

UNIT 8

FAT SOLUBLE VITAMINS |

Structure

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8.1 INTRODUCTION

Vitamins are defined as organic compounds occurring in minute quantities in various natural foods and important for growth and maintenance of good health and protection from deficiency diseases in human beings. They are indispensable factors, which are vital for the proper utilization of the major macro nutrients like carbohydrates, fats and proteins.

In Unit 7 you have already read about the water-soluble vitamins. In Unit 8 you will learn about the fat-soluble vitamins which include A, D, E, and K.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ understand the structure of fat-soluble vitamins;
- ❖ explain the biological role and metabolism of fat-soluble vitamins;
- ❖ know the various dietary sources of the vitamins;
- ❖ know about the daily dietary intake requirement; and
- ❖ explicate the biochemical basis of toxicity and deficiency symptoms.

8.2 Vitamin A

Vitamin A is an essential vitamin required for growth and development, cell recognition, vision, immune function, and reproduction. It also acts as a powerful antioxidant and a hormone in the body, affecting gene expression and consequently influencing phenotype. The other major organs of the human system like heart, lungs and kidneys also function well in the presence of Vitamin A.

8.2.1 Structure

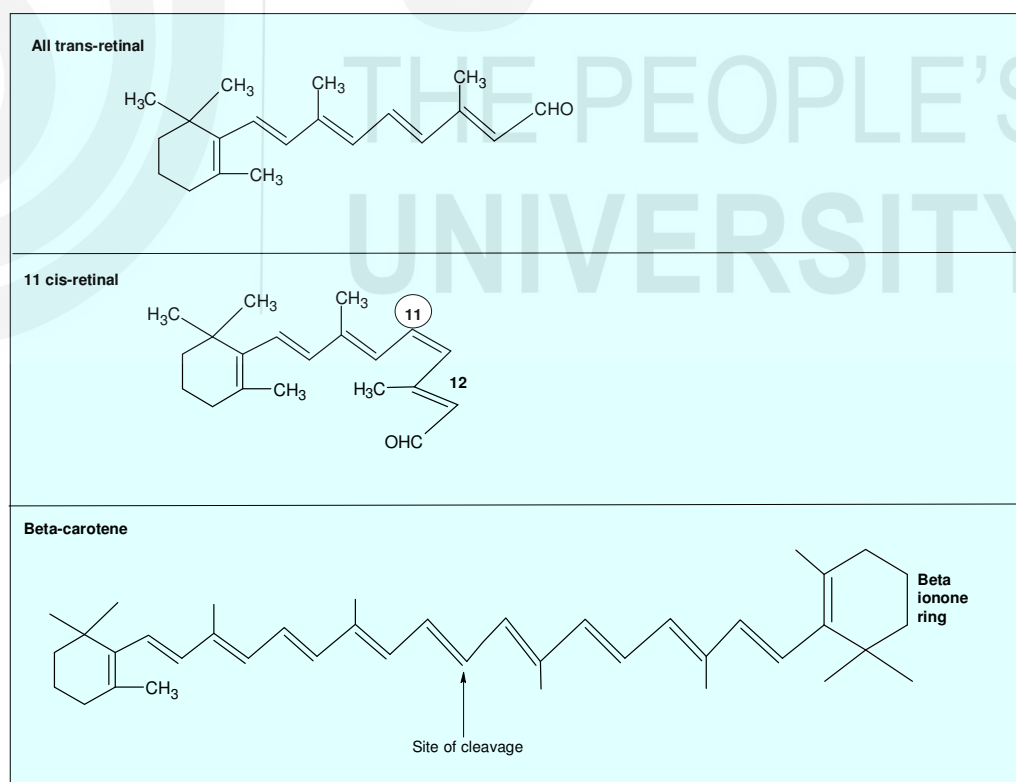


Fig. 8.1: Structures of vitamin A.

The active form of Vitamin A is present only in animal tissues. The precursor of vitamin A (**provitamin**), beta-carotene is available in plant tissues. **Beta-carotene** consists of two beta ionone rings (Fig. 8.1). Retinoids are

compounds depicting vitamin A activity. They have **beta-ionone** (cyclohexenyl) ring system. **Retinol** (vitamin A alcohol), **retinal or retinaldehyde** (vitamin A aldehyde) and **retinoic acid** (vitamin A acid) are the three different compounds having vitamin A activity (Fig. 8.1). The presence of enzyme retinal reductase reduces retinal to retinol. It is a readily reversible reaction. Retinoic acid is obtained by the oxidation of retinal, which cannot be transformed back to the other forms (Fig. 8.2). Since the side chain contains alternate double bonds, many isomers are possible. Vitamin A1, also called the **all-trans** variety of retinal, is most common. Vitamin A2 found in *fish oils*, has an extra double bond in the ring. **11-cis-retinal** is the biologically important compound.

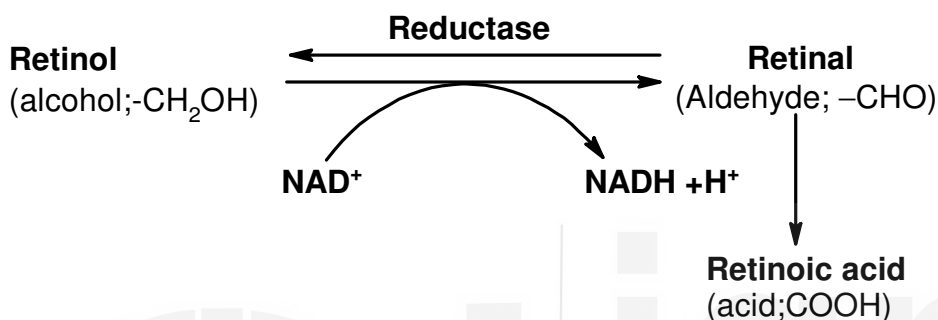


Fig. 8.2: Inter-conversion of Vitamin A molecules.

8.2.2 Biological Role

Vitamin A (retinol) is an essential fat-soluble nutrient needed in small amounts by humans for normal functioning of the visual system; for growth and development; and for maintenance of cellular integrity of the epithelial cells, immune system function, and reproduction.

Wald's Visual Cycle

Generation of Nerve Impulse: In 1967, Nobel Prize was awarded to Wald, for recognizing the role of vitamin A in vision. The photoreceptor cells of the retina consist of a membrane protein called **Rhodopsin (visual purple)**. The two components of Rhodopsin are **the protein opsin and the vitamin A isomer, 11-cis-retinal**. When light falls on the retina, all-trans retinal is formed as a result of the isomerization of the 11-cis-retinal (Fig. 8.3). The photon gives rise to immediate conformational change which leads to the production of opsin + all-trans retinal. The protein then releases the all-trans-retinal. Visual pigments are G-protein-coupled receptors. The receptor protein (Opsin) is locked in its inactive form by 11-cis retinal (Fig. 8.4). The G-protein is activated due to the isomerization and photo excitation and generates cyclic -GMP. The retina consists of **Transducin** as its G-protein. A nerve impulse in the retina is generated by the Cyclic GMP which is carried to visual centers in the brain.

The absorption of light by rhodopsin also causes a series of conformational changes in opsin to form Photorhodopsin, Bathorhodopsin, Lumirhodopsin and finally Metarhodopsin III. In the final step this is converted to opsin and all-trans retinal. Hence, bright light markedly depletes the stores of rhodopsin in the rods.

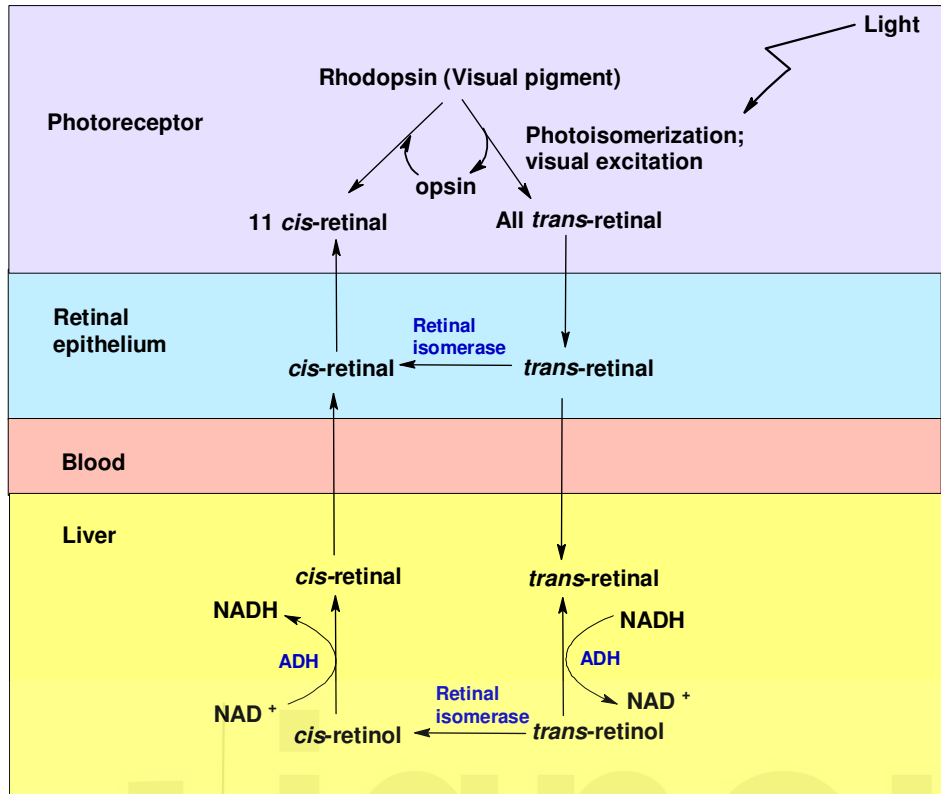


Fig. 8.3: Wald's visual cycle. Purple color represents reactions in photoreceptor matrix. Blue background represents reactions in retinal epithelium. Red depicts blood. Brown shows reactions in liver.

Regeneration of 11-cis-retinal: After dissociation, trans retinal enters the blood circulation but the opsin remains in retina. Later cis-retinal is generated, which reaches retina. The enzyme **retinal isomerase** converts all-trans-retinal to 11-cis-retinal in the retina itself in the dark. **Rhodopsin** can be regenerated by recombination of the 11-cis retinal and opsin. On the other hand, an NADH dependent enzyme, **alcohol dehydrogenase (ADH)** reduces all-trans-retinal to all-trans-retinol in the liver. The all-trans-retinol is isomerized to 11-cis-retinol and then oxidized to 11-cis-retinal in liver, which is further transported to retina. This concludes the Wald's (Rhodopsin) visual cycle (Fig. 8.3 and 8.4).

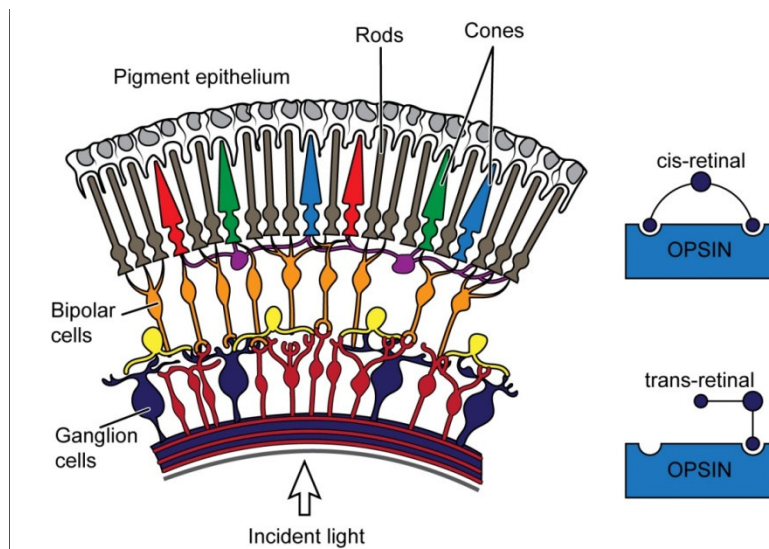


Fig. 8.4: Structure of retina showing rods and cones. The blue box on the right side shows the structural alteration during photo-isomerization.

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. Recollect your experience when you have entered a movie theatre a little late!

Other Biochemical Functions

i. Retinoic acid acts like steroid hormone in the regulation of gene expression and **differentiation** of tissues. The two biologically active isomers, all-trans retinoic acid and 9-cis retinoic acid are transported to the nucleus of the cell and bind to nuclear receptors which in turn bind to specific regions of DNA called response elements. This then causes expression of that gene and synthesis of mRNA (transcription). This will ultimately lead to the synthesis of a specific protein required for growth.

ii. Vitamin A is required for normal **reproduction**. All-trans retinoic acid supports both male and female reproduction as well as embryonic development. In vitamin A deficiency, miscarriages are noticed in female rats.

iii. Since carotenoids are natural antioxidants, they exhibit anticancer activity. Fresh vegetables containing carotenoids were shown to reduce the incidence of cancer. β -carotene is an antioxidant effective at low oxygen concentrations. Oxidation (peroxidation) in tissues produces free radicals which are extremely toxic and damage tissues. β -carotene plays a role in trapping peroxy free radicals and preventing development of cancerous tissues.

iv. Vitamin A is essential for the maintenance of normal epithelium and skin. Retinoyl phosphate functions as a carrier of oligosaccharides across cell membrane and the oligosaccharides are used for synthesis of glycoproteins which in turn are necessary for mucous secretion. Mucous contains the glycoprotein mucin. Hence, in Vitamin A deficiency there is reduction in mucous secretion resulting in keratinization of epithelial tissues of eyes, lungs, gastrointestinal and genitourinary tracts. Figure 8.5 summarizes the actions of vitamin A.

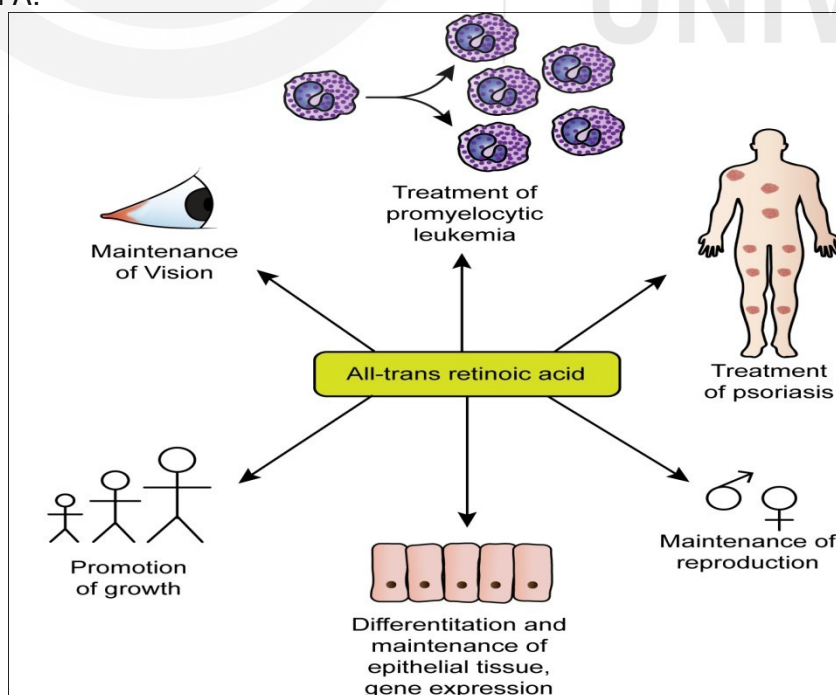


Fig. 8.5: Summary of actions of vitamin A.

8.2.3 Metabolism

Retinal is formed by the cleavage of beta carotene by a dioxygenase. The enzyme retinal reductase present in the intestinal mucosa converts retinol to retinal. Intestine is the major site of absorption (Fig.8.6). Bile salts are required for the absorption along with other fats. Vitamin is incorporated into chylomicrons and transported to the liver. In the liver, vitamin A is stored as **retinol palmitate (80%)**. Adipose tissues contain about 15% vitamin A.

Transport from Liver to Tissues:

The peripheral tissues receive Vitamin A as trans retinol bound to the **retinol binding protein** or RBP. In the case of vitamin A deficiency, the RBP level in blood decreases.

Uptake by Tissues:

The retinol-RBP complex binds to specified receptors on the retina, skin, reproductive organs and other tissues. Vitamin A binds to cellular retinoic acid binding protein (CRBP) and finally to hormone responsive elements (HRE) of DNA. Consequently, genes are activated (Fig. 8.6).

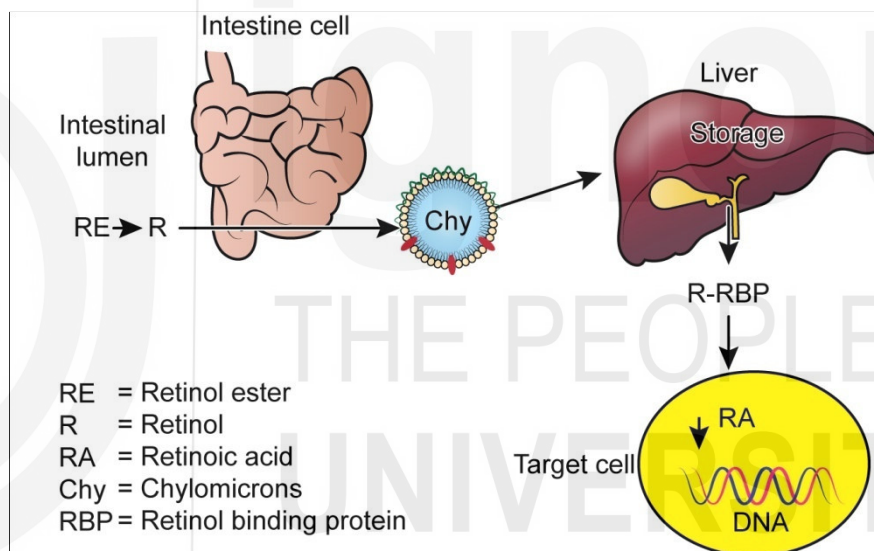


Fig. 8.6: Vitamin A metabolism.

8.2.4 Dietary Sources

Animal sources of Vitamin A include milk, butter, cream, cheese, egg yolk and liver. Fish liver oils (cod liver oil and shark liver oil) are abundant sources of the vitamin. Vegetable sources contain the yellow pigment, carotenoids like beta carotene, a precursor of Vitamin A. **Carrot** contains significant quantities of beta carotene. **Papaya, mango, pumpkin, green leafy vegetables** (spinach, amaranth) are other good sources of vitamin A. IFCT, 2017 gives food sources of all the fat-soluble vitamins. Since Vitamin A is fat soluble, it is not destroyed during cooking.

8.2.5 Dietary Requirements

The conversion ratio suggested for β -carotene: vitamin A is 6:1. Considering the recent studies on vitamin A status carried out in India, an upward revision of retinol has been recommended. The EAR for vitamin A is the daily dietary

intake required to balance its catabolic loss, absorption and efficiency of storage. The EAR for adult man and woman has been calculated to be $7\mu\text{g}$ RAE/kg/day. Thus, a 65 kg man and a 55 kg woman would need EAR of $460\mu\text{g/d}$ and $390\mu\text{g/d}$ respectively of Vitamin A. The RDA is $15\mu\text{g}$ RAE/kg/day and accordingly 65 kg adult man and 55 kg adult woman should have $1000\mu\text{g/day}$ and $840\mu\text{g/day}$ respectively of Vitamin A. For a 55 kg pregnant woman EAR and RDA have been set at $406\mu\text{g/d}$ and $900\mu\text{g/d}$ respectively; during lactation, the requirement is $720\mu\text{g/day}$ and $950\mu\text{g/day}$ respectively. To ensure adequacy at least in vulnerable groups like pregnant and lactating women, the Committee has recommended that a minimum of 50% RE be drawn from animal sources (ICMR-NIN, 2020). The EAR and RDA for infants, children and adolescents are given in Appendices 1.1 and 1.2.

8.2.6 Biochemical Basis of Deficiency Diseases and Toxicity, their Symptoms

Night Blindness or Nyctalopia

Visual sharpness is declined in dim light and is one of the earliest signs. The person is unable to read, write or drive in poor light. Resynthesis of Rhodopsin does not reach optimal levels and/or is delayed. Hence the dark adaptation time is increased.

Xerophthalmia

The conjunctiva becomes dry, thick and wrinkled. It loses its transparency and gets keratinized. The dryness progresses to the cornea. The corneal epithelium gets keratinized resulting in glazy and lusterless cornea. Infections may supersede.

Bitot's Spots

These spots are seen as grey-white triangular plaques adherent to the conjunctiva. It happens due to increased thickness of conjunctiva in certain areas. You have seen picture of Bitot's spot in Unit 1. If the vitamin is supplemented at these stages, the ocular changes are completely reversible.

Keratomalacia

When xerophthalmia continues for a long time, it progresses to keratomalacia (softening of the cornea). The corneal epithelium gets degenerated and then vascularized. Later, blurring of the cornea occurs. In later stages, perforation of cornea, corneal ulceration and total blindness occurs due to bacterial infection.

Preventable Blindness

The most common cause of blindness in Indian children below the age of 5 has been Vitamin A deficiency (VAD). As a prophylactic measure, 6-59 month old children are given mega dose of Vitamin A every six months. Various surveys have indicated that during the past three decades, there has been a sharp decline in prevalence of Bitot's spots and further, keratomalacia and blindness due to VAD in children has almost disappeared. However, sub-clinical VAD may still be a nutritional challenge in many sections of the population.

Skin and Mucous Membrane Lesions

- i) The epithelium lining the follicles get hyper keratinized, which results in follicular **hyperkeratosis** or phrynoderma. The skin becomes rough and scaly. Keratinizing metaplasia of the epithelium of the respiratory, gastro-intestinal and genitourinary tracts have been observed.
- ii) There is increased occurrence of generalized infections due to the alterations in the skin. Therefore, in old literature, vitamin A is referred to as anti-inflammatory vitamin or anti-infective vitamin.
- iii) Isoretinone, a synthetic variant of vitamin A is known to reduce the sebaceous secretions; therefore, it is used to prevent **acne** formation during adolescence.

Hypervitaminosis A or Toxicity:

Hypervitaminosis or toxicity can occur due to excessive intake since the vitamin can be stored in the liver. Symptoms of toxicity include anorexia, irritability, headache, peeling of skin, drowsiness, vomiting, liver damage and birth defects. Hypercarotenemia can result from persistent excessive consumption of foods rich in carotenoids. The skin becomes yellow, but no staining of sclera as in jaundice is observed.

The tolerable upper limit (TUL) of intake of Vitamin A has been set at 3000 µg/day (10,000 IU /day) for adults (ICMR-NIN, 2020). Appendix 1.3 lists TUL for infants, children and adolescents across all age groups.

SAQ 1a

Tick Mark the Correct Option:

- i) RDA of vitamin A for a normal 65 kg adult is:
 - a) 1 microgram
 - b) 5 microgram
 - c) 100 microgram
 - d) 1000 microgram
- ii) Deficiency of vitamin A leads to:
 - a) Night blindness
 - b) Rickets
 - c) Macrocytic anemia
 - d) Microcytic anemia
- iii) All are good sources of vitamin A, *except*:
 - a) Pumpkin
 - b) Carrot
 - c) Mangoes
 - d) Oranges

- iv) The structure of vitamin A contains:
- a) Chromane ring
 - b) Beta ionone ring
 - c) Thiazole ring
 - d) Naphthoquinone ring
-
-

SAQ 16

Fill in the blanks with appropriate words:

- i) The precursor of vitamin A (provitamin),is available in plant tissues.
 - ii) The two component proteins of Rhodopsin are and
 - iii) In the liver, vitamin A is stored as
 - iv) The full form of CRBP is
-

8.3 VITAMIN D (CHOLECALCIFEROL)

McCullum (1919) showed experimental rickets induced by dietary deficiency in rats. In the year 1931, Vitamin D was isolated by Angus and coworkers. It was named as calciferol, and later identified as Vitamin D₃. The structural clarification was done by Otto Diels and Kurt Alder. Both were awarded Nobel Prize in Chemistry in 1950.

8.3.1 Formation

Vitamin D is obtained either from 7-dehydrocholesterol or ergosterol by the action of ultraviolet radiations. **7-dehydrocholesterol**, a derivative of cholesterol, is present in epidermis. In the skin, the bond between positions 9 and 10 of the steroid ring is broken by the ultraviolet rays. So, the provitamin **secosterol** is formed upon the opening of ring B. The isomerization of the cis double bond between 5th and 6th carbon atoms takes place forming a trans double bond (rotation on the 6th carbon atom). This gives rise to vitamin D₃ or **cholecalciferol** (Fig. 8.7). So, vitamin D is called the “**sun-shine vitamin**” since it is produced after exposure of the human body to sunlight. The production of vitamin D in the skin is equivalent to the sunlight received and inversely proportional to the pigmentation of skin. Vitamin deficiency is seen in winter. For profitable use, the vitamin is derived from the fungus, ergot. When ergosterol is treated with ultraviolet light, **ergocalciferol** or vitamin D₂ is obtained. An unsaturation in the side chain and an extra methyl group (C₂₈) makes it different from vitamin D₃.

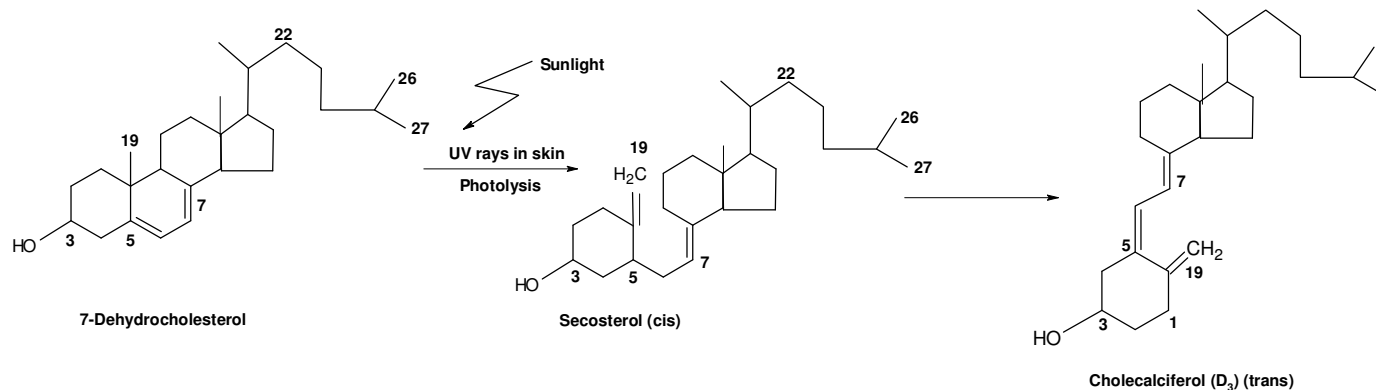


Fig. 8.7: Synthesis of cholecalciferol or vitamin D₃.

8.3.2 Activation

Vitamin D is a **prohormone**. The cholecalciferol is first transported to liver, where it is converted to 25-hydroxy cholecalciferol (25 HCC) by hydroxylation at **25th position** (Fig. 8.8). The 25-Hydroxy cholecalciferol is the major storage form of vitamin D in the liver. The “vitamin D binding protein” (VDBP) binds 25-HCC in the plasma. It is further hydroxylated at the 1st position in the kidney. In this way, generation of 1-25 -dihydroxy cholecalciferol (**DHCC**) takes place. It is also called **Calcitriol** since it contains three hydroxyl groups at 1, 3 and 25 positions (Fig. 8.8). The formation of new product calcitriol is the active form of vitamin D, which is a hormone. Low serum calcium leads to the release of parathyroid hormone (PTH) which induces the production of calcitriol. A complex is formed between calcitriol and its highly specific nuclear receptor- Vitamin D receptor (VDR); which further binds to VDRE (vitamin D response elements) on DNA which regulate the expression of more than 500 genes. This activation takes place rapidly.

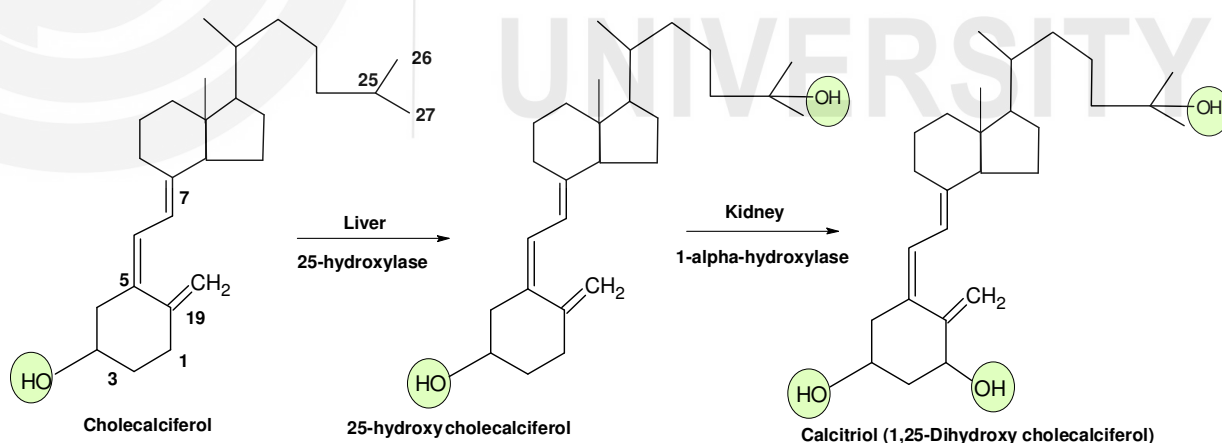


Fig. 8.8: Generation of Calcitriol.

Biochemical Effects

Vitamin D functions like a hormone. Calcitriol acts as a transcription factor promoting the expression of a gene encoding for calcium binding protein which is involved in calcium absorption. Therefore, vitamin D is essential for calcium absorption in the intestine and it also enhances bone growth. Additionally, calcitriol stimulates reabsorption of calcium in the distal renal tubules and it also mobilizes bone mineral.

In addition to calcium, vitamin D also helps in maintaining phosphorous concentration in plasma. Calcitriol also participates in insulin secretion as well as synthesis and secretion of thyroid hormone, inhibition of production of interleukin by activated T-lymphocytes and of immunoglobulin by activated B-lymphocytes, differentiation of monocyte precursor cells and modulation of cell proliferation.

8.3.3 Absorption of Calcium

The absorption of calcium and phosphorous is promoted by Calcitriol in the intestine. Calcium is absorbed passively on the brush-border surface. The absorption of calcium from intestinal cell to blood needs energy. **Calcitriol** functions as a steroid hormone. The specific genes which code for calbindin are activated (Fig. 8.9). The absorption of calcium is enhanced due to the increased availability of calcium binding protein.

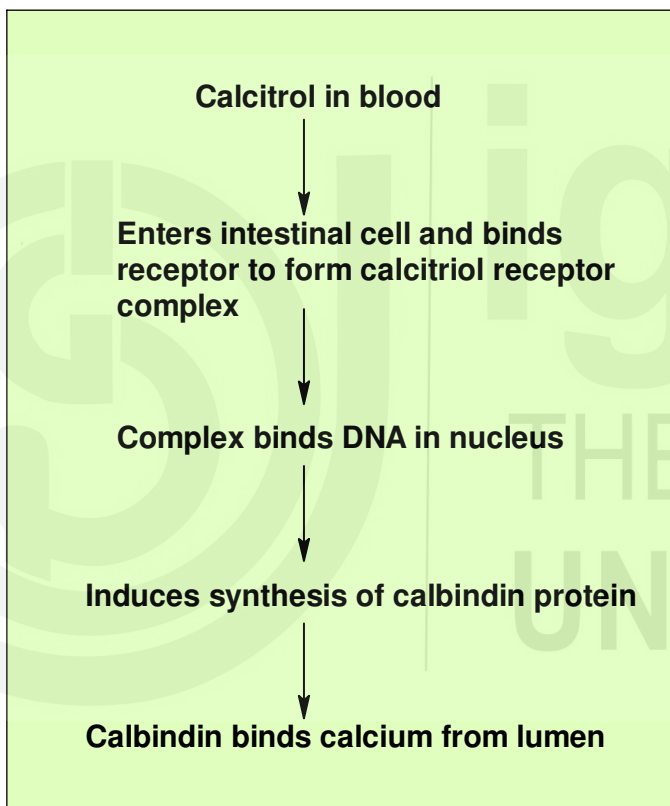


Fig. 8.9: Calcitriol increases calcium absorption.

8.3.4 Dietary Sources

Adequate exposure to sunlight with ultraviolet B radiation of wavelength 290-320 nm, for 20 minutes a day, without the application of a sunscreen is highly recommended. Maximum vitamin D synthesis takes place between 11 AM-3 PM. However, there is now widespread vitamin D deficiency in India due to indoor sedentary lifestyle and limited exposure to sun. Hence, dietary sources of vitamin D become important. Fish liver oil, fish, liver and egg yolk are good sources of the vitamin. Milk contains a moderate quantity of vitamin D. Fortification of dairy products with vitamin D will be beneficial.

8.3.5 Dietary Requirements

As per the report of the expert Group (ICMR-NIN 2020), an EAR of 400 IU/day (10 µg/day) and RDA of 600 IU/day (15 µg/day) is recommended for all the age groups, except infants up to one year of age where the recommended intake (RDA) is 400 IU/day (10 µg/day), while emphasizing the importance of outdoor physical activity as a means of achieving adequate vitamin D status in a tropical country like India.

8.3.6 Biochemical Basis of Deficiency Diseases and Toxicity, their Symptoms

The deficiency diseases caused due to lack of vitamin D are **rickets** in children and **osteomalacia** in adults. Hence vitamin D is known as antirachitic vitamin. The optimal concentration of 25-hydroxy D3 is > 30 ng/ml, whereas 20–29 ng/ml is considered insufficient and 10–19 ng/ml is considered as deficient. A level below 10 ng/mL indicates severe deficiency. Concentration more than 100 ng/mL is toxic. Various studies have shown that 50–80% of elderly and 20–50% of children suffer from deficiency of vitamin D. Even in affluent countries, vitamin D deficiency is very common. Vitamin D deficiency is associated with poor bone health, low calcium concentration, higher systolic blood pressure and lower HDL cholesterol values; all of which are risk factors for heart disease. Additionally, it is being increasingly recognized that many non-calcitropic health promoting effects of Vitamin D are important for chronic diseases like diabetes, psoriasis, arthritis and malignancies (ICMR-NIN, 2020).

Causes for Vitamin D Deficiency

- i) Deficiency of vitamin D occurs in people who are not exposed to sunlight adequately, e.g. inhabitants of northern latitudes in winters, in people who are bedridden for long periods, or those who cover the whole body (*purdah*).
- ii) Nutritional deficiency of calcium or phosphate may also produce lack of vitamin D as these two minerals help in its absorption.
- iii) Malabsorption of vitamin in conditions like obstructive jaundice and steatorrhea may take place. High phytate content in diet reduces the absorption of vitamin D.
- iv) In certain diseases, where liver and renal functions are impaired, abnormality of vitamin D activation takes place. In such cases, the hydroxylation reactions are retarded.
- v) Deficient renal absorption of phosphates.

Clinical Features of Rickets

Rickets is seen in children. The mineralization of bone is insufficient. Bones become soft and pliable. The bone growth is markedly affected. **Bone deformities** are the classical features of rickets. Weight bearing bones are bent. Continued action of muscles also causes bone malformations. The clinical manifestations include bow legs, knock-knee, rickety rosary, bossing of frontal bones, and pigeon chest. An enlargement of the epiphysis at the lower

end of ribs and costochondral junction leads to beading of ribs or **rickety rosary**. **Harrison's sulcus** is a transverse depression passing outwards from the costal cartilage to axilla. This is due to the indentation of lower ribs at the site of the attachment of diaphragm. You have seen in Unit 3, picture of child suffering from rickets and having 'bow legs'.

Clinical Features of Osteomalacia

The term Osteomalacia is derived from Greek "osteon" = bone; and "malakia" = softness. The bones are softened due to inadequate mineralization and increased osteoporosis. Patients are prone to get fractures. It may be noted that vitamin D deficiency never produces severe hypocalcemia. Tetany will not be manifested. Biochemical parameters like serum **alkaline phosphatase**, bone isoenzyme, are highly increased.

Hypervitaminosis D:

Toxicity symptoms include weakness, polyuria, intense thirst, hypertension and weight loss. Hypercalcemia leads to calcification of soft tissues, (metastatic **calcification**, otherwise called **calcinosis**), especially in vascular and renal tissues. Although vitamin D is toxic in higher doses, excessive exposure to sunlight does not result in vitamin D toxicity, because excess D₃ is destroyed by sunlight itself.

The Tolerable Upper Limit (TUL) for vitamin D for adults is 100 µg/day (4000 IU/day). The TUL for infants, children and adolescents is given in Appendix 1.3.

SAQ 2a

Tick Mark the Correct Option:

- i) Cholecalciferol is synthesized in:
 - a) Liver
 - b) Skin
 - c) Kidney
 - d) Intestinal mucosa
- ii) Rickets may occur in all conditions, except:
 - a) Chronic renal failure
 - b) Liver diseases
 - c) Under exposure to sun light
 - d) Prolonged antibiotic therapy
- iii) Richest source of vitamin D is:
 - a) Fresh leafy vegetables
 - b) Fish liver oil
 - c) Egg yolk
 - d) Vegetable oils

- iv) The RDA of vitamin D for an adult is:
- 500 IU
 - 600 IU
 - 300 IU
 - 400 IU

SAQ 26

Fill in the blanks with appropriate words:

- Vitamin D is also called the
- 1-25 -dihydroxy cholecalciferol is also called as
- The deficiency diseases caused due to lack of vitamin D areand.....
- Serum is highly increased in Osteomalacia.

8.4 VITAMIN E (TOCOPHEROL)

The word tocopherol is obtained from Greek words (*tokos* = child birth; *pheros* = to bear; *ol* = alcohol). The active form of vitamin E was isolated from wheat germ oil. It has been called as the anti-infertility vitamin as the initial studies of induced vitamin E deficiency in laboratory animals resulted in infertility. Now vitamin E is known as the most powerful biological antioxidant.

8.4.1 Structure and Chemical Nature

There are eight naturally occurring tocopherols with a chromane ring (tocol) system and an isoprenoid side chain. The greatest biological activity is depicted by **alpha tocopherol** (5, 7, 8-trimethyl tocol) (Fig. 8.10). Paul Karrer, a recipient of Nobel Prize in Chemistry (1937) elucidated the structure of vitamin E.

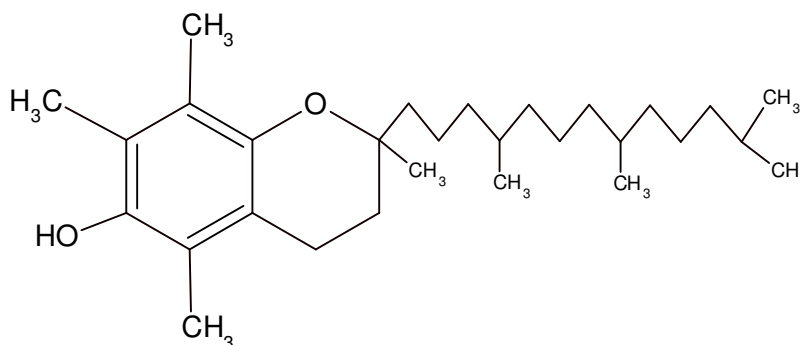
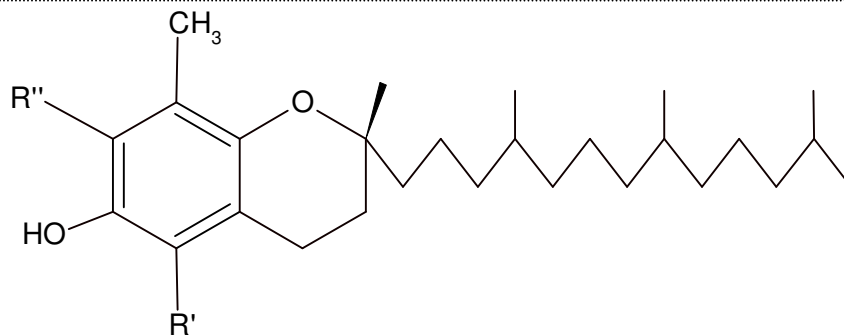


Fig. 8.10: Chemical structure of Alpha tocopherol.

The vitamin is divided into two types - tocopherols and tocotrienols. These compounds are synthesized only by plants and other oxygenic, photosynthetic organisms. In 1936, vitamin E was isolated from wheat- germ oil by Evans and his team. Vitamin E has four main constituents; alpha (5, 7, 8-trimethyl), beta (5,8-dimethyl), gamma (7,8-dimethyl) and delta (8-methyl) (Fig. 8.11).



	R'	R''
alpha-tocopherol	-CH ₃	-CH ₃
beta-tocopherol	-CH ₃	-H
gamma-tocopherol	-H	-CH ₃
delta-tocopherol	-H	-H

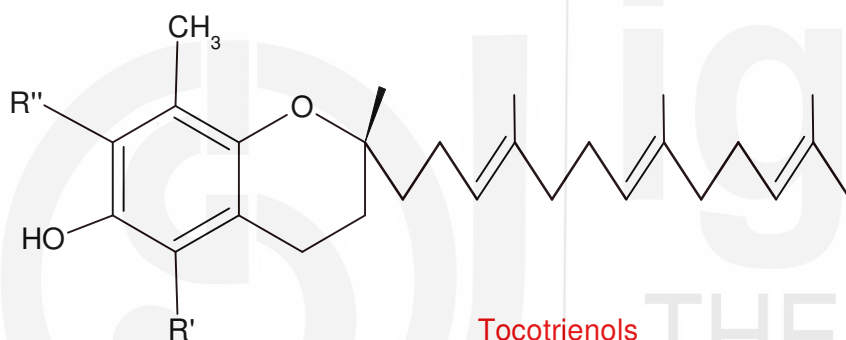


Fig. 8.11: The four main constituents of Vitamin E.

8.4.2 Biochemical Role

- i) Vitamin E has been identified as the **most powerful natural antioxidant**. The living systems generate free radicals continuously. Their instant inactivation is of great importance. Vitamin E is a familiar biological antioxidant which is able to quench the lipid peroxidation chain. Vitamin E acts as a scavenger of free radicals.
- ii) Bio- membranes are attacked by the free radicals. Vitamin E protects RBC from **hemolysis**. The structural and functional integrity of all the cells is maintained as Vitamin E prevents the peroxidation.
- iii) There is a gradual deterioration in **aging** process due to the cumulative effects of free radicals. Immune response is also boosted due to Vitamin E.
- iv) Since Vitamin E reduces the oxidation of LDL, it also reduces the risk of atherosclerosis.
- v) As per the results revealed by many research studies, vitamin E reduces the risk of cancer such as breast and lung cancers.

8.4.3 Inter-relationship with Selenium

Selenium is a constituent of **glutathione peroxidase**; a vital enzyme that destroys the free radicals. Selenium has been found to decrease the requirement of vitamin E and vice versa.

They act synergistically to minimize lipid peroxidation.

8.4.4 Dietary Sources

Wheat germ oil, nuts and seeds, sunflower oil, safflower oil, cotton seed oil and many such **vegetable oils** are richest sources of vitamin E; Fish liver oils are devoid of vitamin E. Green leafy vegetables are also good sources. Vitamin E can be synthesized only by plants and photosynthetic organisms. The synthesis is facilitated by enzymes to produce stereospecific tocopherols. Vegetable oils and other higher plant materials are among the best sources from which tocopherols and tocotrienols can be extracted and purified. Dietary supplements are another vital source of vitamin E. Dietary supplements and fortified foods contain vitamin E in esterified form to extend its shelf life, simultaneously preserving its antioxidant properties.

8.5.5 Dietary Requirements

Data on Vitamin E intake and requirements are limited. Information available suggests that Indians have blood α -tocopherol levels of 0.5 mg/kg/ml which is considered as satisfactory. α -tocopherol requirements are related to its protective antioxidant property on essential fatty acids (EFA), linoleic and linolenic acid content of the diet. The requirement of alpha tocopherol suggested is 0.8 mg/ g of dietary essential fatty acids. This figure suggests that at least 7.5-10 mg α -tocopherol per day should be taken, depending on the edible oil used (ICMR-NIN, 2020).

8.6.6 Biochemical Basis of Deficiency Diseases and Toxicity, their Symptoms

Dietary deficiency of vitamin E is not normally seen. Vitamin E deficiency may be seen in the following: (a) In people where dietary fat cannot be absorbed, and (b) in premature infants (birth weight less than 1500 grams). Vitamin E deficiency causes neurological problems due to poor nerve conduction. These include neuromuscular problems such as spinocerebellar ataxia, retinopathy, peripheral neuropathy and myopathies. Anemia may also develop as a result of deficiency of vitamin E, due to oxidative damage to red blood cells.

Hypervitaminosis E

At very high doses it may cause tendency to hemorrhage, as it is a mild anticoagulant.

SAQ 3a**Tick Mark the Correct Option:**

- i) The most important biological role for vitamin E is:
- a) To produce clotting factors
 - b) Antidote of selenium poisoning
 - c) Anticoagulant
 - d) Antioxidant
- ii) The structure of vitamin E contains:
- a) Chromane ring
 - b) Beta ionone ring
 - c) Thiazole ring
 - d) Naphthoquinone ring
- iii) Among the following, this has the greatest biological activity:
- a) Alpha (5, 7, 8-trimethyl)
 - b) Beta (5, 8-dimethyl)
 - c) Gamma (7, 8-dimethyl)
 - d) Delta (8-methyl)
-

SAQ 3b**Fill in the blanks with appropriate words:**

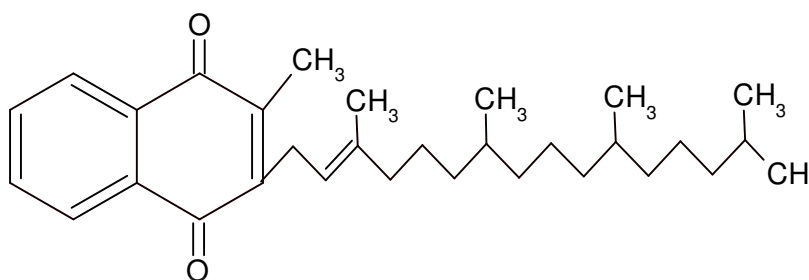
- a) Vitamin E is known as the most powerful
.....
- b) Selenium is a constituent of enzyme.
- c) elucidated the structure of vitamin E.
- d) The recommended intake of tocopherol is suggested as
..... per day.
-

8.5 VITAMIN K**8.5.1 Chemistry**

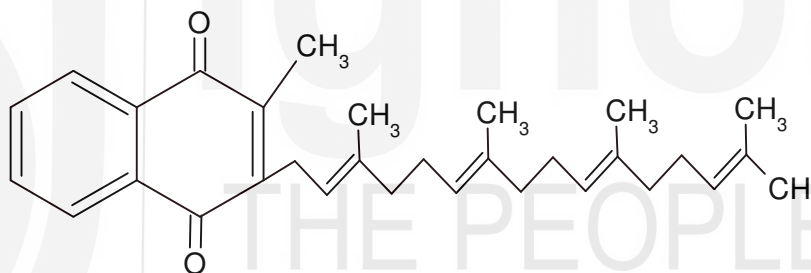
The letter “K” in vitamin K is derived from the German word “koagulation”. It is a **naphthoquinone** derivative. The structure of vitamin K consists of quinone ring linked with an isoprenoid side chain. The length of side chain differs. There are two naturally occurring forms of vitamin K (Fig. 8.12).

1. Vitamin K₁ (phylloquinone) is synthesized by plants.
2. Vitamin K₂ (menaquinone) is synthesized by intestinal bacteria.

Vitamin K₁ has 20C side chain and it is also known as **Phylloquinone** (Fig. 8.12). Vitamin K₂ has a 30C side chain which is known as **Menaquinone**. Yet another structurally similar synthetic compound having vitamin K activity is **Menadione** and is water soluble, which is widely used in clinical practice. Vitamin K₁ was isolated in 1929 by a Danish biochemist, Henrik Dam. Edward Doisy, who was an American biochemist, discovered vitamin K₂ in 1939. Both of them were awarded Nobel Prize in 1943 in the field of Physiology.



Vitamin K₁ (Phylloquinone)



Vitamin K₂ (Menaquinone)

Fig. 8.12: Vitamin K.

8.5.2 Biochemical Role

The blood clotting process requires Vitamin K as an essential constituent. It is required for the post-translational modification to produce γ -carboxy glutamic acid (GCG) from glutamic acid (Fig. 8.13). These modified residues can bind Ca^{2+} , which is important for blood clotting cascade.

Factors dependent on vitamin K are Factor II (prothrombin); Factor VII (SPCA); Factor IX (Christmas factor); Factor X (Stuart Prower factor). The liver synthesizes all these factors as inactive zymogens. A **zymogen**, also called a proenzyme, is an inactive precursor of an enzyme. These inactive precursors go through **post-translational** modification in which glutamic acid (Glu) residues present in their structure are carboxylated to form γ -carboxy glutamate (Gla) residues which are good chelators. The calcium ions bind to these sites and permits the binding of the blood-clotting proteins to membranes.

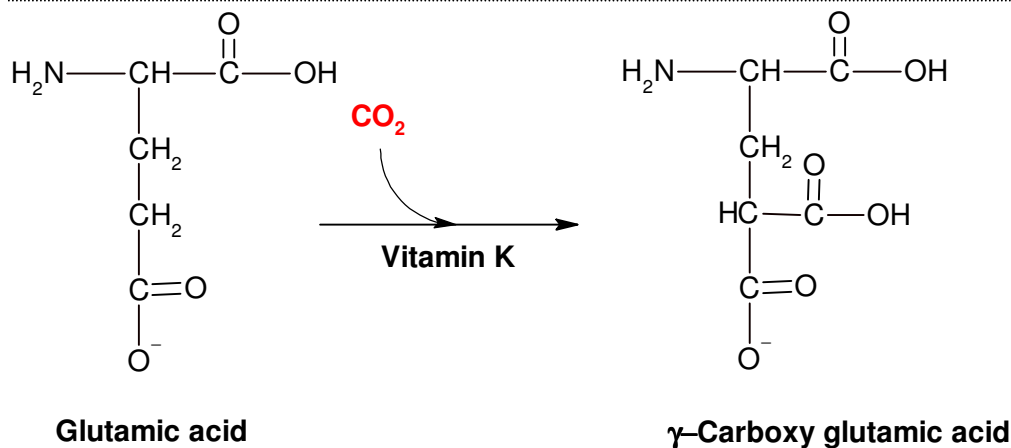


Fig. 8.13: Vitamin K as co-factor in GCG synthesis.

During this carboxylation process, Vitamin K in the hydroquinone form is oxidized to the epoxide by Vitamin K epoxidase. Vitamin K epoxide is reduced to the quinone form by Vitamin K epoxide reductase. The quinone form is reduced to the active hydroquinone form by Vitamin K quinone reductase requiring NADPH. This cyclic process is called Vitamin K cycle (Fig 8.14).

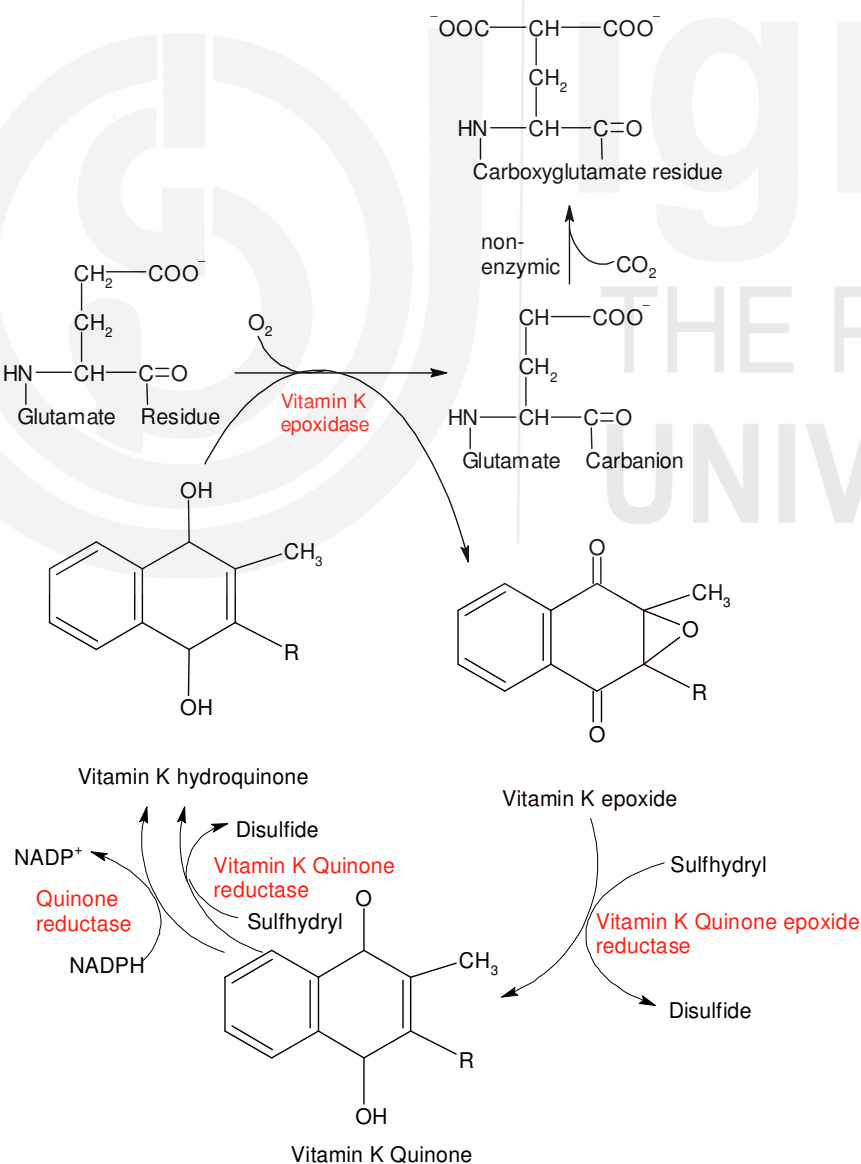


Fig 8.14: Vitamin K cycle.

Vitamin K is also important in the synthesis of γ -carboxy glutamate residues in calcium binding proteins in the bone (osteocalcin and bone matrix protein).

8.5.3 Causes for Deficiency

Vitamin K deficiency is generally not seen in India. Intestinal bacterial synthesis may contribute to a significant fraction of Vitamin K in humans. However, malabsorption of lipids can cause a deficiency of the vitamin. Obstructive jaundice, chronic pancreatitis, sprue are among such conditions that may cause lipid malabsorption. Extended use of antibiotic therapy and gastrointestinal infections with diarrhea will destroy the bacterial flora and can lead to vitamin K deficiency.

8.5.4 Sources

Dietary sources of Vitamin K include **green leafy vegetables** which are considered as good dietary sources. Other vegetables like beans, peas and some spices contain moderate amounts.

8.5.5 Dietary Requirements

RDA for vitamin K is 55 $\mu\text{g}/\text{day}$ for adults (ICMR-NIN, 2020).

8.5.6 Biochemical Basis of Deficiency Diseases and Toxicity, their Symptoms

Hemorrhagic disease of the newborn is due to vitamin K deficiency. The premature infants have relative vitamin K deficiency. The reason for this deficiency is lack of hepatic stores, limited oral intake (breast milk has very low levels) and absence of intestinal bacterial flora. It is often advised that preterm infants be given prophylactic doses of vitamin K (1 mg Menadione). In children and adults, bruising tendency, ecchymotic patches, mucous membrane hemorrhage, post-traumatic bleeding and internal bleeding are the major symptoms of vitamin K deficiency. Prolongation of prothrombin time and delayed clotting time are also features of vitamin K deficiency. The liver function index is measured as **prothrombin time (PT)**. When liver function is considerably lowered, prolongation of PT occurs due to deficient synthesis of the coagulation factors. In such cases, administration of vitamin fails to restore PT to normal levels. Hence before undertaking any surgery on jaundiced patients, PT before and after administration of vitamin K should be done. **Warfarin** and **dicoumarol** are extensively used as anticoagulants. for therapeutic uses as they competitively inhibit the gamma carboxylation system due to structural similarity with vitamin K.

Hypervitaminosis K

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

SAQ 4a**Tick Mark the Correct Option:**

- i) Biochemical function of vitamin K is for:
- a) Converting proline to hydroxyproline
 - b) Conversion of prothrombin to thrombin
 - c) Gamma carboxylation of clotting factors
 - d) Inhibition of lipid peroxidation in biomembranes
- ii) Vitamin K is inhibited by:
- a) Isoniazid (INH)
 - b) Methotrexate
 - c) Dicoumarol
 - d) Avidin
- iii) The Recommended Dietary Allowance for Vitamin K is:
- a) 55 µg
 - b) 45 µg
 - c) 50 µg
 - b) 60 µg
- iv) Following are the causes for deficiency of vitamin K, except:
- a) Obstructive jaundice
 - b) Chronic pancreatitis
 - c) Sprue
 - d) High phytate content in the diet

SAQ 4b**Fill in the blanks with appropriate words:**

1. Vitamin K is obtained from derivative.
 2. The liver function index is measured as
 3. A, also called a proenzyme, is an inactive precursor of an enzyme.
 4.and..... are extensively used as anticoagulants for therapeutic uses.
-

8.6 SUMMARY

- The active form of vitamin A is present only in animal tissues whereas provitamin A (beta carotene) is a constituent of plant tissues.
- Retinols have the β -ionone ring system and are polyisoprenoid compounds with Vitamin A activity.
- Active forms of vitamin A include; Retinol, Retinal, Retinoic acid. The two important isomers are all trans-retinal and 11-cis retinal.
- The Retinal Binding Protein helps in the transportation of vitamin A and this retinal–RBP complex has specific receptors in various tissues.
- The membrane protein important in visual cycle is Rhodopsin which is made up of opsin and 11-cis-retinal.
- Vitamin D is derived from 7 dehydrocholesterol by the action of UV rays.
- Vitamin D deficiency results in rickets and osteomalacia. Vitamin D resistant and renal rickets are different types of rickets.
- Vitamin E is also named as tocopherol. It is absorbed along with fats with the help of bile salts. It is transported as chylomicrons and stored in adipose tissue.
- Vitamin E is the most important biological antioxidant.
- Vitamin K is absorbed in intestine along with chylomicrons. They are also synthesized by intestinal flora.
- Vitamin K is involved in blood coagulation. It is required for post-translational modification of coagulation factors.

8.7 TERMINAL QUESTIONS

1. Discuss the structure of Vitamin A.
2. What are the functions of Vitamin A?
3. How does activation of vitamin D take place?
4. Describe the sources and requirement of Vitamin E.
5. Explain the biological role of vitamin K.

8.8 ANSWERS

Self Assessment Questions

1. a) i) d)
 ii) a)
 iii) d)
 iv) b)
 - b) i) Beta Carotene
 - ii) Opsin, 11-Cis-Retinal
 - iii) Retinol Palmitate
 - iv) Cellular Retinoic acid Binding Protein

2. a) i) b)
 ii) d)
 iii) c)
 iv) b)
- b) i) Sun Shine Vitamin
 ii) Calcitriol
 iii) Rickets and Osteomalacia
 iv) Alkaline Phosphatase
3. a) i) d)
 ii) a)
 iii) a)
- b) i) Biological Antioxidant
 ii) Glutathione Peroxidase
 iii) Paul Karrer
 iv) 7.5-10 mg
4. a) i) c)
 ii) c)
 iii) a)
 iv) d)
- b) i) Naphthoquinone
 ii) Prothrombin Time
 iii) Zymogen
 iv) Warfarin and Dicoumarol

Terminal Questions

1. The active form of Vitamin A is present only in animal tissues. The precursor of vitamin A (**provitamin**), beta-carotene is available in plant tissues. **Beta-carotene** consists of two beta ionone rings. Retinoids are the compounds depicting vitamin A activity. They have **beta-ionone** (cyclohexenyl) ring system. **Retinol** (vitamin A alcohol), **retinal** (vitamin A aldehyde) and **retinoic acid** (vitamin A acid) are the three different compounds having vitamin A activity. The presence of enzyme retinal reductase reduces retinal to retinol. It is a readily reversible reaction. Retinoic acid is obtained by the oxidation of retinal, which cannot be transformed back to the other forms. Since the side chain contains alternate double bonds, many isomers are possible. Vitamin A1, also called the **all-trans** variety of retinal, is most common. Vitamin A2 found in *fish oils*, has an extra double bond in the ring. **11-cisretinal** is the biologically important compound.
2. Following are the important functions of vitamin A:
- It is of utmost importance in vision.
 - Retinoic acid acts like steroid hormones in the regulation of gene expression and **differentiation** of tissues.

- iii) Vitamin A is required for normal **reproduction**. In vitamin A deficiency, miscarriages are noticed in female rats.
 - iv) Since carotenoids are natural anti-oxidants, they exhibit anticancer activity. Fresh vegetables containing carotenoids were shown to reduce the incidence of cancer.
 - v) Vitamin A is essential for the maintenance of normal epithelium and skin.
3. The cholecalciferol is first transported to liver, where it is converted to 25- hydroxy cholecalciferol (25 HCC) by hydroxylation at **25th position**. The 25-Hydroxy cholecalciferol is the major storage form of vitamin D in the liver. The “vitamin D binding protein” (VDBP) binds 25-HCC in the plasma. It is further hydroxylated at the 1st position in the kidney. In this way, generation of 1-25 -dihydroxy cholecalciferol (**DHCC**) takes place. It is also called **Calcitriol** since it contains three hydroxyl groups at 1, 3 and 25 positions. The formation of new product calcitriol is the active form of vitamin D, which is a hormone. Low serum calcium leads to the release of parathyroid hormone (PTH) which induces the production of calcitriol. A complex is formed between calcitriol and its highly specific nuclear receptor- Vitamin D receptor (VDR); which further binds to VDRE (vitamin D response elements) on DNA which regulates the expression of more than 500 genes. This activation takes place rapidly.
4. **Sources of Vitamin E:** Wheat germ oil, sunflower oil, safflower oil, cotton seed oil and many such **vegetable oils** are richest sources of vitamin E; Fish liver oils are devoid of vitamin E. Green leafy vegetables are also good sources. Vitamin E can be synthesized only by plants and photosynthetic organisms. Vegetable oils and other higher plant materials are among the best sources from which tocopherols and tocotrienols can be extracted and purified. Dietary supplements are another vital source of vitamin E. The Recommended Daily Allowance of Vitamin E suggested is 0.8 mg/ g of dietary essential fatty acids. This figure suggests that at least 7.5-10 mg tocopherol per day should be taken, depending on the edible oil used.
5. The blood clotting process requires Vitamin K as an essential constituent. It is required for the post-translational modification to produce γ -carboxy glutamic acid from glutamic acid. These modified residues can bind Ca^{2+} , which is important for blood clotting cascade. Factors dependent on vitamin K are Factor II (prothrombin); Factor VII (SPCA); Factor IX (Christmas factor); Factor X (Stuart Prower factor). The liver synthesizes all these factors as inactive zymogens. A **zymogen**, also called a proenzyme, is an inactive precursor of an enzyme. They go through **post-translational** modification in which glutamic acid residues are carboxylated. The calcium ions bind to these sites. Vitamin K is required as a co factor in the gamma carboxyglutamic acid (GCG) synthesis.