UNIT 10 VITAMINS AND MINERALS

Structure

10.1 INTRODUCTION

We have already studied the chemical nature of the various vitamins earlier in Unit 3. 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 are catalyzed by protein molecules called *enzymes*. We have read about the characteristics of enzymes in Unit 4. We learnt 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.

Objectives

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 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 synthesize 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 β-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.

10.3 FAT-SOLUBLE VITAMINS

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 all 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 from 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 of toxicity, 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.

10.3.1 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 red coloured carotenoid pigments found in plants. These include carotenes (α, β and γ) 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 (CH_2OH)
- 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 retinol 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. You may have already learnt about these functions in the Advance Nutrition Course in Unit 7. Here we shall look at these functions from the nutritional biochemistry point of view.

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 *retinaldehyde* 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 function in dim light.

You have seen in Unit 3 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 *11-cis*. In all-*trans* isomer, all the double bonds have groups on both sides of the double bond. In 11-*cis* isomer, the double bond between carbons 11 and 12 has groups on one side of the double bond. In rhodopsin, the amino acid *lysine* in the protein opsin forms a complex with 11-*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 11-*cis* retinaldehyde to all-*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, 11-*cis* retinaldehyde is converted to all-*trans* retinaldehyde and the complex photorhodopsin is formed. Next,

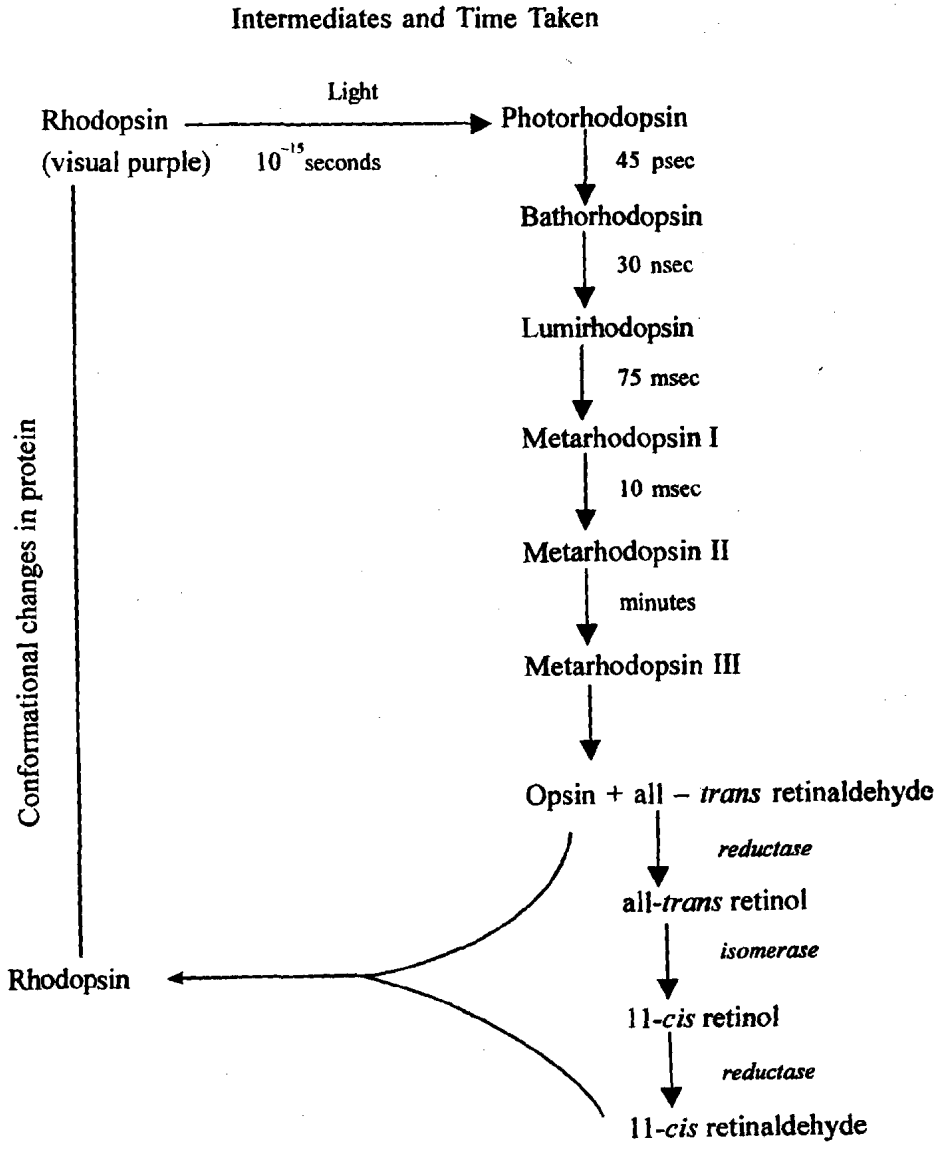


Figure 10.1: Light activation of rhodopsin

bathorhodopsin is formed within pico second of illumination. This is followed by a series of conformational changes and ultimately metarhodopsin III 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 11-*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 isomerized to the specific 11-*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 11-*cis* retinol. This is then converted to 11-*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.

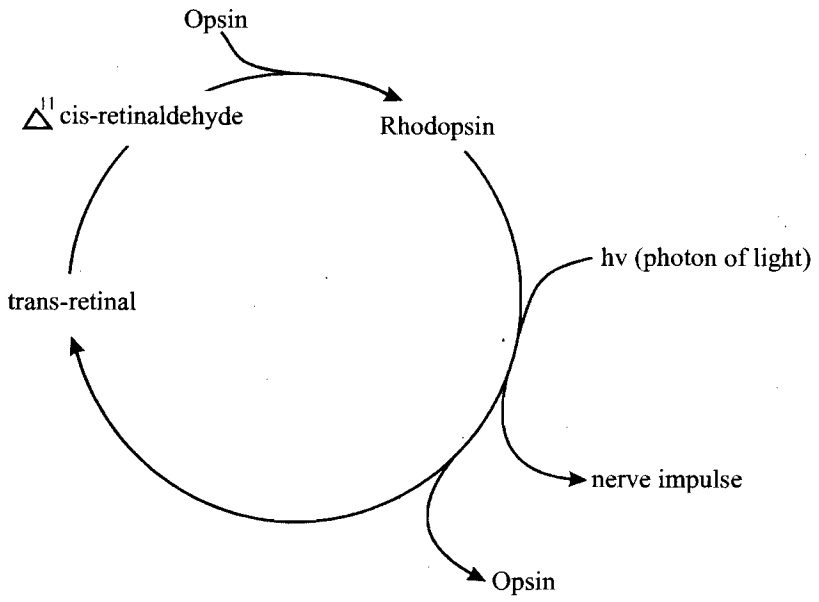


Figure 10.2: Visual cycle (Rhodopsin Cycle)

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. We will read about how steroid hormones act in Unit 11.

iii) *Retinoic acid participates in glycoprotein synthesis.* This may partly explain how retinoic acid helps in promoting growth 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) *β-carotene is an antioxidant*, effective at low oxygen concentrations. Compounds like unsaturated fatty 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. β-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-à-vis its functions.

10.3.2 Vitamin D

You have already studied earlier that there are two forms of vitamin D called D_2 *(ergocalciferol)* and D_3 *(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 D_2 and D_3 are formed by irradiation of plant sterol, ergosterol and animal sterol 7-dehydrocholesterol, respectively.

For vitamin D to be physiologically active, it has to be converted into the active form. 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.

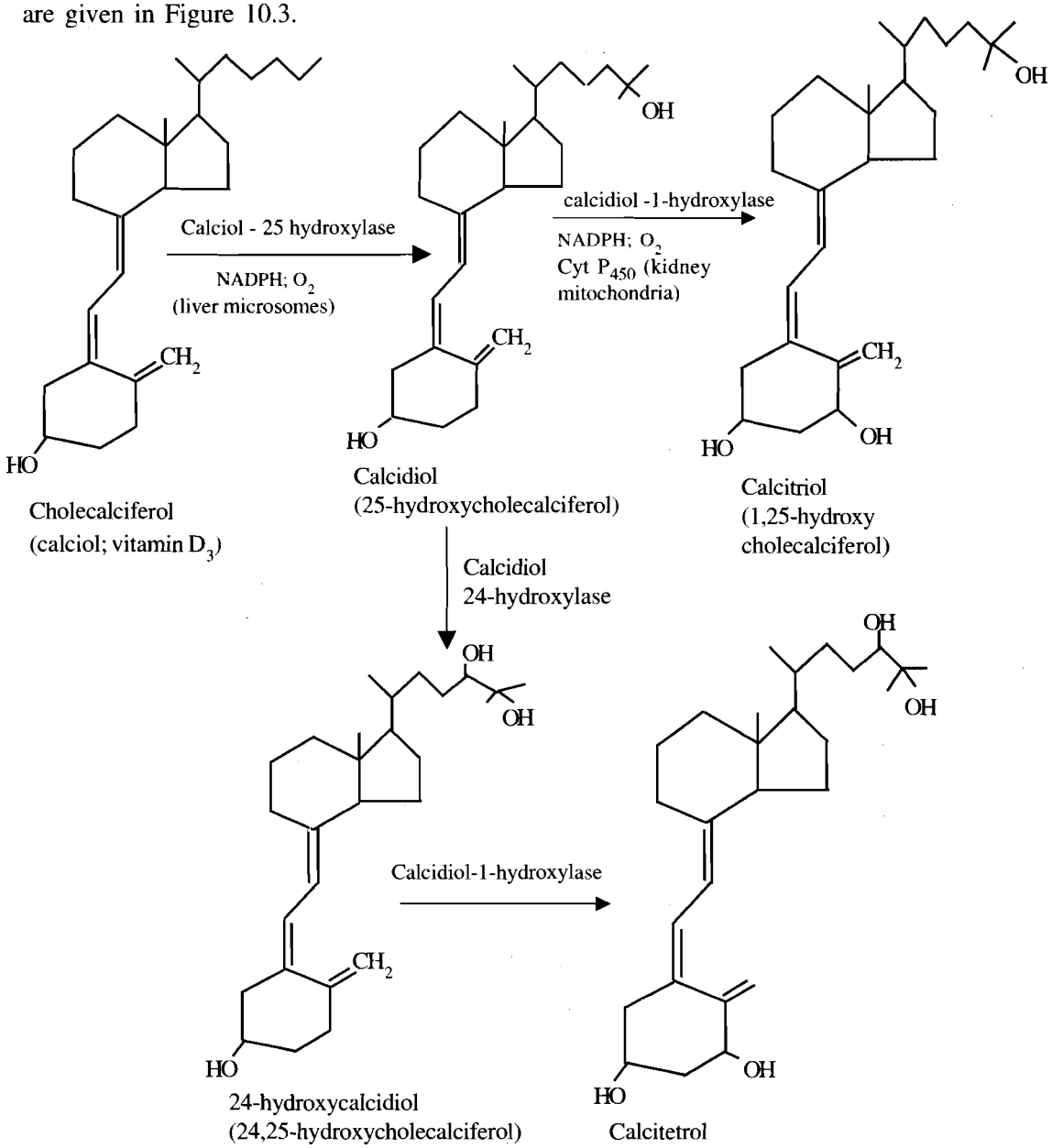


Figure 10.3: Metabolism of vitamin D

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,

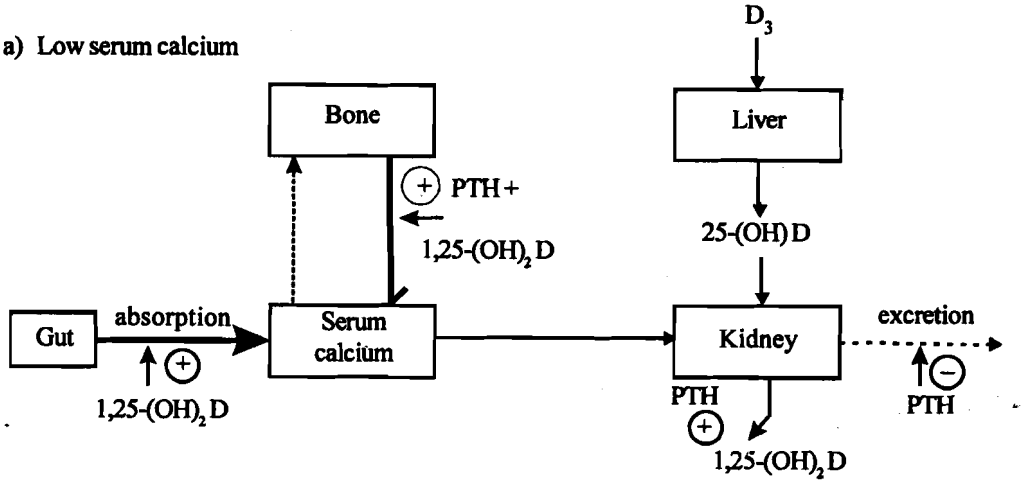
ii) reduces excretion of calcium by stimulating resorption in the distal tubules (in the kidney), and

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. You will read about steroid hormones in detail in Unit 11. We will have a brief look at it here.

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 (demineralization). Finally, PTH 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 PTH 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



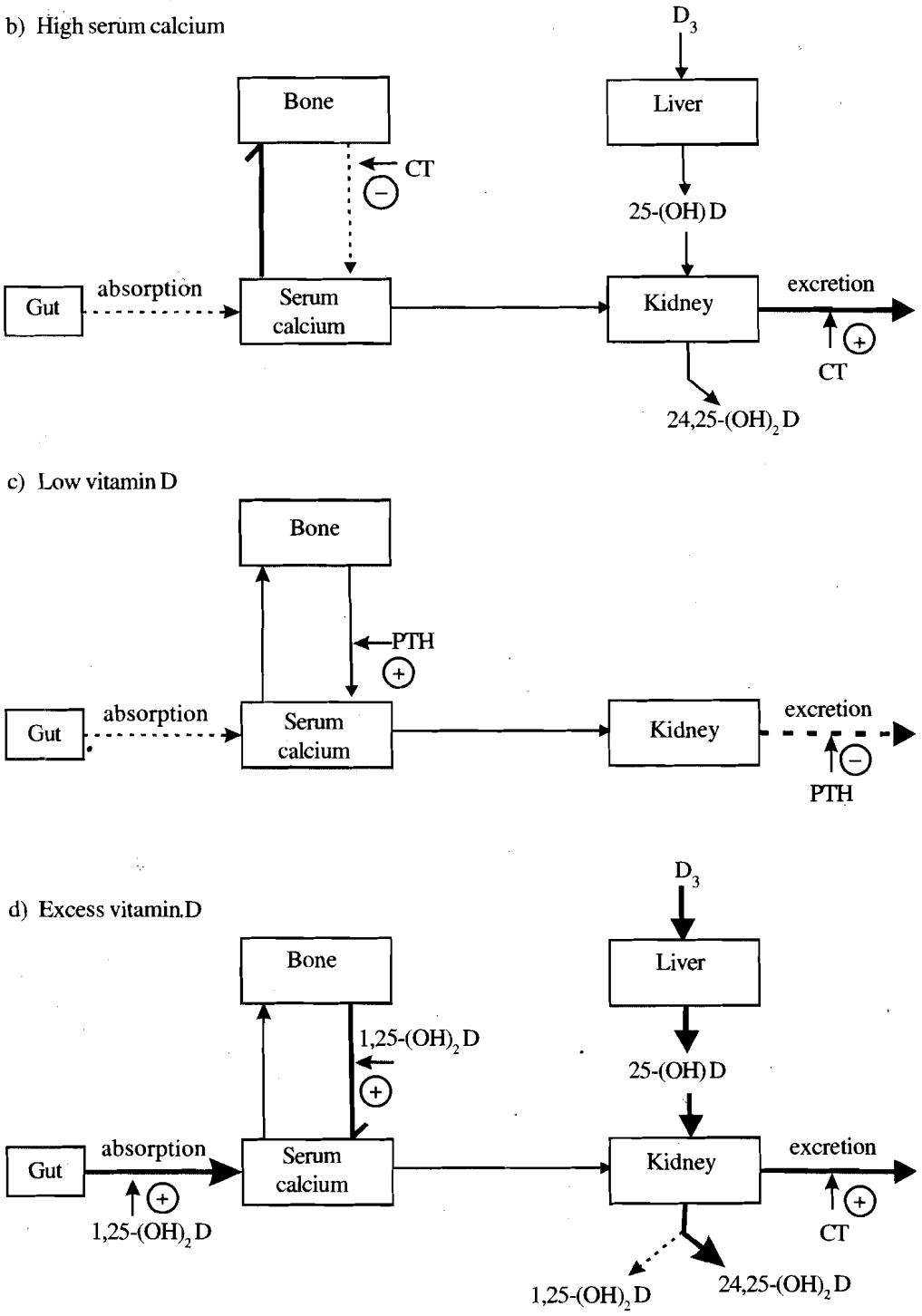


Figure 10.4: Vitamin D and calcium homeostasis

10.3.3 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:

$$C{=}C + O_2 \rightarrow \begin{matrix} C{-}C \\ | \quad | \\ O{-}O \end{matrix}$$

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 of tocopherols was known for sometime, information regarding tocotrienols has been obtained in recent 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.

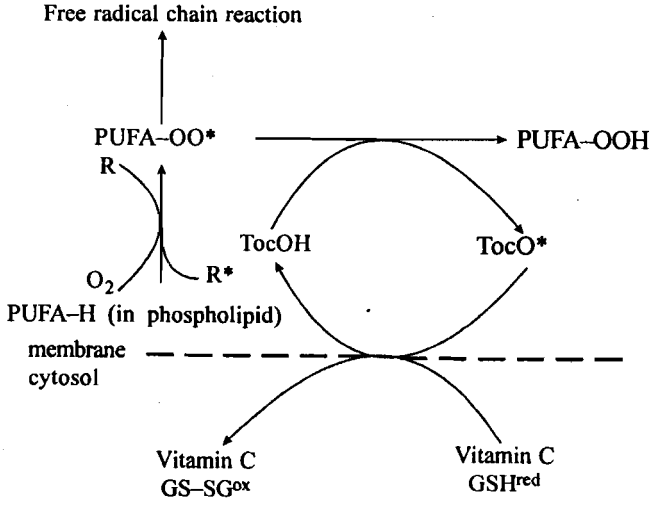


Figure 10.5: Biological role of vitamin E

10.3.4 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 in Unit 3. Hope you are familiar with these forms. List down these forms herewith.

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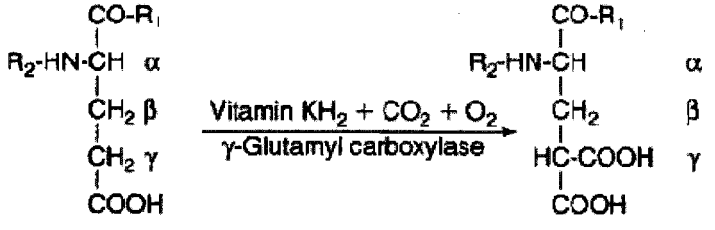
Next, let us look at the functions of vitamin K.

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 γ-carbon (4th carbon) of glutamate residue is carboxylated to form γ-carboxy glutamate, as illustrated in Figure 10.6.



glutamate residue (present in preprothrombin)

γ-carboxy glutamate residue (present in prothrombin)

R_1 and R_2 represent the other amino acids in the protein sequence.

Figure 10.6: Vitamin K dependent carboxylation

The γ-carboxy glutamate 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 *fibrin*. Fibrin has a mesh-like structure and traps red and white blood cells and other plasma proteins to form the blood clot. The biological role of vitamin K as discussed above is presented in Figure 10.7.

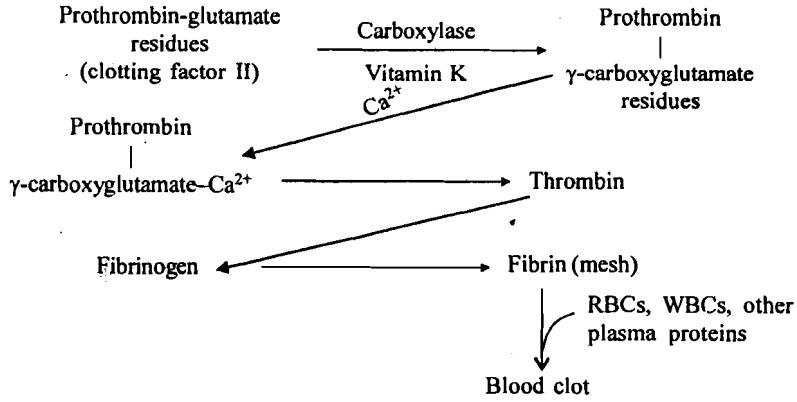


Figure 10.7: Biological role of vitamin K

Next, we shall learn about the vitamin K cycle, which you will see is the salvage pathway for vitamin K.

Vitamin K cycle

Figure 10.8 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 (sulfhydryl) 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 catalyzed by *vitamin K quinone reductase* and vitamin K hydroquinone is regenerated, as can be seen in Figure 10.8. 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.

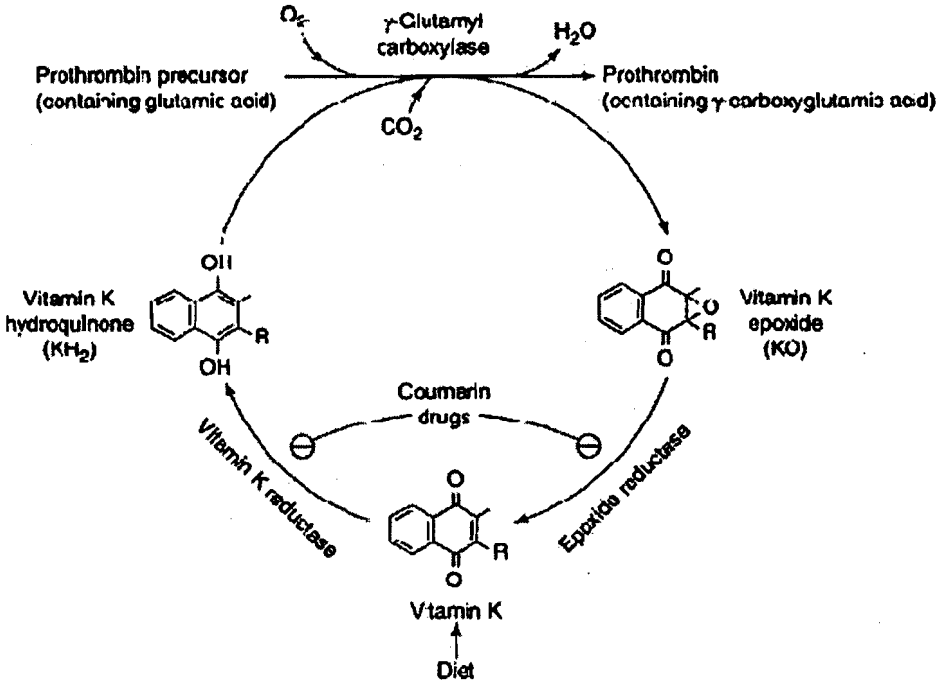


Figure 10.8: Vitamin K cycle

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 γ-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. With vitamin K, we end our discussion on fat-soluble vitamins. Let us now recapitulate what we have learnt so far.

Check Your Progress Exercise 1

1) How are fat-soluble vitamins transported? What are the factors that lead to deficiency of these vitamins in our body?

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2) List the active forms of vitamin A and vitamin D.

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3) Enumerate the various functions of vitamin A. Give the mechanism by which rods function in dim light.

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4) Discuss the role of:

a) Vitamin E as anti-oxidant.

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b) Vitamin K as anti-coagulant.

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c) Calcitriol in calcium metabolism.

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5) Explain the 2-step conversion process of vitamin K to hydroquinone.

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Having looked at the biological role of different fat-soluble vitains, we now move on to water-soluble vitamins.

10.4 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.

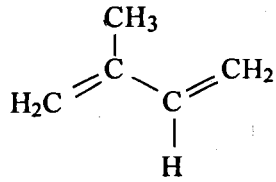


Figure 10.9: Isoprene unit

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 : B_1, B_2, B_6, niacin, pantothenic acid and biotin
- hematopoietic water-soluble vitamins : folic acid, vitamin B_{12}
- other water-soluble vitamins : ascorbic acid (vitamin C).

We begin our study of water-soluble vitamins with energy releasing water-soluble vitamins.

10.4.1 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 - B_1, B_2, B_6, 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_1.

10.4.1.1 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.

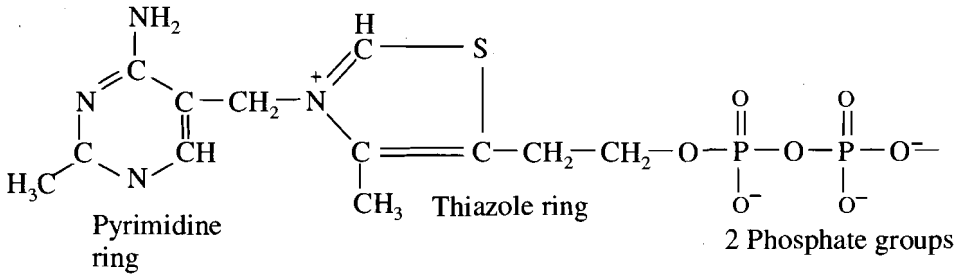


Figure 10.10: Active form of thiamin

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
- α-*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 α-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 NADH 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. So please read that section for this role of TDP in Unit 6.

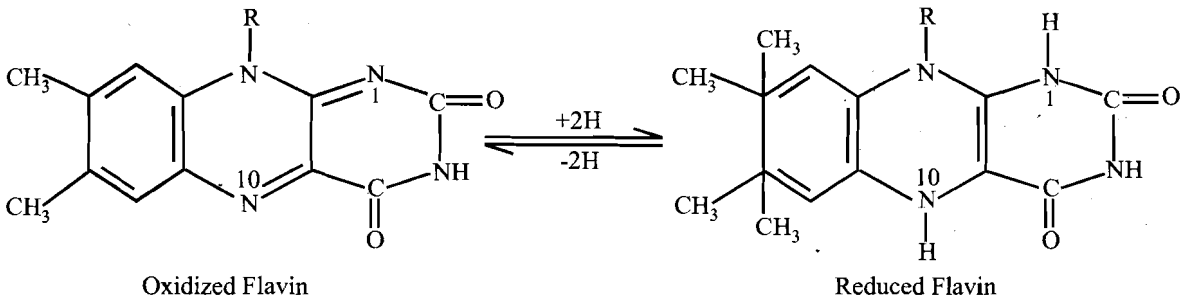
10.4.1.2 Riboflavin (Vitamin B_2)

This vitamin which functions as a coenzyme in energy-yielding metabolism has 2 active forms – FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide). We have already studied their structures in Unit 4 section 4.9. 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:

$$FMN \longrightarrow FMNH_2;\ FAD \longrightarrow FADH_2$$



Oxidized Flavin

Reduced Flavin

Figure 10.11: Oxidation-reduction property of flavin coenzyme

After sometime, all the FAD will exist in the cell as $FADH_2$. 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 $FMNH_2$ 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 (Fe^{3+}) and molybdenum (Mo^{6+}) 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 NH_3 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 carbons 2 and 3 forming Δ^2 *trans* enoyl CoA. You may recall reading about this reaction in β-oxidation of fatty acids, Unit 7 section 7.2.

- *Xanthine oxidase* participates in the last two steps of catabolism of the purine bases adenine and guanine, finally forming uric acid. We learnt about this in Unit 8, sub-section 8.3.3.

10.4.1.3 Pyridoxine (Vitamin B_6)

It is a generic name for six compounds that have vitamin B_6 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 B_6 functions as a coenzyme for many enzymes involved in amino acid metabolism. There are 2 such reactions which require vitamin B_6. These are:

- *Transamination*-transfer of 'NH_2' groups from amino acids by enzymes called transaminases (or aminotransferases)

- *Decaboxylation*-removal of 'COOH' group of amino acids as CO_2 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).

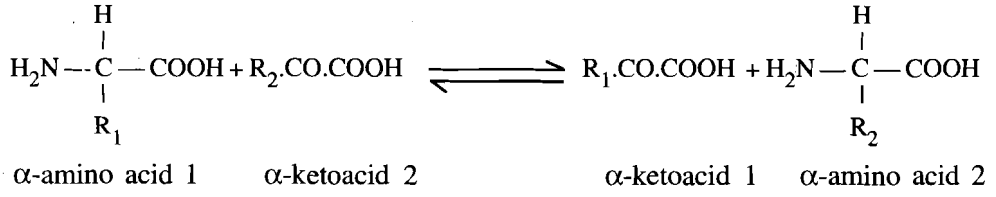
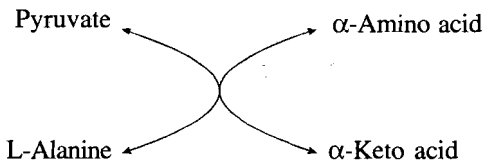


Figure 10.12: General reaction of transamination

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-α-ketoglutarate aminotransferase (or glutamate transaminase) present in most mammalian tissues catalyze the transfer of amino groups to pyruvate (forming alanine) or to α-ketoglutarate (forming 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.



Alanine transaminase

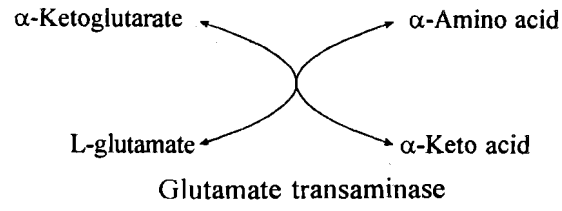


Figure 10.13: Action of transaminases

Approximately 60 specific reactions of amino acids involving pyridoxal phosphate have been discovered. Because B_6 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 B_6 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 CO_2. 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.

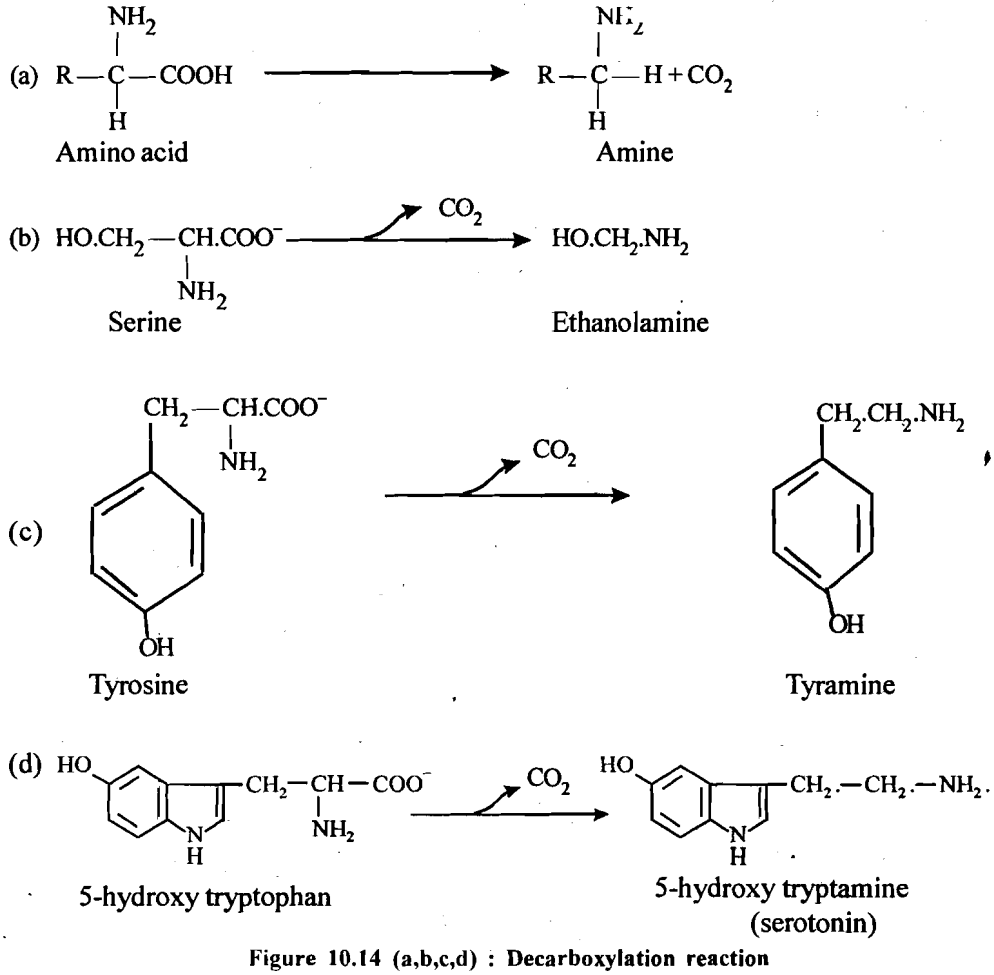


Figure 10.14 (a,b,c,d) : Decarboxylation reaction

Another important enzyme which requires vitamin B_6 phosphate is L-glutamate decarboxylase, converting glutamate to γ-aminobutyrate (GABA) which is an important 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.

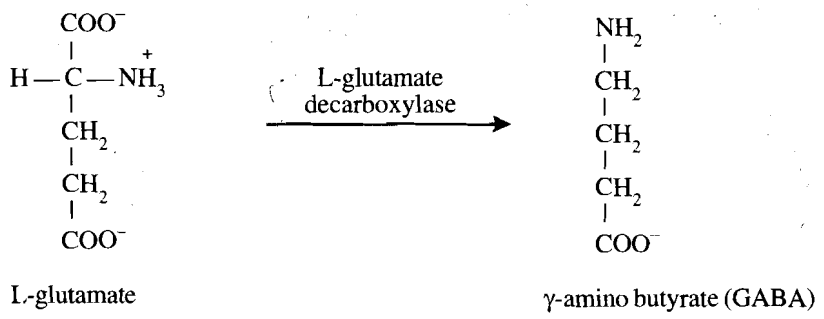


Figure 10.15: L-gluatamate decarboxylase reaction

Pyridoxal phosphate acts as a coenzyme in other physiologically important reactions. It is required for the synthesis of δ-amino levulinic acid, a precursor of heme. Hence B_6 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 which breaks down glycogen in the body. Hence decreased tolerance to exercise is associated with B_6 deficiency.

Pyridoxal phosphate is one of the cofactors required for the conversion of the amino acid tryptophan to the coenzyme NAD^+.

10.4.1.4 Niacin

It is not a vitamin in the strictest sense of the word, since some niacin can be synthesized from tryptophan. However conversion of tryptophan to niacin is very small (60 mg of tryptophan forming 1mg of niacin) requires other vitamins like B_1, B_2 and B_6 and it is also very inefficient on a marginal diet. Niacin is also called *nicotinic acid* or sometimes vitamin B_3. Besides nicotinic acid, the diet also provides us nicotinamide which is the amide ($CO.NH_2$) 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 (AH_2) as a hydrogen nucleus with two electrons forming the hydride ion H^-. 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.

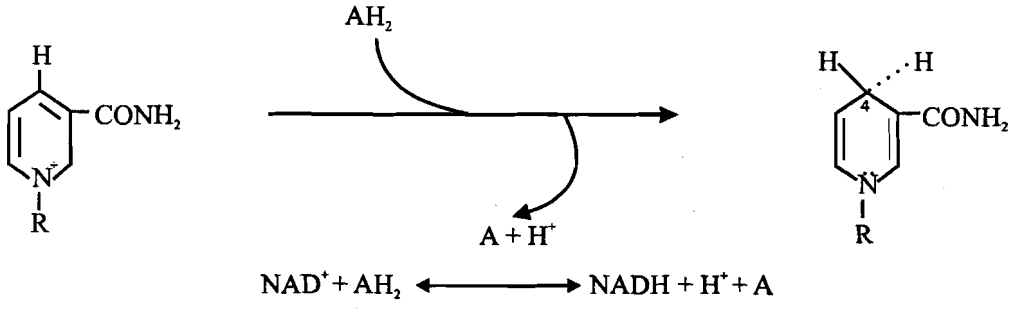


Figure 10.16: Mechanism of oxidation and reduction of nicotinamide coenzyme

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)
  - — malate dehydrogenase (look up citric acid cycle in Unit 6)
  - — L-3-hydroxyacyl CoA dehydrogenase (look up β-oxidation in Unit 7)
  - — L-glutamate dehydrogenase (look up oxidative deamination of glutamate in Unit 8).
- *$NADP^+$*
  - — glucose-6-phosphate dehydrogenase (look up pentose phosphate pathway in Unit 6)
  - — 3-ketocyl reductase (look up fatty acid biosynthesis in Unit 7)
  - — squalene synthetase (look up cholesterol biosynthesis in Unit 7)
  - — malic enzyme (look up fatty acid biosynthesis in Unit 7).

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.

10.4.1.5 Pantothenic acid

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 as you have seen in Unit 4. 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:

$$\underset{\text{(carboxyl group)}}{R\text{-}COOH} + CoA\text{-}SH \longrightarrow \underset{\text{(thioester)}}{R.Co.S.CoA} + H_2O$$

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)
- α-ketoglutarate dehydrogenase complex (in citric acid cycle in Unit 6)
- acyl-CoA synthetase (thiokinase) (in β-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 (as you may recall reading in Unit 7, lipid metabolism).

The last vitamin in this list is biotin. Let's look at its function as a cofactor in our body.

10.4.1.6 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 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 (in gluconeogenesis, Unit 6)
- propionyl CoA carboxylase (in gluconeogenesis, Unit 6)
- acetyl CoA carboxylase (in fatty acid biosynthesis, Unit 7).

The carboxylation takes place stepwise as given in Figure 10.17.

Carbon dioxide takes part in the form of bicarbonate (HCO_3^-) ion as shown herewith:

$$CO_2 + H_2O \longrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

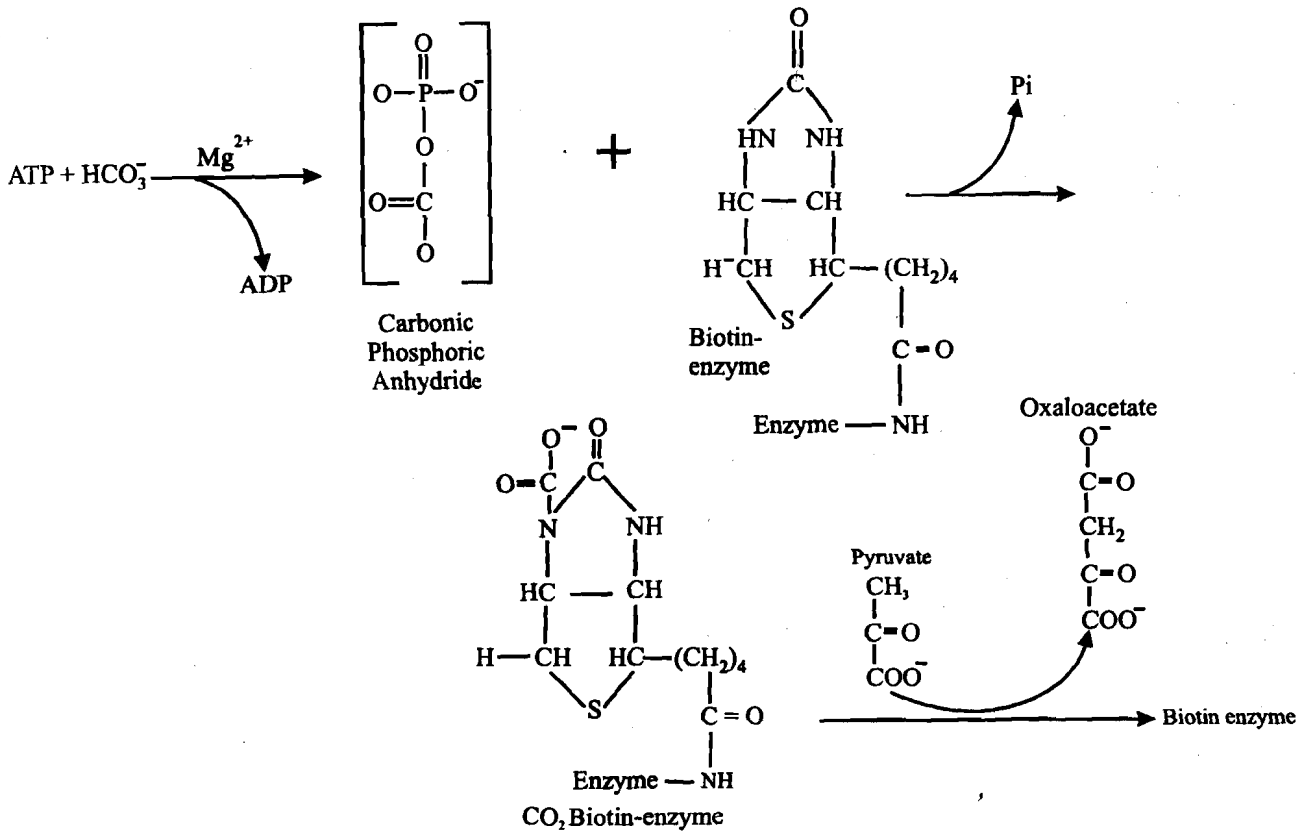


Figure 10.17: Biological role of biotin

In the first step, CO_2 is added to biotin catalyzed by the enzyme *biotin carboxylase* forming CO_2- biotin enzyme. This step is called *activation of* CO_2. The CO_2 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. β-oxidase complex and fatty acid synthase complex about which you may recall reading earlier in Unit 7.

With biotin, we end our discussion on energy-releasing water-soluble vitamins. Next group of vitamins we shall discuss now are the hematopoietic water-soluble vitamins. But first let us recall what we have learnt so far.

Check Your Progress Exercise 2

1) What are the major points of difference between the water-soluble and fat-soluble vitamins? Classify the water-soluble vitamins.

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2). Indicate the active forms of the following vitamins along with the reactions catalyzed by them:

a) Thiamin

.....

b) Niacin

.....

c) Biotin

.....

3) What are the 'metalloflavoproteins? Name any two of these giving appropriate examples?

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4) Discuss the role of :

a) Vitamin B_6 in transamination reaction.

.....

b) Pantothenic acid in metabolism of fat, protein and carbohydrate.

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5) Name the two important neurotransmitters that require pyridoxine as a coenzyme for their synthesis.

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Earlier in this section we studied about the energy-releasing water soluble vitamins. We have seen that some water soluble vitamins also have hematopoetic function. Let us get to know about these vitamins next.

10.4.2 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. Two such vitamins have been identified, which are folic acid and vitamin B_{12}. Let's look at their metabolic role next.

10.4.2.1 Folic acid

Folacin, as you may already know, is the generic form of folic acid and related substances, having folic acid activity. Look up the structure of folic acid in Unit 3, sub-section 3.3.8. 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 H^+ 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 (H_4) folate and these polyglutamate derivatives are the active form. Folic acid is also stored as tetrahydrofolate polyglutamate in liver.

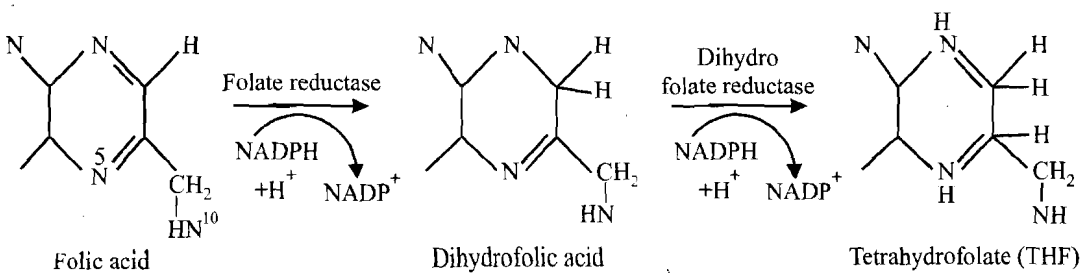

Figure 10.18: Synthesis of tetrahydrofolate

After a basic understanding of its structure, let us look at its metabolic role in our body.

Functions of Folic Acid

H_4 folate functions in what is referred to as *'one-carbon metabolism'*. It transfers one-carbon groups (groups containing only one carbon). These include methyl (CH_3), methylene (CH_2), 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.

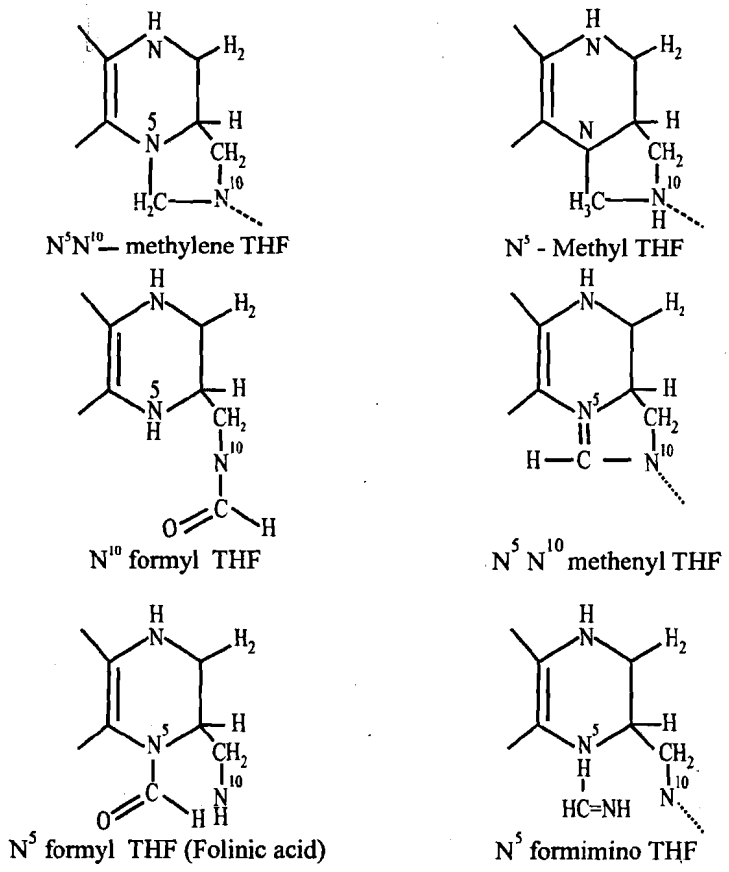

Figure 10.19: One-carbon units attached to tetrahydrofolate

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 anaemia. 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 N^5-formimino derivative.
- *Synthesis of purines-*
  N^{10}-formyl THF is the source of the carbon in position 2 of the purine ring while N^5,N^{10} 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.

You have already studied the structures of the intermediates involved in the above reactions. So revise them and write these folic acid reactions with structures in the space given herewith.

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Now, let us move on to vitamin B_{12}.

10.4.2.2 Cyanocobalamin (Vitamin B_{12})

Vitamin B_{12} 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 B_{12} 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_{12} deficiency 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.

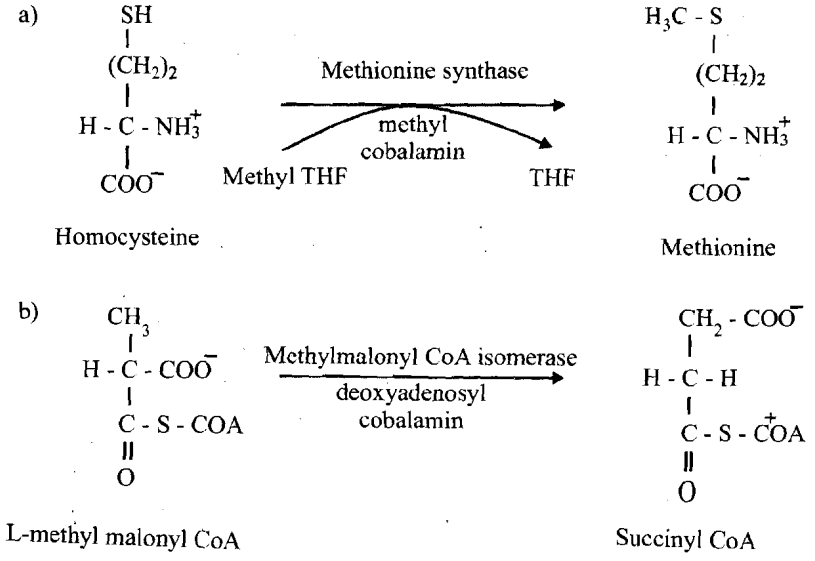

Figure 10.20 : Action of B_{12} coenzymes

Next, we shall look at the other water soluble vitamins, mainly the role of vitamin C

10.4.3 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.

10.4.3.1 Ascorbic Acid (Vitamin C)

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 reducing 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 (Fe^{2+}). During hydroxylation, Cu^+ (cuprous ion) is oxidized to Cu^{2+} (cupric ion) and Fe^{2+} (ferrous ion) to Fe^{3+} (ferric ion). Reduction back to Cu^+ or Fe^{2+} specifically requires ascorbate, which in the process, is oxidized to dehydroascorbic acid. In the body both forms are biologically 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 acids, 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

  Dopamine ⟶ 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 the 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 7α-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 (Fe^{2+}) form is more soluble and hence easily absorbed as compared to the ferric (Fe^{3+}) 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.

Check Your Progress Exercise 3

1) What are the active forms of folic acid and cyanocobalamin?

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2) Discuss the role of folate in 'one-carbon metabolism reactions'. Give any two examples.

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3) What is meant by 'folate trap'?

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4) Enumerate the various reactions catalyzed by ascorbic acid.

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The first section in this unit focussed on the study of vitamins. Minerals are the other important inorganic compounds required by our body for metabolism. Next, let us get to learn about their role in body metabolism.

10.5 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,

each of the other four nutrients has generally at least one common function in the body:

- carbohydrates – best source of energy
- proteins – structural component
- 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 faeces.

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 microminerals (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 function

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.

10.5.1 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.

10.5.1.1 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* [$Ca_{10}(PO_4)_6(OH)_2$]. 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 γ-carboxy glutamyl residues (Glu→Gla). These residues serve as high affinity binding sites for Ca^{2+}

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 VII, IX and X and Proteins C and S) are activated by calcium ions. Each contains between four and six γ-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 γ-carboxy glutamate residues in functions of vitamin K in this unit in sub-section 10.3.4. Milk clotting also requires Ca^{2+}. This is important in the digestion of milk in infants.

- Calcium ion is directly related to muscle contraction and nerve impulse transmission. In the absence of calcium, all types of muscles lose their ability to contract.
- 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
  - phospholipase A_2
  - calbindin
  - calmodulin
  - Ca^{2+}-dependent protein kinases
  - phosphorylase kinase
  - calsequestrin
  - 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. *Calsequestrin* is again 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 (Ca^{2+}) 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, glandular 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 (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) 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 about magnesium.

10.5.1.3 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 $Mg_3(PO_4)_2$ 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.

Let us look at its functions next.

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 (Mg^{2+}) 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. Mg^{2+} is essential for many enzymatic reactions and has two general interactions as highlighted herewith-

  a) Mg^{2+} 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) Mg^{2+} 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_2 to biotin in carboxylation reactions requires Mg^{2+}. Glutathione, a key intracellular antioxidant has Mg^{2+} 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.

Check Your Progress Exercise 4

1) Why are minerals considered essential for us?

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2) Discuss the role of calcium in our body. Highlight the significance of calcium as a signal transmitter.

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3) Enumerate the physiological functions of phosphorous.

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4) Describe the role of magnesium in-

a) Metabolic reactions

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b) Hormone action

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We move on to the next group of minerals classified as micro minerals.

10.5.2 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.

10.5.2.1 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.

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, CO_2 formed as a waste product in catabolic reactions

in the tissues, enters RBC and combines with haemoglobin to form *haemoglobin carbamate*. On reaching lungs, haemoglobin carbamate dissociates, releasing CO_2, 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 protein, *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 part 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 membranes and 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, c_1, c, a and a_3 (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 b_5. In liver microsomes, these two molecules have an important role in *detoxification* (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 Fe^{2+} and Fe^{3+}. *Succinic dehydrogenase*, an enzyme of the citric acid cycle, contains Fe:S and oxidizes succinate to fumarate. Another metalloflavoprotein 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 form 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 δ-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.

10.5.2.2 Iodine

You are all aware that it is important to use iodized salt i.e. salt to which the microelement iodine is added. In 1997, Parliament passed law making sale 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 general 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 70-80% 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 1mg 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-*triiodothyronine (T_3)* and *tetraiodothyronine (T_4* 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. T_3 binds with approximately 10 times the affinity of T_4. The role of thyroid hormone include :

- *Thermogenesis* 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. Obesity in some patients has been suggested to be the result of decreased energy and heat production due to diminished ATPase activity.

- *Metabolic effects of T_3 and T_4* – these include alterations in metabolism of carbohydrates, proteins, lipids, electrolytes and water. Thyroid hormone effects on carbohydrate metabolism 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. T_3 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 T_3 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 tract 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.

10.5.2.3 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 60 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

1.5% 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 (Zn^{2+}). 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 300 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 impairment of sexual development. Mild zinc deficiency may also affect the quality of growth. In both, children and adults, zinc deficiencies result in 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 hormone, 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 deficiency also leads to decreased taste acuity (hypogeusia).

10.5.2.4 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 insufficiency) 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 (H_2O_2) 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 H_2O_2 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 normal methionine.

10.5.2.5 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 ceruloplasmin 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, C_{18}, Δ^9 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}, Δ^9 desaturase – This enzyme is responsible for converting stearic acid (C_{18} saturated fatty acid) to oleic acid (C_{18} 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 11 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 Cu^{2+}. Hence copper is a cofactor for certain enzymes. Copper accepts 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 important physiological role in oxidation-reduction reactions, connective tissue formation, iron metabolism, central nervous system, melanin (pigment) formation and blood clotting.

10.5.2.6 Chromium

All plant and animal tissues contain chromium. Dietary chromium occurs in multiple valence states. Most of the chromium in the food supply is in the trivalent state (Cr^{3+}). Chromium is poorly absorbed in the intestine. Some of the chromium ingested with the food and inhaled as dust finally reaches the tissues. However, the 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 cross-linking agent for *collagen* (a protein present in connective tissue).
- Chromium is a component of a low molecular weight protein called *chromodulin* which 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 chromium as an *ergogenic* (muscle-building) *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.

10.5.2.7 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 occurring 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_{12}. You have already studied the functions of this vitamin. It functions as a coenzyme. The B_{12} coenzymes are called *cobamides* because of the presence of cobalt. You should revise the functions of vitamin B_{12} done in the earlier section under metabolic pathways.

10.5.2.8 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 tends 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 Mn^{2+} activates a number of plant and animal enzymes including oxidoreductases, lyases, ligases, hydrolases, kinases, transferases and various decarboxylases. While Mn^{2+} is specific for glucosyltransferase and xylosyltransferase, other divalent ions may replace Mn^{2+} as a cofactor in the case of other enzymes. Mitochondrial superoxide dismutase contains Mn^{2+}. 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).

Check Your Progress Exercise 5

1) What are microminerals or trace elements? How are these different from macro minerals?

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2) Enumerate the physiological roles of iron.

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3) What are the two active forms of thyroid? Discuss their metabolic effects.

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4) Briefly discuss:

a) Consequences of increased and decreased thyroid activity.

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b) Any four functions of Zn.

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5) Discuss the role of following:

a) Copper in blood clotting

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b) Chromium in glucose tolerance

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c) Manganese as enzyme activator

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6) Match the following:

	A		B
(i)	Selenium	(a)	Chromodulin
(ii)	Copper	(b)	Cytochromes
(iii)	Zinc	(c)	Glutathione peroxidase
(iv)	Chromium	(d)	Gustin
(v)	Iron	(e)	Ceruloplasmin

10.6 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 – macrominerals, required in much larger quantities than the other class called as micro – minerals. Here we dealt with an exhaustive list of these minerals and got to know about their structure and functions.

10.7 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 deficiencies 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.

Metalloflavoproteins : 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.

10.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) Fat-souble vitamins are transported in blood as constituents of lipoprotein molecules (molecules containing lipid plus protein) or attached to specific binding proteins. Conditions affecting the digestion and absorption of the fat-soluble vitamins such as steatorrhea (fatty diarrhoea) and disorders of the biliary system can all lead to deficiency syndromes associated with that particular vitamin.

2) The active forms of vitamin A are retinol, retinal, retinoic acid; and vitamin D is 1, 25 dihydroxy cholecalciferol (calcitriol).

3) The various functions of vitamin A are normal vision, control of cell differentiation and turnover, glycoprotein synthesis, synthesis of the protein transferrin, anti-cancer activity, antioxidant.

   For the rods to function in dim light, two events have to take place. All-*trans* retinaldehyde in the rods has to be first isomerized to the specific 11-*cis* isomer and then it has to combine with the protein opsin to form rhodopsin.

4) a)Vitamin E 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.

   b) The mechanism of vitamin k as anticoagulant is through the proteins (clotting factor) called *prothrombin* or blood clotting factor II. Prothrombin is synthesized as an inactive precursor called preprothrombin. 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.

   c) When calcitriol 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, which 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. 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.

5) The 2-step conversion process of vitamin k to hydroquinone involves a first reduction results in loss of the epoxide oxygen forming the quinone structure. This reaction is catalyzed by vitamin *K epoxide reductase* and needs any compound having 2 SH (sulfhydryl) groups. The compound now has –S–S– (disufide) group. Vitamin K quinone undergoes the second reduction reaction catalyzed by *vitamin K quinone reductase* and vitamin K hydroquinone is regenerated.

Check Your Progress Exercise 2

1) The major points of difference between water-soluble and fat-soluble vitamins are :

Water-soluble vitamins	Fat-soluble vitamins
a) Soluble in water	Insoluble in water

b)	No common structure	Isoprene is common structure
c)	Excess is excreted in urine and no toxic effects are seen	Excess is stored in body and can lead to toxicity
d)	Mostly function as coenzyme	Varying biological roles.

Water-soluble vitamines may be classified as energy-releasing, hematopoietic, others.

2) a) Thiamine pyrophosphate (TPP) or Thiamine diphosphate (TDP) is the active form of thiamin. The reactions catalyzed by it are: Pyruvate dehydrogenase in carbohydrate metabolism, α-ketoglutarate dehydrogenase in the citric acid cycle, branched-chain keto-acid dehydrogenase in metabolism of branched-chain amino acids (leucine, isoleucine and valine).

b) NAD^+ and $NADP^+$ are the active forms of Niacin. It acts as coenzyme in several redox reactions and dehydrogenases reactions.

c) Biotin catalyzes the following reactions: pyruvate carboxylase (in gluconeogenesis), propionyl CoA carboxylase (in gluconeogenesis) and acetyl CoA carboxylase (in fatty acid biosynthesis).

3) Metalloflavoproteins are the flavoproteins containing metal ions. FMN and FAD are the 2 metalloflavoproteins. Examples of FMN are L-amino acid oxidase, NADH dehydrogenase and of FAD are succinate dehydrogenase, Acyl CoA dehydrogenase and Xanthine oxidase.

4) a) Vitamin B_6 acts as a coenzyme for many enzymes involved in transamination reactions, that is, transfer of NH_2 groups from amino acids.

b) The active form of Pantothenic acid is coenzyme A, which is required for the metabolism of fat, protein and carbohydrates. For instance, in pyruvate dehydrogenase complex, α-Ketoglutarate dehydrogenase, Acyl-CoA synthetase and ACP in biosynthesis of fatty acid.

5) Serotonin and γ-aminbutyrate (GABA) are the two important neurotransmitters that require pyridoxine as a coenzyme for their synthesis.

Check Your Progress Exercise 3

1) The active form of Folic Acid is folacin and Cyanocobalamin is methylcobalamin and deoxyadenosyl cobalamin.

2) Folate transfers one-carbon groups including methyl, methylene, methenyl groups which are obtained from various compounds in the body e.g. serine glycine inter-conversion and synthesis of methionine.

3) In case of vitamin B_{12} deficiency, the conversion of L-methyl malonyl CoA to succinyl CoA cannot take place. Subsequently, propionate can't be used for glyconeogenesis and all THF is trapped as methyl THF. This is folate trap.

4) The various reactions catalyzed by ascorbic acid can be enumerated as :

- hydroxylation of basic amino acids required for the synthesis of the protein collagen
- synthesis of the hormones- norepinephrine and epinephrine from tyrosine
- catabolism of tyrosine
- formation of bile acid from cholesterol
- absorption of iron
- steroidogenesis
- antioxidant

Check Your Progress Exercise 4

1) Minerals are essential to many vital processes, such as they 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.

2) Calcium in our body plays role in bone development and teeth formation, blood clotting, muscle contraction and nerve impulse transmission, decreases membrane permeability and increases capillary permeability, and mediator of hormone action, regulator of several enzymes and proteins. The role of calcium as signal transmitter can be discussed as :

   When a cell is activated, calcium channels in the plasma membrane open to admit a few calcium ions into the cytosol. These bind immediately to a wide array of intracellular activator proteins, release a flood of calcium from the intracellular storage vesicles. This second step quickly raises cytosol calcium concentration and leads to activation of the contraction complex. Troponin C and Calmodulin are the two calcium binding proteins involved in signal transmission.

3) The physiological functions of phosphorous are: formation of bone and dental tissue, participation in the structure of all body cells as phospholipids, participation in reactions resulting in the liberation of energy, influencing acid-base balance of blood, regulation of metabolic pathways, constituent of various physiologically important molecules.

4) a) Magnesium in metabolic reactions forms complexes with a variety of organic molecules having biologic activities. Mg^{2+} is essential for many enzymatic reactions for example Mg^{2+} binds to the substrate, thereby forming a complex with which the enzyme interacts as in the reaction of the kinase enzymes with Mg ATP, and Mg^{2+} 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

   b) Cyclic AMP (cAMP) which acts as a second messenger in hormone action is formed from Mg ATP and the enzyme adenylyl cyclase which is activated by magnesium through its two binding sites.

Check Your Progress Exercise 5

1) Microminerals or trace elements are a group of minerals that are required in amounts less than 100 mg/day. These are different from macro minerals as their concentrations can't be measured precisely and these occur in traces and are required in less amounts as compared to macronutrients.

2) Iron has an important role in oxygen transport by haemoglobin, the high metabolic activity of muscle cells is ensured by myoglobin- a Fe^{2+}-containing muscle protein. It is a constituent of heme and enzymes peroxidase and catalase. It is also constituent of cytochromes and flavoproteins.

3) The two active forms of thyroid are traiodothyronine (T_3) and tetraiodothyronine (T_4). Thyroid hormone effects on carbohydrate metabolism 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 positive nitrogen balance. Thyroid hormones induce or repress proteins by increasing or decreasing gene transcription mechanism. It act in conjunction with pituitary growth hormone (GH) as the principal anabolic agents during growth and maintaining protein stores. T_3 enhances transcription of the GH gene so that more GH is produced.

4) a) 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 tract and in the vascular system. 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 of the skin gives rise to the adult hypothyroid state called myxedema.

b) Functions of Zn are as follows :

- cell multiplication and cell growth
- stimulator of trans-acting factors responsible for regulating gene expression
- reduces the risk of acquiring degenerative disease
- maintains the structure and function of biomembranes.

5) a) Blood clotting factor V contains one atom of copper per molecule. This indicates that copper may be required for blood clotting.

b) Chromium is a component of a low molecular weight protein called chromodulin which 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.

c) Manganese as Mn^{2+} activates a number of plant and animal enzymes including oxidoreductases, lyases, ligases, hydrolases, kinases, transferases and various decarboxylases. Glucosyltransferase links carbohydrate to protein during the synthesis of glycoproteins.

6) (i) - (c)

(ii) - (e)

(iii) - (d)

(iv) - (a)

(v) - (b)