine

Increasingly homocystinuria is being linked as a high risk factor for atherogenesi Excess homocysteine forms homocysteine thiolactone, a highly reactive intermediat that thiolates free amino groups in low density lipoproteins (LDL) and causes the to aggregate and be endocytosed by macrophages (phagocytic cells). The lipi deposits form atheromas (cysts). Homocysteine also causes lipid oxidation and platele aggregation which in turn leads to fibrosis and calcification of atherosclerotic plaques

Some dietary tips for management of this disorder have been highlighted here:

- Foods to be excluded — Meat, chicken, fish, eggs, milk and dairy products, whea flour, bajra, maize, rice, pulses, nuts and dried fruits, peas etc.
- Foods to be consumed in moderate amounts — Most vegetables and fresh fruits
- Unrestricted foods — Sugars, fats and oils, tea, coffee, salt, spices, arrowroot cornflour, sago

You will realize how diffcult it wili be to fomulate a satisfactory diet.

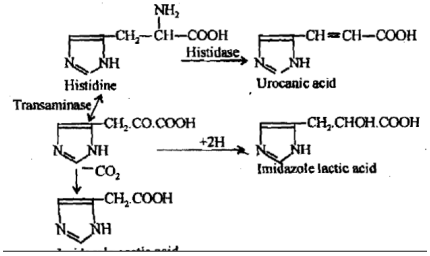
If there is some residual enzyme activity, the condition improves after treatment wit large amounts (supraphysiologic) of pyridoxine. Hence both vitamin Bcresponsiv and vitamin Bcunresponsive forms are known (see Box 3). However excess vitamil B6 for prolonged periods may cause peripheral neuropathy and liver injury. Consequentl regular monitoring is needed.

12.4.7 Histidinemia

Histidinemia is a rare hereditary metabotic disorder characterized by a deficiency o the enzyme histidase, which is necessary for the metabolism of the amino aci histidine. It is a disorder of histidine metabolism due to inadequate activity of th hepatic enzyme histidase (also called histidine a-deaminase). This enzyme normall converts histidine to urocanic acid as can be seen in Figure 12.4. Due to the metaboli block, histidine levels increase in blood. The alternate rout

e is transamination to fo imidazole pyruvic acid, which now being in excess, is

excreted in the urine. Imidazol lactic acid and imidazole acetic acid are formed by reduction and oxidation repectivel of imidazole pyruvic acid and have also been detected in urine. Very often speec development is retarded. Some cases also show signs of mental retardation. Screenin of over 20 million newborn infants has revealed a worldwide incidence of I : 10,000 Diagnosis can be done by assaying (estimating) for histidase. The assay for histidas uses skin which produces urocanate as a constituent of sweat.



12.4.8 Primary Hyperoxaluria

Primary hyperoxaluria is a rare metabolic disease characterized by high levels of)xalate in the blood and urine. The amount of oxalate excreted is unrelated to the dietary intake of oxalate. The excess oxalate arises from deamination of glycine orming glyoxylate which is not catabolized further and instead is oxidized to oxalate vhich is excreted in urine as shown in Figure 12.5. Oxalate cannot be further netabolized in the body. Normally glyoxylate and a-ketoglutaric acid undergo synergistic lecarboxylation catalyzed by a-ketoglutarate:glyoxylate carboligase to form a-hydroxy I-keto adipate. In primary hyperoxaluria, there is a deficiency of this enzyme in liver, pleen and kidney. Solubility ofoxalate is poor, hence excess of oxalate in urine leads o supersaturation. This results in precipitation of calcium oxalate leading to stone formation (oxalate calculi, nephrolithiasis). There is a recurrent infection of the trinary tract. In addition, there is a widespread deposit of oxalate crystals throughout he body called oxalosis. Death occurs in childhood or early adult life from renal failure or hypertension. Hence early diagnosis is essential.

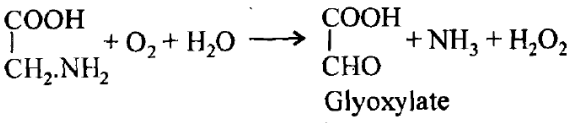


Figure 12.5: Metabolism of glycine

The major approaches to therapy have been directed either towards reduction of oxalate synthesis and excretion or towards prevention of calcium oxalate stone formation at a given level of urinary oxalate. Trapping of glycine as hippurate with benzoate administration has been attempted. Maintenance of a large urine volume is d so recommended.

12.4.9 Cystinuria (Cystine-Lysinuria)

Cystinuria is an inherited metabolic disorder characterized by the abnormal movement in the intestines and kidneys, of certain amino acids. These include cystine, lysine, arginine and ornithine. Excessive amounts of undissolved cystine in the ur ine (cystinuria) cause the formation of stones (calculi) in the kidney, bladder and/ or ureter.

In this inborn error of metabolism, there is a defect in the renal reabsorptive mechanisms as a result of which four amino acids — cystine, lysine, arginine and omithine are not reabsorbed in the kidney tubules. Hence they are found in excessive amounts in urine. Urinary excretion of cystine increases upto 30 times normal. Cystine is relatively insoluble leading to cystine calculi. Apart from this, cystinuria is benign (harmless).

12.4.10 Cystinosis

Cystinosis is a rare inherited disorder characterized by the impaired transport of cystine out of lysosomes. Cystine, as-you know, is an amino acid found in many different proteins in the body. Lysosomes, on the other hand, are membrane bound articles within cells, which aid in intracellular digestive function. Cystinosis is haracterized by the accumulation of cystine in tissues throughout the body, which can cause certain organs to malfunction.

ystinosis, therefore, is a rare lysosomal disorder characterized by the excessive eposition of cystine crystals in various tissues of the body. Hence it is also called ys!ine storage disease. Normal transport of cystine brought about by carTier molecules s defective. Kidney is particularly affected and patients usually die young from acute renal failure. Prenatal diagnosis is an established procedure. Affected foetuses have 0 to 100 times the usual amount of free cystine in most internal organs.

12.4.11 Maple Syrup Urine Disease (MSUD)

MSUD is an inherited metabolic disease caused by the inability of the body metabolize the branched-chain amino acids— valine, leucine and isoleucine. These 3 amino acids show a branch point in their structure and hence are commonly referred to as branched-chain amino acids. The disease is called MSUD because urine from affected people has a smell resembling maple sugar or burnt sugar. In the catabolism of these 3 amino acids, they first undergo transamination as shown in Figure 12.6. Transamination, you already know, is a process undergone by various amino acids during their breakdown.

The enzyme catalyzing this reaction is transaminase (or also known as amino transferase). One transaminase can act on all these 3 amino acids. Here the branched-chain amino acid reacts with any a-keto acid (like a-keto glutaric acid) and gets converted to the corresponding branched-chain a-keto acid. In normal individuals, this branched-chain a-keto acid is acted upon by a-keto acid decarboxylase complex.

This is an oxidative decarboxylation reaction similar to the reaction catalyzed by pyruvate dehydrogenase complex (following glycolysis) or a-ketoglutarate dehydrogenase complex (in citric acid cycle) . You will recollect that all such reactions require 5 cofactors-thiamin diphosphate (TDP), lipoic acid, coenzyme A, FAD and NAD+. The end product is the corresponding branched-chain coenzyme derivative. However, in MSUD, this a- keto decarboxylase is defective. Hence branched-chain a-keto acids accumulate in blood and are excreted in urine. The keto acid of L-leucine called a-keto isocaproic acid is found in highest amounts. Thus BISUD is also known as branched- chain ketonuria. In addition, the branched-chain amino acids also accumulate in blood. As all the three branched chain amino acids are excreted, it suggests that one enzyme complex is involved in the decarboxylation step.

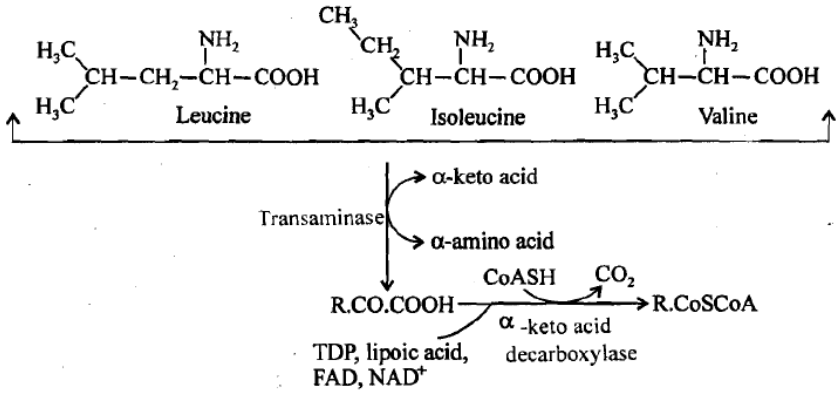


Figure 12.6 : Catabolism of branched chain amino acids

Affected infants begin to show clinical symptoms, as highlighted in Table 12.4, between the third and fifth days of life. Infants die within a few weeks or months. Hence early diagnosis, especially prior to I week is essential. This is done by measuring the level of the decarboxylase complex.

Treatment has to be started immediately after birth. MSUD is much more diffcult to treat as compared to PKU since three essential amino acids are involved. Intake of all the 3 branched-chain amino acids must be restricted. Hence.very little of natural protein can be given. Appropriate IV therapy must be initiated to correct acidosis and electrolyte imbalances which may occur by the time diagnosis is made.

Further, constant fluctuation in the tolerance for the amino acids involved, requires frequent monitoring of blood for the 3 branched-chain amino acids (plasma aminograms). The levels for leucine must be maintained between 100-700 prnoVL and between 100-400 gmoVL for valine and isoleucine. Even with strict dietary regimen, height and weight lie in the lower normal range with some mental retardation.

Administration of vitamin cofactors (cofactor therapy) is beneficial in many patients.

The basis of use of cofactors in the diseases is given in Box 3.

Table 12.3: Disorders of non-aromatic amino acid metabolism

Name of disease	Defective enzyme	Amino acid involved	Amino acid/metabolite accumulated	Reported incidence	Beneficial diet therapy
5) Cystinuria	Renal reabsorptive mechanism	Cystine	–	–	–
6) Cystinosis	Cystine transporter of lysosomal membranes	Cystine	Cystine	1:100,000	Providing adequate fluid intake for increasing cystine solubility
7) MSUD	α-keto acid decarboxylase	Branched chain amino acids	Leucine Isoleucine valine	1:185,000	Restriction of intake of all 3 branched chain amino acids. High caloric intake to prevent breakdown of endogenous protein. Correction of metabolic acidosis and electrolyte imbalances through appropriate IV therapy

Table 12.4: Clinical symptoms of disorders of non-aromatic amino acid metabolism

Name of disease	Clinical symptoms
1) Arginemia	Vomiting in infancy, avoidance of high protein foods, intermittent ataxia, irritability, lethargy, mental retardation, respiratory distress, convulsions and coma.
2) Homocystinuria	Thrombosis, osteoporosis, dislocated lenses in the eyes, other ocular abnormalities and frequently mental retardation.
3) Histidinemia	Speech development often retarded. Some patients show signs of mental retardation.
4) Primary hyperoxaluria	Calcium oxalate urolithiasis, recurrent infection of the urinary tract, widespread deposits of oxalate crystals throughout the body called oxalosis.
5) Cystinuria	Cystine calculi
6) Cystinosis	Cystine crystals deposited in tissues and organs particularly reticuloendothelial system, various renal functions impaired.
7) MSUD	Difficulty in feeding, vomiting, failure to thrive, absence of Moro reflex, extensive brain damage, retarded physical and mental development.

Box 3 : Use of cofactors (cofactor therapy) in inborn errors of amino acid metabolism

Use of nutritional intervention (wherever possible) is the first form of therapy. A second mode oftherapy, which works in some cases, is the use of vitamin cofactors.

A number ofenzymatic disturbances of amino acid metabolism have been identified, which respond to supraphysiologic (upto 100 times the physiologic dose) amounts of various water soluble vitamins including B , B6, B 12, folic acid, biotin and niacinamide.

What is the biochemical basis for this?

This could be explained by 2 general classes of mutation:

- Those that involve vitamin metabolismperse. This could occur at various steps (in intestinal absorption, plasma transport, cellular entry, conversion ofvitamin to active coenzyme form), with a different protein functioning at each step and hence subject to genetic mutation resulting in defective vitamin activity. Without the vitamin cofactor, the enzyme will not be able to work.
- Vitamin metabolism is normal but the defect involves a specific apoenzyme (protein part of the active enzyme) that needs a vitamin cofactor for normal activity.

If as a result of genetic mutation, there is loss of complete activity of any of these proteins, then the condition will not respond to vitamin therapy. But if the mutations involved are 'leaky', i.e. there is some residual activity, then administration of supraphysiologic amounts of vitamin may allow for enhanced residual activity according to mass action principles. The more residual enzyme (protein) activity present, the more dramatic the response to the vitamin therapy.

However, a word of caution must be introduced here. The side effects of these supraphysiologic doses ofthe vitamins have to be looked into. And this is as important as their therapeutic value.

12.5 DISORDERS OF CARBOHYDRATE METABOLISM

Here in this section, we will have a look at the defects involving both pentose (5-carbon) and hexose (6-carbon) sugars. The diseases which we will study, however, occur rarely. They all have an autosomal recessive pattern of inheritance. Nutritional intervention is possible in many of these diseases. As you read the text given below, follow the other characteristics listed in Tables 12.5 and 12.6. Many of the chemical reactions affected in the following disorders have already been described in Unit 6. So you should have a look at them again.

We start with the disease pentosuria.

12.5.1 Pentosuria (Essential Pentosuria)

Pentosuria is an inborn error of carbohydrate metabolism, characterized by the excessive urinary excretion of the sugar xylitol. It is caused by a defect in the enzyme xylitol dehydrogenase, by which xylitol is normally metabolized. No disabilities are incurred, and no dietary or other measures are necessary.

Since in this condition the enzyme NADP-dependent xylitol dehydrogenase is defective, the conversion of L-xylulose (which is a pentose sugar with a ketonic group) to xylitol (the corresponding alcohol formed when ketone group is reduced to CHOH group) does not take place. Persons excrete as much as 4g L-xylulose/ day in urine. However, it is simply a harmless biochemical anomaly with luckily no clinical symptoms.

12.5.2 Fructosuria (Essential Fructosuria)

Fructosuria is a genetic condition with inability to process fructose i.e. fruit sugar. It is characterized by the excretion of fruit sugar (fructose) in the urine. Nomally, no fructose is excreted in the urine. This condition is caused by a deficiency of the enzyme fructokinase in the liver.

The dietary fructose absorbed in the intestine is transported by portal vein to the liver. Here this enzyme fructokinase phosphorylates fructose to form fructose-1-phosphate. Since fructokinase is defective, conversion of fructose to fructose-1-phosphate does not take place and fructose absorbed in the intestine accumulates and is excreted in urine. However, this condition too is completely harmless.

12.5.3 Hereditary Fructose Intolerance

Hereditary Fructose Intolerance (HFI) is an autosomal recessive disorder of fructose metabolism due to a deficiency of fructose-I-phosphate aldolase activity, which results in an accumulation of fructose-1 -phosphate in the liver, kidney and small intestine.

The enzyme fructose-1-phosphate aldolase normally converts fructose-I-phosphate to dihydroxyacetone phosphate and glyceraldehyde as you have already learnt earlier in the glycolysis pathway in Unit 6. In the absence of the enzyme, fructose- 1 -phosphate accumulates. The accumulated fructose-I-phosphate inhibits glycogen breakdown and glucose synthesis, thereby causing severe hypoglycemia following ingestion of fructose. Clinical symptoms include severe abdominal pain, vomiting and hypoglycemia following ingestion of fructose or other sugars metabolized through fructose- 1 -phosphate.

Unlike fructosuria, in this condition severe symptoms are encountered since all the inorganic phosphate (Pi) is tied up as fructose-I -phosphate and thus is not available for phosphorylation of ADP to form AT P. Thus there is depletion of Pi (inorganic phosphate) and ATP. During breast feeding, no metabolic derangement occurs since breast milk does not contain fructose or sucrose (as you know sucrose is made up of glucose and fructose). But if the newborn infant receives a milk formula with fructose or sucrose, the infant develops various symptoms as highlighted in Table 12.6. But at weaning, with the intake of vegetables and fruits containing fructose, symptoms appear. There is fructosemia (high levels of fructose in blood), fructosuria (fructose in urine), hypophosphatemia (low phosphate level in blood)

and hypoglycemia (low blood sugar) despite the presence of high glycogen reserves.

This is because accumulation of fructose-1-phosphate inhibits the activity of liver phosphorylase (which breaksdown liver glycogen to glucose) by allosteric mechanisms. Hence fructose/sucrose free diet should be initiated as soon as diagnosis is made. Adequate fructose restriction is reported to normalize liver and renal function. Foods of animal origin (meat and dairy products) are free of fructose unless they have been processed using fructose/sucrose. Older children have been reported to develop an aversion to sweets and fruits. The diet, free of fructose, obviously eliminates many pleasant tasting foods normally enjoyed by children.

12.5.4 Galactosemia

Galactosemia is an inherited disorder characterized by an inability of the body to utilize galactose. Galactosemia means "galactose in the blood".

In this hereditary disease, patients are not able to metabolize galactose. As a result, galactose accumulates leading to galactosemia (high levels of galactose in blood) which is accompanied by galactosuria (excretion of galactose in urine). This may be caused due to defect in any of the enzymes involved in metabolism of galactose..

The most common type of galactosemia is due to deficiency of the enzyme galactose-I-phosphate uridyl transferase which converts galactose- 1 -phosphate to glucose- 1 -phosphate. Hence galactose- 1 -phosphate and galactose accumulate in the body causing various symptoms. The galactose absorbed following intestinal digestion of milk lactose remains unutilized in the infant. Unavailability of sufficient carbohydrate as a source of energy leads to usage of tissue proteins and fats as sources of energy. This can lead to amino aciduria (high level of amino acids in urine) and ketosuria (presence of ketone bodies in urine). Excess galactose is reduced by aldose reductase in the eye to the corresponding alcohol (galactitol) which accumulates causing cataract. In untreated cases, symptoms as listed in Table 12.6 usually begin days to several weeks after birth. Some infants are even born with cataracts and cirrhosis due to maternal ingestion of galactose. Those who survive are usually malnourished and dwarfed at 2 or 3 months of age and are mentally retarded.

Treatment should begin as early as possible, in the first week of life since breast milk contains lactose (made up of glucose and galactose). Lactose free formulae are available in our country and hence it has been reported that this disordå can be managed with ease. However, even with excellent nutrition control, children may have a lower IQ, difficulty with language, abstract thinking and visual perception. Females may have ovarian failure. These may be related to intrauterine damage due to maternal blood galactose crossing the placenta into the vulnerable foetus. Restriction of pulses, beans and peas has been recommended due to presence of galactose containing oligosaccharides (cabohydrates containing 2-10 monosaccharide units). However since they are not digested in human GI

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tract, galactose may not be released for absorption. With increased intake of solid foods, dietary regimen becomes easier. However, total relaxation in diet restriction is not recommended even in adult life.

12.5.5 Hereditary Lactose Intolerance

Lactose intolerance is an inability to digest milk sugar due to a specific deficiency of the enzyme lactase, and not, as commonly believed, a "milk allergy."

Majority of Asians, Africans and American Blacks are affected by this hereditary conclition. Lactose intolerance appears to develop in healthy young children at about 3 years of age in those population groups having a high prevalence rate. In others, it may not occur until 13 years of age.

The condition is due to a gradual decline in activity of lactase enzyme owing to reduction in synthesis of the enzyme. Lactase is an intestinal enzyme which hydrolyzes dietary lactose to glucose and galactose during the process of digestion. The decrease in activity occurs probably because there. is a failure in translation of lactase mRNA. Hence the enzyme is not synthesized and there is accumulation in the intestine of undigested lactose which is osmotically active, so that it holds water leading to diarrhoea. Additionally intestinal bacteria bring about fermentation of lactose with the formation of short-chain acids and gases like H_2 and CO_2. This causes flatulence. All foods containing lactose should be eliminated from the diet. This would include milk and milk products (ice creams, milk shakes).

12.5.6 Glycogen Storage Disease

In 1929, Von Gierke first described a glycogen storage disease in which as the name suggests, large amounts of glycogen were stored in the liver. Glycogen storage disease (glycogenosis) is a generic term used to describe a group of inherited disorders in which either the synthesis or breakdown of glycogen is defective.

This results in deposition of an abnormal type or quantity of glycogen in the tissues. Accumulation of large amounts of glycogen disrupts the normal functions of the cell. As you already know, glycogen is a polymer of glucose units and thus is the storage form of glucose in the body. Whenever blood sugar level goes down, liver glycogen is broken down to glucose units which then enter the blood and raise the sugar level. Muscle glycogen is used to supply glucose units for muscular activity. Hence in glycogen storage diseases, several clinical symptoms occur due to defective glycogen metabolism.

In some of the cases, nutritional intervention can reduce the severity of the symptoms. Eight different types have been described which differ with respect to the enzyme which is defective. Accordingly the symptoms and management also vary'. For easy understanding of the symptoms (of the eight different types), the information has been tabulated in Table 12.7.

NOTES

Table 12.5: Disorders of carbohydrate metabolism

Name of disease	Defective enzyme	Amino acid involved	Amino acid/metabolite accumulated	Reported incidence	Beneficial diet therapy.
1) Alcaptonuria	Homogentisate oxidase	Phenylalanine Tyrosine	Homogentisate	2-5 per million live births	Phenlyalanine and tyrosine restriction Ascorbic acid supplementation
2) Phenylketonuria (PKU)	Phenlyalanine hydroxylase	Phenylalanine	Phenylalanine, phenylpyruvate, phenyllactate, phenylacetate	1:10,000 white newborns 1:132,000 black newborns	Phenylalanine restricted diet Tyrosine supplementation
3) Tyrosinemia Type I (Tyrosinosis)	Fumaryl acetoacetate hydrolase and maleyl acetoacetate hydrolase	Tyrosine Phenylalanine ± Methionine	Tyrosine, phenylalanine, succinyl acetone, fumaryl acetoacetate, maleyl acetoacetate, δ-amino levulenate, ± methionine	–	Diet low in tyrosine, phenylalanine, ± methionine High carbohydrate feeds (65-75% of calories), hematin supplementation
4) Tyrosinemia Type II (Richner-Hanhart Syndrome)	Tyrosine transaminase	Tyrosine	Tyrosine, p-hydroxy phenylpyruvate, p-hydroxy phenyllactate, p-hydroxy phenylacetate, N-acetyl tyrosine, tyramine	–	Phenylalanine and tyrosine restriction required less severe than Type I Early but transient dietary protein restriction
5) Neonatal tyrosinemia	p-hydroxy phenyl pyruvate hydroxylase	Tyrosine	Tyrosine, phenylalanine, p-hydroxy phenyl acetate, N-acetyl tyrosine, tyramine	–	–
6) Albinism	Tyrosine hydroxylase (tyrosinase)		–	1:20,000	–

Table 12.6: Clinical symptoms of disorders of carbohydrate metabolism

Name of disease	Clinical symptoms
1) Pentosuria	No Symptoms. Condition compatible with health and well being.
2) Fructosuria	No clinical symptoms reported. Only delayed fructose intolerance reported.
3) Hereditary fructose intolerance	Frequent vomiting, poor feeding, fever, poor growth, pallor, diarrhoea, lethargy, jaundice, hepatomegaly, oedema, ascites, coma, convulsions
4) Galactosemia	Vomiting, failure to thrive, fever, jaundice, hepatomegaly, liver cirrhosis, cataracts, mental retardation
5) Hereditary lactose intolerance	Abdominal cramps, diarrhoea, flatulence. Production of gases like H_2 and CO_2 and short chain acids, all of which serve as intestinal irritants.

Table 12.7: Glycogen storage diseases

Glycogenosis	Name	Defective enzyme	Prevalence	Characteristics
Type I	Von Gierke's disease	Glusose-6-phospatase	~1:100,000	Liver and renal tubule cells loaded with glycogen leading to organomegaly. Anorexia, weight loss, vomiting, hypoglycemia, convulsions and coma. Low response of blood glucose to injection of epinephrine. Hyperuricemia (high uric acid levels in blood) causing clinical gout. Death ensues within 2 years. Frequent feeding, high protein diet, nasogastric infusion of glucose, oral uncooked starch acting as slow release form of glucose beneficial.
Type II	Pompe's disease	Lysosomal α-1→4 and α-1→6 glucosidase (acid maltase)	~1:100,000	There is massive cardiomegaly and heart failure by 1 year of age. Prenatal diagnosis by enzyme assay offers the only form of management.
Type III	Limit dextrinosis, Forbe's or Cori's disease	Debrancher	~1:100,000	Glycogen with enormous branches and very short outer chains stored in liver and muscle. Symptoms milder than those seen in Type I. Similar dietary management indicated.
Type IV	Andersen's disease	Brancher	~1:500,000	Glycogen with few branch points accumulates leading to cirrhosis, hepatosplenomagaly with bleeding tendencies. Death due to cardiac or liver failure in first year of life.
Type V	McArdle's disease	Muscle phosphorylase	~1:500,000	Muscles have abnormally high content of glycogen (2.5-4.0%). Little or no lactate in blood after exercise since muscle glycogen unavailable as fuel. Hence markedly diminished tolerance to exercise. Clinically patients are well developed, normal and no abnormalities at rest. Avoidance of extreme exercise advocated.
Type VI	Her's disease	Liver phosphorylase	~1:200,000	High glycogen content in liver with hepatomegaly. Tendency towards hypoglycemia.
Type VII	Taur's disease	Phosphofructokinase in muscle and erythrocytes	~1:500,000	Glycogen storage in muscle due to activation of glycogen synthase combined with inhibition of phosphorylase by accumulated glucose-6-phosphate. Also promotes PRPP synthesis leading to hyperuricemia and gout. Exercise intolerance and possibility of hemolytic anaemia.
Type VIII	–	Liver phosphorylase kinase	–	Due to kinase deficiency, liver phosphorylase cannot be activated leading to high glycogen content in liver.

12.6 DISORDERS OF LIPID METABOLISM

We will be discussing here three diseases involving lipid metabolism. In fact all

the. 3 conditions result in storage of lipid -in the cell, i.e. lipidosis. Nutritional intervention is not passible in these three diseases. At the present time, no effective treatment is available. Administering the defective enzyme by a process called enzyme replacement therapy has been tried, but it has not been very successful. The difficulty is in obtaining a highly purified human enzyme along with administration [(has to be intravenous/intrathecal (injected into the fluid surrounding the spinal cord)]. Hence gene therapy of the future is the only hope. All these three diseases have recessive autosomal pattern of inheritance. Table 12.8 gives other details related to the diseases. Before going through the details it would be worthwhile to revise the structure of these molecules as given in Unit 2.

12.6.1 Gaucher's Disease (Glucosyl Ceramide Lipidosis)

Gaucher's Disease was observed by Gaucher in 1882 and is the most common inherited metabolic disorder of glycolipid (combination of carbohydrate and lipid) metabolism. In this disease, the defective enzyme is glucocerebrosidase. In normal individuals, this enzyme catabolizes glucocerebroside. Glucocerebroside or glucosyl ceramide is composed of equimolar portions of long-chain amino alcohol (alcohol containing amino group) called sphingosine, a long-chain fatty acid and glucose. The enzyme glucocerebrosidase breaks glucocerebroside into glucose and another compound containing sphingosine and long-chain fatty acid called ceramide. Hence, in Gaucher's disease, since this reaction cannot take place, glucocerebroside accumulates in the cells of the reticuloendothelial (blood forming) system. Thus these ceils become enlarged. The cytoplasm of such cells is replaced entirely by the lipid and under the microscope the cells appear as large pale cells (cells do not take up the aqueous dye used for staining the cells). The cytoplasm of such cells when observed under the microscope looks like 'wrinkled tissue paper' or 'crumpled silk'. These cells are called Gaucher cells.

The disorder has been detected in patients of all ages, symptoms may appear at any time. Accordingly, three different types have been described. At this point, see Table 12.8 for the clinical symptoms. As you would notice, Type I is the chronic type in which visceral organs like liver, spleen etc. are affected. But CNS is not involved. In Type 2, CNS 'is involved and hence it is an acute type with death occurring by 2 years of age. In Type 3 visceral organs and CNS are involved, but to a less severe extent. In Gaucher's disease since cells of the reticuloendothelial system are affected, blood abnormalities occur. Hence, surveillance by haematologists is necessary throughout life along with treatment for anaemia. When the spleen gets enlarged to a great extent, splenectomy (surgical removal of spleen) is necessary in many cases. Strong analgesics are needed for pain in bones and joints. The different types are genetically distinct. Type 1 is more common in Jews with a prevalence of 1:2500 births.

12.6.2 Niemann-Pick Disease (Sphingomyelin Lipidosis)

Niemann-Pick Disease, the hereditary metabolic disease, was first described by

Niemann in 1914 and was later confirmed in 1927 by Pick. In this disease, there is an excessive storage of the lipid, sphingomyelin, which is a phospholipid. It is made up of the amino alcohol sphingosine, fatty acid, phosphoric acid and choline. The defective enzyme is sphingomyelinase which normally cleaves sphingomyelin forming phosphorylcholine and ceramide (sphingosine + long-chain fatty acid). Hence sphingomyelin accumulates in cytoplasm of cells of spleen, liver, bone marrow and. lymph nodes. Here too because of excessive accumulation of lipid, the cells appear large and pale when seen under a microscope. They are called Niemann-Pick cells. Here too 3 clinically different types have been described. Type A develops in infancy with severe CNS damage. Type B is subacute and chronic with only visceral involvment. In Type C, both visceral organs and CNS are involved. Types D and E have also been described. At the present time, no treatment is available. Only prenatal diagnosis is possible by assaying (estimating) for sphingomyelinase activity in cultured amniotic cells. Therefore genetic counseling is important to prevent the birth of the affected baby. The disorder is panethnic (in people of different ethnic groups) in prevalence. Type A is found in people of Ashkenazic Jewish ancestry with a very high prevalence of 1:100.

12.6.3 Tay-Sach's Disease (TSD) (Ganglioside Lipidosis)

Tay-Sachs disease is an inherited disease caused by an abnormal gene. People with this abnormal gene lack an important enzyme called hexosaminidase A (HEXA) that helps to break down a fatty material called ganglioside. This material builds up in the brain, and eventually damages nerve cells and causes neurological problems. In this disease, there is a severe deficiency of the enzyme Hexoaminidase A, which in normal persons catabolizes the lipid called gangliosides. Ganglioside is a complex glycosphingolipid (fatty acid+sphingosine+oligosaccharide chain). The oligosaccharide chain also contains sialic acid which is a 9-carbon sugar derivative. Gangliosides are present in high concentration in ganglion cells (neurons or nerve cells), hence the name. There are different types of gangliosides depending upon the number of sialic acid units and are accordingly called GMI, GM2, GM3. In Tay-Sachs disease, GM2 accumulates and hence is also referred to as GM2 ganglioside. Normally hexoaminidase A cleaves N-acetyl hexosamine from GM2. TSD is the most common ganglioside storage disease Very severe symptoms are encountered in TSD.

There is motor weakness and mental and motor deterioration progresses rapidly after I year of age, with death occurring by 3 years of age. For detailed symptoms of this disorder, look up Table 12.8. At present, no treatment is available and gene therapy is the only future hope.

Mass screening programs have been carried out in 73 cities in 13 different countries with over 312,000 individuals tested since 1970 for TSD. These community based voluntary screening programs have led to the identification of over 250 at risk carrier (having one defective gene) couples who have had no history' of TSD in their families. Prenatal diagnosis in such couples has led to the identification of

TSD in utero. Thus TSD is the first example of a genetic disease in which the birth of an affected child has been prevented by mass screening for heterozygotes (having one defective gene) in at risk populations.

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For GM2 TSD, an extremely high frequency of I :24 in Jewish individuals has been reported. Prenatal diagnosis can be done by estimating the enzyme Hexoaminidase A in amniotic fluid. Hence at the present time, proper recognition, early diagnosis and immediate genetic counseling followed by contraception is the simplest, most effective means available for preventing the conception and birth of children with ganglioside storage disease.

Table 12.8: Disorders of lipid metabolism

Name of disease	Defective enzyme	Type of lipid stored	Normal action of enzyme	Clinical Symptoms
1) Gaucher's disease	Glucocerebrosidase (β-Glucosidase)	Glucocerebroside	Cleaving ceramide and glucose	*Type 1*-Adult, chronic, non-neuronopathic form manifesting at any time from birth till old age. Organomegally, haemetologic abnormalities due to hyperspleenism and bone lesions. CNS not involved. *Type 2*-Acute, neuronopathic, infantile form. Usually apparent before 6 months of age, hepatosplenomegally, Gaucher's cells in bone marrow, CNS acutely involved, fatal by 2 years. *Type 3*-Subacute, neuronopathic, juvenile form. Visceral organs and CNS involved. Signs of neuorologic damage appear later than in Type 2 patients.
2) Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Cleaving ceramide and phosphoryl choline	*Type A*- Develops in infancy. Severe CNS damage. Feeding difficulties, emaciation with protuberant abdomen. Fatal by 3-4 years. *Type B*- Becomes apparent in infancy or childhood. Visceral involvement extensive but CNS normal, splenomegaly, respiratory involvement. Condition is subacute and chronic. *Type C*- Both CNS and visceral involvement, condition is subacute and chronic. Ataxia, loss of speech, seizures. Fatal before 20 years.
3) Tay-Sach's disease	Hexoaminidase A	GM2 Ganglioside	Cleaving N-acetyl hexosamine from ganglioside	Motor weakness manifesting between 3 and 6 months of age. Startle reaction is a characteristic early symptom. Infants cannot walk. Feeding difficulty, poor muscle tone, generalized paralysis, deafness, blindness, convulsions, spasticity appearing by about 18 months. Death occurs from bronchopneumonia by 3 years of age.

12.7 HAEMOGLOBINOPATHIES

Haemoglobinopathies are those conditions where haemoglobin (Hb) is unable to perform its function. The function of haemoglobin, as we all know, is the transport of oxygen from the lung to all the tissues of the body.

To understand these disorders better, we shall first look at the structure of haemoglobin. Haemoglobin consists of heme, a non-protein part and globin, which

is a protein. The heme portion contains iron. The protein globin is made up of four polypeptide chains (polymer of amino acids joined together by peptide bonds). Each chain is designated by a Greek letter. The amino acid composition of 2 chains is identical and these 2 chains are called a-chains. The amino acid composition of the remaining 2 chains is different from a-chains, but identical to each other and is called ß-chains. Hence normal adult Hb called HbA has ct2ß2 structure (2 a-chains and 2 ß-chains). Thus Hb has a tetramer structure. The teüamer structure is essential to the efficiency of this process. Hb in the foetus has a different structure. While it also contains 2 a-chains, the amino acid composition of the other 2 chains is different from that of ß-chain and is called y-chaifi. Therefore it is 02b. At birth , foetal Hb (HbF) (0272) predominates and is rapidly replaced by HbA @2ß2), which is the normal adult Hb, by about 6 months of age. There is also HbA2 (0262), a minor adult Hb, which constitutes about 2.5% of the total. HbA is a globular protein with a molecular weight of 68,000. Each a-chain has 141 amino acids while each ß-chain has 146 residues. Several .mutations (changes) in the structure of the gene coding for Hb are known, each leading to a specific disease. We will have. a look at these diseases now, basically sickle-cell anaemia and the thalassemias.

12.7.1 Sickle Cell Anaemia

Sickle cell anaemia is an inherited blood disease. As the name suggests, in this disease RBCs assume sickle or cresent shape. The sickling is dependent on the removal of oxygen and it is reversible. You may recall reading about this in the Applied Physiology Course in Unit 12.

Due to genetic mutation, in this disorder, the globin chain synthesis is not normal. In the ß-chain at position 6, amino acid valine replaces glutamic acid. Sickle cell anaemia is thus the prime example of a 'molecular disease'. Is it not remarkable that a single amino acid substitution in the Hb molecule leads to severe disease in homozygous (having both defective genes) individuals?

The S (sickle-cell) gene occurs throughout tropical Africa, as well as, in blacks in the US and other countries to which Africans were exported during the slave trade. It is also found in the Middle East and may occur in Caucasians (light coloured racial groups). About 8% American blacks are carriers. It is also prevalent in various states in India, particularly among tribal groups.

A given cell can undergo reversible sickling several times but during each 'sickle-unsickle' cycle, it probably loses a small portion of membrane. This results in loss of cell water with an increase in intracellular Hb concentration and an increased tendency to sickle. Finally, it is no longer able to unsickle and becomes an irreversibly sickled cell. These have very short survival period and very low oxygen affinity. HbF (which does not contain ß-chain) when present in the cells of persons carrying HbS is beneficial. Thus the newbom is not affected until HbF synthesis declines. An unusually high level (about 18%) of HbF found in the Middle East offers a protective effect. Clinical symptoms include vasooclusion (blockage of blood flow in blood vessels), pain and tissue death. Reticulocytosis (synthesis

of reticulocytes-immature erythrocytes), sickling and extensive haemolysis (destruction of red blood cells) are seen by 10-12 weeks of age. By 5-6 months, splenomegaly (enlargement of the spleen) is seen. There is a swelling of the dorsum (back) of the hands and feet (hand- foot syndrome). There is a relentless gnawing pain in the long bones and joints.

Children are susceptible to various infections.

There is no effective treatment at present. Good nutrition and personal hygiene, early diagnosis and treatment of infections, prophylaxis (prevention) against malaria and folic acid administration are beneficial. Many antisickling agents are constantly being tested.

12.7.2 Thalassemias

Thalassemias are a heterogenous group of hereditary diseases characterized by anaemia. In thalassemias, due to genetic mutation, synthesis of the protein globin is affected. Normally u and ß globin chain production is balanced to form globin tetramers (with 4 polypeptides) about which you learnt above. Adult HbA is 042. In thalassemia, the impaired production of one or more of these globin components causes deficient Hb molecule. The unaffected chain continues to be produced in normal amounts and in the homozygous (having both defective genes) state excessive accumulation of the unaffected chain may disrupt erythroid (red) cell maturation and function causing premature destruction of the RBC.

Thalassemia occurs throughout the world and constitutes one of the most common hereditary disorders. It has an autosomal recessive inheritance.

When synthesis of a-globin chain is defective, the condition is called a-thalassemia, while in ß-thalassemia, synthesis of ß-globin chain is affected. Let us see the important features of both these conditions.

a-Thalassemia

The defect ranges from mild to complete suppression of a-chain synthesis. This is due to mutations in the genes which carry the information for the synthesis of a-globin qhain. Four clinical syndromes of increasing severity are recognized. These are.

- Silent carrier state — here the person is a carrier having one defective gene. a-globin chain production is very mildly impaired. Even making a firm clinical diagnosis is often impossible. Red cell morphology is normal and anaemia is absent.
- a-Thalassemia trait — here the dtsease is present in a more severe forrn. Hb levels may be slightly below normal. However, the affected person is not usually anaemic. RBCs are characteristically microcytic (small in size).
- Hb-H disease — here distinctly there is mild to moderate degree of haemolytic anaemia which can become severe in young children, during pregnancy or

whenever the person gets some infection. Hb levels average 10 g/dl. There is reticulocytosis of about 5%. This is a compensatory mechanism by the body in an effort to improve the anaemic condition. Spleen is often enlarged. Occasional bone abnormalities are present. RBCs are microcytic.

- Hydrops faetalis — this is the most severe form of a-thalassemia and the condition is invariably lethal. As the name suggests, the affected foetus dies during the third trimester of pregnancy or if born alive within hours or at most lor 2 days after birth. At the present time, no treatment is available. Hence it is important to have a timely diagnosis along with genetic counseling for prevention/ termination of pregnancy which is doomed to fail.

a-thalassemia occurs predominantly in people of Mediterranean, African and Asian origin. Hydrops faetalis occurs exclusively in South-East Asia e.g. in Chinese, Thai, Vietnamese etc. It has rarely been found in people of Greek and Cypriot origin and also has never been detected in pedple of African descent. Hb-H disease however is common in Mediterranean area and in Asia but rare in Africa.

ß-Thalassemia

This occurs due to a very wide variety of mutations in the ß-globin gene affecting every aspect of its structure. There are 2 variations of the disease depending on whether the individual is heterozygous (one defective gene) or homozygous (both defective genes). The 2 variants are discussed below :

- ***ß-Thalassemia frait (A-Thalassemia Minor):*** This is the carrier or heterozygous state, which is usually asymptomatic. Hb levels are normal or slightly decreased. In times of stress, precipitated by pregnancy or infection, the patient may become anaemic. In children, Hb levels may. be below 10 g/ dl. Occasionally, there is hepatosplenomegaly. There is elevated HbA2 (ct2ö2) (minor adult Hb component) level which is protective since HbA2 does not contain ß-chain. Incidence of thalassemia trait is about 240 million around the world. In India it is about 30 million, being more common in North India (3-15%) as compared to South India (1-2%).
- ***ß-Thalassemia Major:*** This is the homozygous state and is also known as Cooley's Anaemia. Every year about 8,000-10,000 children in India are born with this disease. It occurs widely in people of Mediterranean origin, Middle East, the Indian subcontinent, South East Asia and Africa. In India, prevalence is high in the northern and eastern parts of the country. It is believed to have originated here following Alexander's invasion when there was intermingling of his soldiers with the local population. The Indian Council of Medical Research (ICMR) has conducted a study to determine the incidence of the disease in India. It afflicts communities like Sindhis, Punjabis and Gujaratis. The study has also carried out a religion-wise and a caste-wise breakup of the subjects.

The affected child is not anaemic at birth due to the high levels of foetal haemoglobin HbF (CGY„) which does not contain ß-chain. However as Y-globin chain synthesis gradually diminishes after a few months to be replaced by ß-chain synthesis,

anaemia becomes increasingly evident. Symptoms include pallor, listlessness and failure to thrive. Hb levels fall to 3-5 g/dl. RBC is severely hypochromic (less coloured) and varies greatly in size and shape. There is hepatosplenomegaly. Bone marrow proliferation causes deformities in bone structure (e.g. there is typical mongoloid face) and fractures occur. Lymph nodes get hypertrophied (excessive activity) due to erythropoiesis (synthesis of RBCs). Physical and sexual development is retarded. Female patients often have delayed onset of menarche or some do not menstruate at all. Infection is the common cause of childhood mortality.

lb-thalassemia has been traditionally treated by giving the affected person blood transfusion as and when required, depending upon the severity of the disease. In very severe cases, this could be once in 3-4 weeks. The cost of this is very high. Further with continous blood transfusions, there is accumulation of iron in the body tissues. This is called hemosiderosis. Hence to prevent hemosiderosis (accumulation of iron in tissues), thalassemics have to take expensive iron-chelation drugs (desferal or kelfer) to drain the excess iron out of the system. These drugs combine (chelate) readily with iron and the complex is excreted. These drugs are called iron-chelating drugs. The cost of medication could exceed Rs.2.5 lakhs per annum. However a bone manow or cord blood stem cell transplant confers permanent cure and eliminates all necessity of blood transfusion. This is now performed in various major hospitals in the country and the one- time estimated cost of the one-time treatment is Rs.10- 12 lakhs. Blood transfusion also poses the additional danger of contacting infections like hepatitis and HIV. Hence it must be ensured that blood transfusion is safe. Thalassemics India, which is a registered body regularly, organizes camps to create public awareness and initiate detection and prevention program.

Some of the steps that would go a long way in the prevention of this disease are listed in Box 4. Read them for better understanding of the topic.

Box 4: Steps in prevention of Thalassemia

- Awareness about the disease should be created in high risk populations.
- High risk communities and affected families should undergo blood tests.
- Ideally marriage should be avoided between two known thalassemia carriers.
- In case both partners are carriers, prenatal test must be undergone by the pregnant woman at each pregnancy.
- All married women must be screened for thalassemia carrier status before planning their family.
- All patients/parents should be educated about all aspects of blood transfusion.

12.8 LET US SUM UP

In this unit we leamt about the various inborn errors of metabolism. The discussion was presented under the three headings — disorders of protein metabolism, carbohyåyate metabolism and lipid metabolism. Some of the common specific biochemical in each of these disorders along with their symptoms were highlighted. The last part of the unit focussed on haemoglobinopathies within which sickle cell anaemia and thalassaemias were discussed.

The Unit presented an interesting discussion (of course not interesting to be suffering from!) on inborn errors of metabolism. The salient features are summarized herewith:

- These diseases are congenital, present throughout life and hereditary.
- A genetic mutation results in the synthesis of a defective enzyme/protein molecure.
- Inherited deficiencies in specific enzymes/protein molecules are the causes of the disorders.
- Autosomal recessive pattern of inheritance is observed in all the diseases considered in this presentation.
- As a result ofdefective enzyme/protein molecule a metabolic block occurs at that step in the metabolic pathway. This leads to accumulation of the metabolite (intermediate).
- In an attempt to reduce the amount of the accumulated metabolite, it goes through alternate/secondary pathways forming additionally various other compounds.
- All the accumulated metabolites are generally excreted in the urine.
- Identification of these metabolites affords a mechanism for diagnosis of the disease.
- Very often one or more of the accumulated metabolites are extremely toxic causing mild/severe clinical symptoms. This could result in physical and mental retardation.
- Clinical symptoms may appear within the first few days of life. Hence early diagnosis in neo-natal life is very important. With poor health care
- system in our country, the diseases remain routinely undiagnosed leading to death of the infant.
- Ideal way to treat the disorder would be to introduce the correct gene so
- that the right type of the gene product (protein) is synthesized. This constitutes gene therapy. However this is not available at the present moment.
- A Itematively only symptomatic treatnent is possible-to reduce the ill effects/ pain or to prevent the disease from progressing to more severe consequences. Nutritional intervention does provide reliefin many instances
- However nutritional management is difficul , needs constant biochemical

monitoring, very often expensive and requfres one-to-one monitoring for becoming successful.

- Apart from gene therapy, a large number of the diseases have no known meü10d oftreatrnent, leading to inevitable end, death ofthe patien in infancy or early childhood.
- Hence prenaml diagnosts followed by counseling for medical termination of
- pregnancy will help the mother from going through die stress of pregnancy and childbirth.
- Prenatal diagrosis is currently possible for many diseases which involves analysis of amniotic fluid cells for the defective enzyme, the • nfetabolit
- which accumulates or for the defective product.
- Mass screening programs conducted in at risk populations to ascertain the carriers of the disease.

12.9 GLOSSARY

Allosteric mechanisms	:	regulation of enzyme activity by exerting effect at a site other than the catalytic site.
Amniotic fluid	:	fluid protecting the embryo.
Ascites	:	accumulation of fluid in peritoneal cavity.
Autosomal	:	a chromosome that is not a sex chromosome.
Bacteriophage	:	a virus that infects a bacterium.
Calculi	:	stones.
Ceramide	:	a combination of sphingosine (alcohol) and fatty acid.
Chimeric DNA	:	DNA containing sequences derived from two different species (eg. bacteria and human being).
Chromosomes	:	long pieces of DNA contained in the nucleus of cells.
Cloning	:	a process of obtaining a large number of cells or molecules that are identical with a single parent cell or molecule.
Congenital	:	existing at birth.
Duplex	:	twofold.
Gene	:	a segment of DNA chain that contains the instructions for the complete protein.

Genetic engineering	:	process of altering chemical structure of genes.
Genetic mutation	:	a mutation occurs when a DNA gene is damaged or changed in such a way as to alter the genetic message carried by that gene.
Gout	:	hereditary condition of uric acid metabolism.
Hepatomegaly	:	enlargement of liver.
Hyperkeratosis	:	disease of the skin characterized by an excessive overgrowth of the cornified epithelium.
Keratitis	:	inflammation of the cornea.

12.10 CHECK YOUR PROGRESS

1) What do you understand by the term 'inborn errors of metabolism'? Discuss its etiolog.

2) List any three aromatic amino acids and their mention the defective enzymes involved.

3) Comment on the following statements:

   a) Tyrosine becomes an essential amino acid for PKU patients.

   b) In Alcaptonuria, urine upon standing becomes dark in colour.

   c) Inclusion of hematin is beneficial iri Tyrosinemia 1

   d) PKU is a neuro-metabolic disorder.

e) Patients with albinism suffer from photophobia.

5) Which sugars are involved in disorders of carbohydrate metabolism? List any three disorders.

6) What are haemoglobinopathies? Name the two disorders caused due to it.

7) Briefly justify the following statements

a) Large pale cells are seen in Gaucher's disease.

b) There is an accumulation of sphingomyelin in cytoplasm of cells in Niemann-Pick disease

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