Introduction:

In addition to an adequate source of metabolic fuels such as carbohydrates, fats and proteins, there is a requirement for very much smaller amounts of other nutrients called: vitamins and minerals.

collectively vitamins and minerals are referred to as micronutrients because of the small amounts that are required.

The determination of requirements and reference intakes

For any nutrient, there is a range of intakes between inadequate which leading to a clinical deficiency disease, and the excess of the body's metabolic capacity that may be lead to a toxicity.

Therefore, between these two extremes, there is a level of intake that is adequate for normal health and the maintenance of metabolic integrity, which are adequate may be used to determine requirements and appropriate levels of intake.

The reason for specifying the reference of these requirements is for the several cases, including:

1. **Clinical deficiency disease,** with clear anatomical and functional lesions, and severe metabolic disorder, may be deadly. The minimal goal for the prevention of the deficiency disease by determining requirements
2. **Hidden deficiency,** in which there are no signs of deficiency under normal conditions but any trauma or stress reveals the precarious state of the body reserves and may precipitate clinical signs. For example, an intake of 10 mg of vitamin C per day is adequate to prevent clinical deficiency, but at least 20 mg/day is required for healing of wounds.
3. **Metabolic abnormalities under normal conditions,** such as impaired carbohydrate metabolism in thiamin deficiency.
4. **Low plasma concentration of the nutrient,** indicating an inadequate amount in tissue reserves to permit normal transport between tissues.
5. **Possibly beneficial effects of intakes more than adequate to meet requirements:** There is evidence that relatively high intakes of vitamin E and possibly other antioxidant nutrients may reduce the risk of developing cardiovascular disease and some forms of cancer
6. **Abnormal accumulation in tissues and overloading of normal metabolic pathways,** leading to signs of toxicity and possibly irreversible lesions. Such as vitamins A and D are all known to be toxic in excess.

Dietary Reference Values

Individuals do not all have the same requirement for nutrients, even when calculated on the basis of body size or energy expenditure.

There is a range of individual requirements for of a population. Therefore, in order to set population goals, and assess the adequacy of diets, it is necessary to set a reference level of intake that is high enough to ensure that no-one will either suffer from deficiency or be at risk of toxicity.

As shown in the Figure 11.1, if it is assumed that individual requirements are distributed in a statistically normal fashion around the observed mean requirement. A range of ± 2 times the standard deviation (SD) around the mean will include the requirements of 95% of the population.

The 95% range of the requirement is conventionally used as:

1- the 'normal' or reference range (e.g. in clinical chemistry)
2- assess the normality or otherwise of a test result
3- and is used to define three levels of nutrient intake:

a- Estimated Average Requirement (EAR): This is the observed mean requirement.

b- Reference Nutrient Intake (RNI): This is above the observed mean requirement.

c- Lower Reference Nutrient Intake (LRNI): This is below the observed mean requirement.

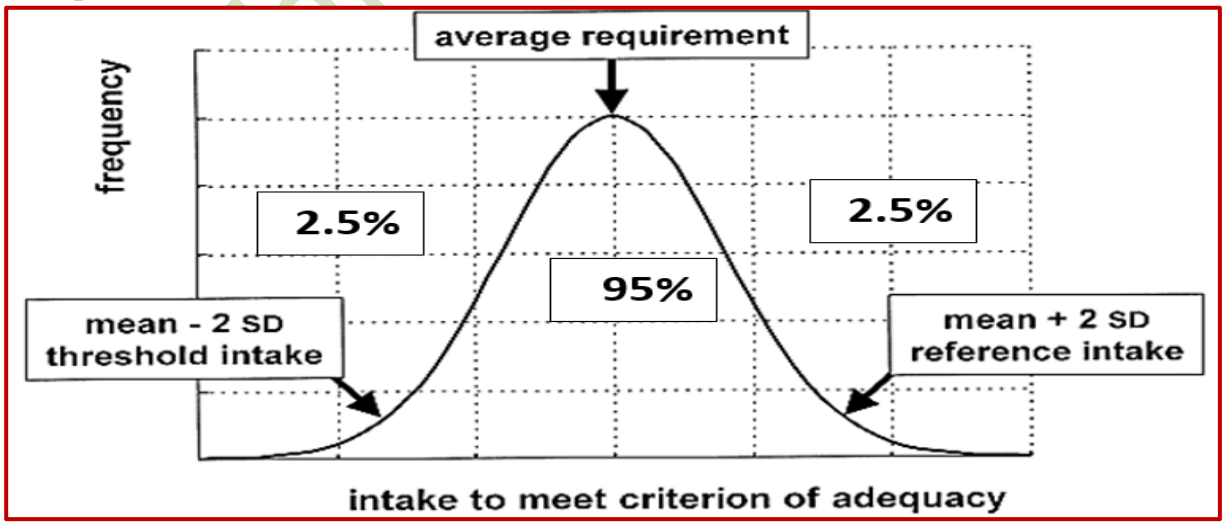


Figure 11.1 *The derivation of reference intakes of nutrients from the distribution around the observed mean requirement.*

The reference intakes of vitamins and minerals published in the USA, UK and European Union are shown in Tables 11.1–11.3.

TABLE 11.1 *Reference nutrient intakes of vitamins and minerals, UK 1991*

Age	Vitamin B_1 (mg)	B_2 (mg)	Niacin (mg)	B_6 (mg)	B_{12} (μg)	Folate (μg)	C (mg)	A (μg)	D (μg)
0–3 months	0.2	0.4	3	0.2	0.3	50	25	350	8.5
4–6 months	0.2	0.4	3	0.2	0.3	50	25	350	8.5
7–9 months	0.2	0.4	4	0.3	0.4	50	25	350	7
10–12 months	0.3	0.4	5	0.4	0.4	50	25	350	7
1–3 years	0.5	0.6	8	0.7	0.5	70	30	400	7
4–6 years	0.7	0.8	11	0.9	0.8	100	30	500	–
7–10 years	0.7	1.0	12	1.0	1.0	150	30	500	–
Males									
11–14 years	0.9	1.2	15	1.2	1.2	200	35	600	–
15–18 years	1.1	1.3	18	1.5	1.5	200	40	700	–
19–50 years	1.0	1.3	17	1.4	1.5	200	40	700	–
50+ years	0.9	1.3	16	1.4	1.5	200	40	700	10
Females									
11–14 years	0.7	1.1	12	1.0	1.2	200	35	600	–
15–18 years	0.8	1.1	14	1.2	1.5	200	40	600	–
19–50 y	0.8	1.1	13	1.2	1.5	200	40	600	–
50+ years	0.8	1.1	12	1.2	1.5	200	40	600	10
Pregnant	+0.1	+0.3	–	–	–	+100	+10	+100	10
Lactating	+0.1	+0.5	+2	–	+0.5	+60	+30	+350	10

TABLE 11.2 *Population reference intakes of vitamins and minerals, European Union, 1993*

Age	Vitamin A (mg)	B_1 (mg)	B_2 (mg)	Niacin (mg)	B_6 (μg)	Folate (μg)	B_{12} (mg)	C (μg)
6–12 months	350	0.3	0.4	5	0.4	50	0.5	20
1–3 years	400	0.5	0.8	9	0.7	100	0.7	25
4–6 years	400	0.7	1.0	11	0.9	130	0.9	25
7–10 years	500	0.8	1.2	13	1.1	150	1.0	30
Males								
11–14 years	600	1.0	1.4	15	1.3	180	1.3	35
15–17 years	700	1.2	1.6	18	1.5	200	1.4	40
18+ years	700	1.1	1.6	18	1.5	200	1.4	45
Females								
11–14 years	600	0.9	1.2	14	1.1	180	1.3	35
15–17 years	600	0.9	1.3	14	1.1	200	1.4	40
18+ years	600	0.9	1.3	14	1.1	200	1.4	45
Pregnant	700	1.0	1.6	14	1.3	400	1.6	55
Lactating	950	1.1	1.7	16	1.4	350	1.9	70

TABLE 11.3 *Recommended dietary allowances and acceptable intakes for vitamins and minerals, USA and Canada, 1997-2001*

Age	Vitamin A (μg)	D (μg)	E (mg)	K (μg)	B_1 (mg)	B_2 (mg)	Niacin (mg)	B_6 (mg)	Folate (μg)	B_{12} (μg)	C (mg)
0–6 months	400	5	4	2.0	0.2	0.3	2	0.1	65	0.4	40
7–12 months	500	5	5	2.5	0.3	0.4	4	0.3	80	0.5	50
1–3 years	300	5	6	30	0.5	0.5	6	0.5	150	0.9	15
4–8 years	400	5	7	55	0.5	0.6	8	0.6	200	1.2	25
Males											
9–13 years	600	5	11	60	0.9	0.9	12	1.0	300	1.8	45
14–18 years	900	5	15	75	1.2	1.3	16	1.3	400	2.4	75
19–30 years	900	5	15	120	1.2	1.3	16	1.3	400	2.4	90
31–50 years	900	5	15	120	1.2	1.3	16	1.3	400	2.4	90
51–70 years	900	10	15	120	1.2	1.3	16	1.7	400	2.4	90
><t>70 years	900	15	15	120	1.2	1.3	16	1.7	400	2.4	90
Females											
9–13 years	600	5	11	60	0.9	0.9	12	1.0	300	1.8	45
14–18 years	700	5	15	75	1.0	1.0	14	1.2	400	2.4	65
19–30 years	700	5	15	90	1.1	1.1	14	1.3	400	2.4	75
31–50 years	700	5	15	90	1.1	1.1	14	1.3	400	2.4	75
51–70 years	700	10	15	90	1.1	1.1	14	1.5	400	2.4	75
> 70 years	700	15	15	90	1.1	1.1	14	1.5	400	2.4	75
Pregnant	770	5	15	90	1.4	1.4	18	1.9	600	2.6	85
Lactating	900	5	16	90	1.4	1.6	17	2.0	500	2.8	120

The vitamins

- Vitamins are organic nutrients of low molecular weight that play a central role in metabolism. It biosynthetic precursors of physiologically active forms called coenzymes.
- Most vitamins are either not synthesized or are synthesized in inadequate amounts by the human organism; therefore, they must be obtained from an exogenous source, such as diet or bacterial flora in the gut.
- Presently 13 different vitamins are known to be required in the diet of the humans and many animal species for normal growth and function.

Vitamins are classification in two broad groups:

1- **Fat –Soluble Vitamins Included:** vitamins A, D, E, and K

2- **Water-Soluble Vitamins Included:** vitamins B-complex group, vitamin C and folic acid.

Water -Soluble Vitamins Included: vitamins B-complex group, vitamin C and folic acid. Water -Soluble Vitamins cannot accumulate to toxic levels in the body as the excessive intake results in their excretion in the urine; except for vitamin B_{12} there is no storage capacity for the water-soluble vitamins, so their intake has to be more frequent than fat-soluble vitamins that are stored.

Some vitamins of the B-complex group are known as the energy-releasing vitamins because they participate in the energy-yielding catabolic pathways. Examples include vitamins B1 , B2 , B3 , B5 , B7 , etc..

Correctly, for a compound to be classified as a vitamin, it should be a dietary essential **that cannot be synthesized in the body.** By this strict definition, two vitamins should not really be included, as they can be made in the body.

However, both were discovered as a result of investigations of deficiency diseases, and they are usually considered as vitamins:

1. **Vitamin D** is made in the skin after exposure to sunlight and should really be regarded as a steroid hormone rather than a vitamin. It is only when sunlight exposure is inadequate that a dietary source is required.
2. **Niacin** can be formed from the essential amino acid tryptophan. Indeed, synthesis from tryptophan is probably more important than a dietary intake of preformed niacin.

Fat-soluble Vitamins:

The fat-soluble vitamins (A, D, E, and K or their precursor pro-vitamins). Despite limited chemical similarity, they share many properties:

1. Absorption: They are absorbed into the intestinal lymphatics along with other dietary lipids (or their digestion products).

2. Transport: The absorbed vitamins are delivered to liver (by chylomicrons), from where they are transported to other organs; the interorgan transport is affected by either plasma lipoproteins or specific transport proteins.

4. Storage: Because they are predominantly non-polar, they cannot be excreted by the kidneys, and so tend to be stored in the body. Deficiency disease (except in the case of vitamin K) is difficult to produce in adults because of the storage in liver (A, D, K) or adipose tissue (E).

5. Toxicity: Excessive intake leads to accumulation of these vitamins to toxic (as in case of vitamins A and D).

Vitamin A

The term vitamin A refers to some poly-isoprenoid compounds found only in animals. However, their precursors, carotenoids are found in plant, mainly as β- carotene. The latter consists, effectively, of two molecules of vitamin A joined end to end.

Vitamin A and pro-vitamin A (carotenoid) are poly-isoprenoid compounds comprising two distinct components (Fig. 18.12). :

(a) a cyclohexene ring, and (b) a sidechain made up of several isoprene units, which is attached to the cyclohexene ring (Fig. 18.12).

The pro-vitamin carotenoids are also included in the vitamin A family. They are present in a variety of plants. The most important, quantitatively, is β-carotene (Table 18.3) (responsible for the orange colour of carrots).

Chemistry and Nomenclature of Vitamin A

Vitamin A refers to three biologically active vitamins and Related compounds (collectively known as carotenoids):

A- retinol, retinal and retinoic acid, all of which are found only in animals.

1- retinol (an alcohol): is found only in foods of animal origin and a small number of bacteria, mainly as the ester retinyl palmitate.

2- retinal (an aldehyde).

3- retinoic acid (an acid): is a metabolite of retinol and has important biological activities in its own right. The **oxidation of retinaldehyde** to retinoic acid **is irreversible**, and retinoic acid cannot be converted to retinol in vivo, and does not support either vision or fertility in deficient animals.

Note: Since retinol and retinal are interconvertible and can also be converted to retinoic acid, they can perform all functions of vitamin A. However, retinoic acid cannot perform other functions of vitamin A.

B- Related compounds (collectively known as carotenoids), which can be cleaved oxidative to yield retinaldehyde, and hence retinol and retinoic acid. Those carotenoids that can be cleaved to yield retinaldehyde are known as are known as **pro-vitamin A carotenoid**.

The term retinoid has been used to define these three compounds (and other associated synthetic compounds with vitamin A-like activity).

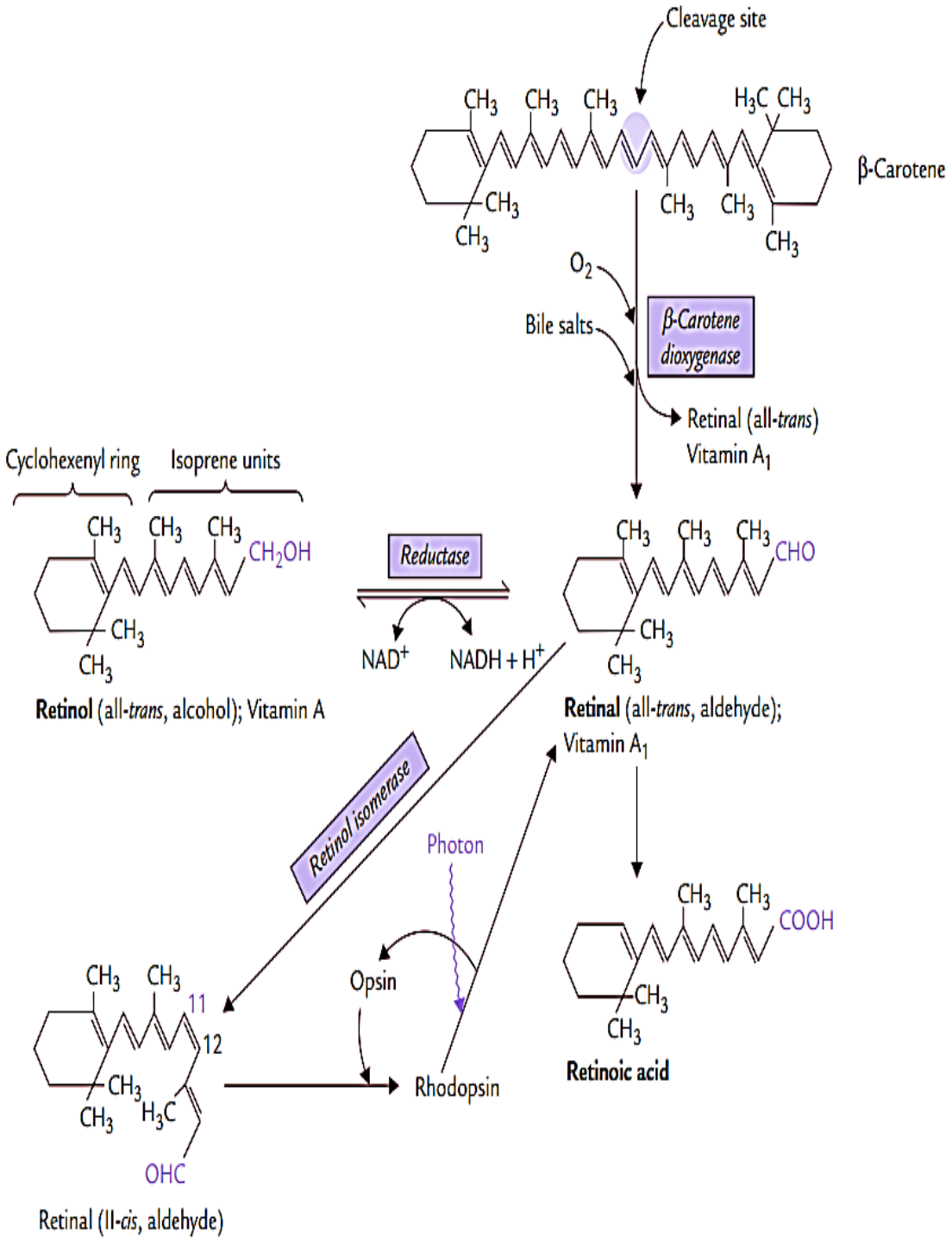


Fig. 18.12. Structures and interconversions of various members of the vitamin A family.

Metabolism of vitamin A and pro-vitamin A carotenoid

Lipid-dissolved retinol is absorbed from the small intestine. The absorption of 70–90% of dietary retinol is typical. 5–60% of dietary carotene is absorbed, depending on food type and cooking method. Retinol and carotene absorption are inhibited in diets with less than 10% fat, and particularly low-fat diets are linked to vitamin A insufficiency.

Absorption

Carotenes and retinol esters are treated differently.

(a) β-carotene is absorbed in small intestine and enters mucosal cells where it is cleaved into two molecules of trans-retinal by a **dioxygenase** and **molecular oxygen; bile salts facilitate the reaction.** Retinal is reduced to retinol **by an NADH or NADPH-dependent retinal reductase.** Retinol is esterified with a fatty acid, incorporated into chylomicrons together with dietary lipids, and secreted into lacteals (Fig. 18.13).

Note: Chylomicrons are composed of a main central lipid core that consists primarily of triglycerides,

(b) Retinol esters of animal foods are treated differently. They are hydrolyzed in the intestinal lumen **by a pancreatic enzyme (carboxylic ester hydrolase).** then generated of free retinol is transferred across the intestinal mucosal cell. It is then esterified, incorporated into chylomicrons and secreted into lacteals. The presence of lipids in the intestine ensures efficient absorption of retinol, up to 80% of the intake.

Carotenoid absorption is less efficient, about 40% of the intake. Its activity is 1/6th that of retinol.

Note: Lacteals are small lymphatic vessels located in the lining of the small intestine. They are responsible for absorbing dietary fats and fat-soluble vitamins from the intestine and transporting them to the bloodstream. This process is essential for proper nutrient absorption and overall health.

Transport

The liver parenchymal cells hydrolyze retinol esters after chylomicrons transport it into circulation. In a one-to-one ratio, the released retinol is reversibly bound to RBP and released into the circulation for delivery to other organs. RBPspecifi c receptors help target cells absorb retinol-RBP, which binds to CRBP. It may then be oxidised to retinal or retinoic acid (Fig. 18.13).

Note: In the liver, parenchymal cells (i.e., mainly hepatocytes) utilize autophagy to provide amino acids, glucose, and free fatty acids as sources of energy and biosynthesis functions, but also for recycling.

Chylomicrons carry the absorbed retinol into the circulation and then to liver parenchymal cells where the retinol esters are hydrolyzed. The retinol so released is reversibly bound to retinol-binding protein (RBP) in a one-to-one proportion and released into the circulation for transport to other organs. The target cells take up the retinol-RBP by a RBPspecifi c receptor-mediated process, and the retinol is bound to cellular retinol-binding protein (CRBP). Subsequently it may be oxidized to retinal or retinoic acid (Fig. 18.13).

What is the Retinol-binding protein (RBP)?

Retinol-binding protein (RBP) in human plasma is a monomeric polypeptide which has a single binding site. Being of low molecular weight (MW 20,000), RBP (**Retinol-binding protein (RBP)** can be cleared by kidneys, so it circulates after being

reversibly complexed with a plasma protein. Retinoic acid is transported in association with albumin.

Storage

The hepatocytes not only dispatch retinol with RBP, they can also store the surplus in the form of retinol esters. More than 90% of the body's supply of vitamin A is usually stored in the liver cells, which contain a year's supply of the vitamin.

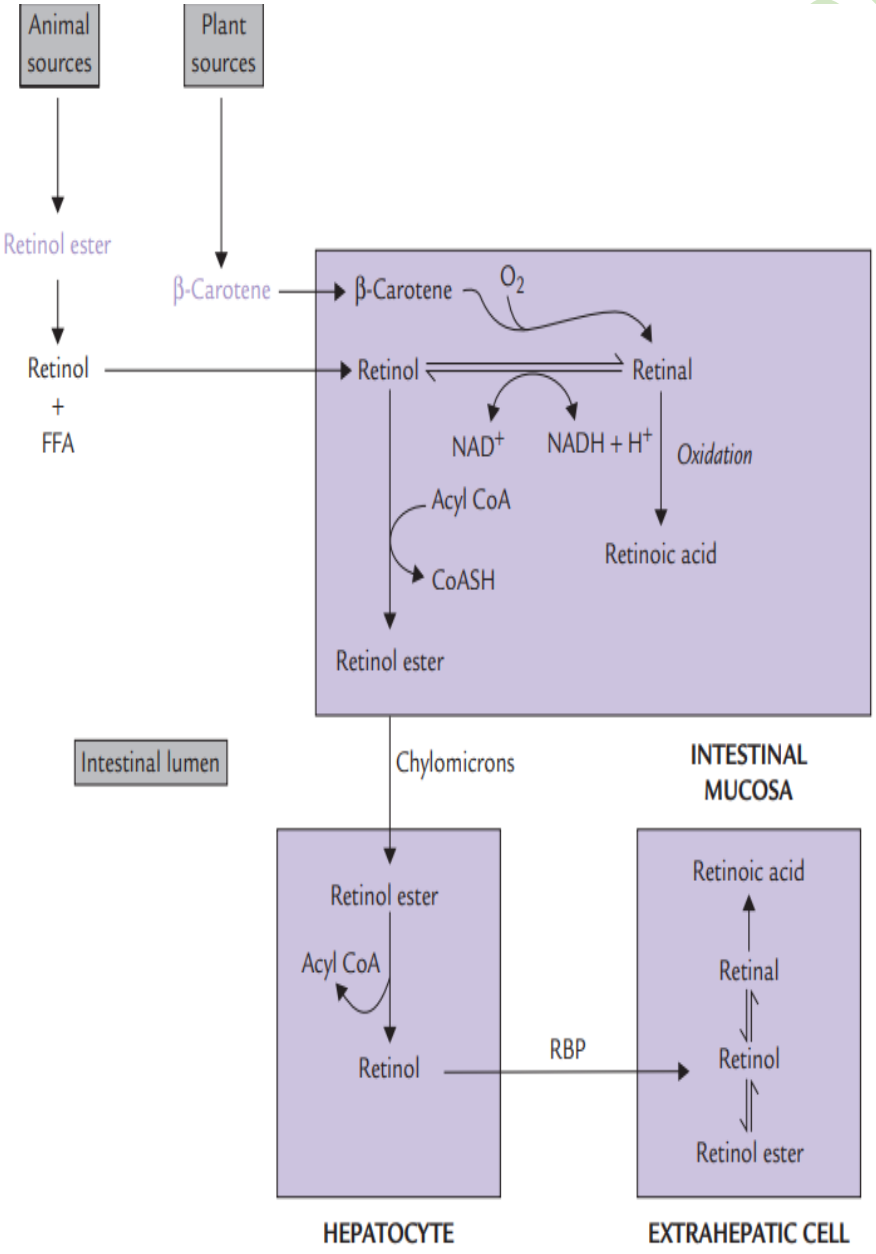


Fig. 18.13. Absorption, transport and metabolism of retinoids (RBP = retinol-binding protein, FFA = free fatty acid).

Functions of vitamin A

The role of vitamin A in vision has been known. Also, its importance in various other cellular functions, unrelated to visual process is also being recognized:

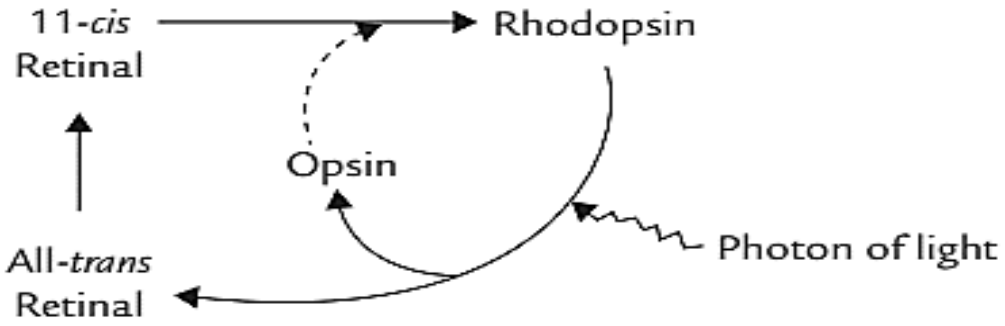
1. Retinal is involved in vision.
2. Retinoic acid is involved in growth and cellular differentiation.
3. Retinol is necessary for the reproductive system.
4. Carotenoids have an antioxidant role per se (not through their conversion into vitamin A).

1- Vitamin A and Vision:

Vitamin A is the prosthetic group of rhodopsin, the light sensing protein in retinal rod cells. Rhodopsin is located in a membrane system in the outer segment of the rod cell.

(a) Rhodopsin synthesis: Rhodopsin is made up of the protein opsin and 11-cis-retinal. The 11-cis retinal serves as the light absorbing part of rhodopsin, and opsin is an integral membrane protein with seven transmembrane helices. In the pigment epithelium of retina, all trans-retinal is isomerized to 11-cis retinal. The aldehyde group of the 11-cisretinal then spontaneously links with a lysyl residue in the apoprotein to form **rhodopsin.** This rhodopsin reaction places the 11-cis-retinal at the center of the molecule.

Note: In the cone cells, opsin is somewhat different and the rhodopsin equivalent is iodopsin



(b) Wald's visual cycle:

Rhodopsin is a photosensitive pigment, which plays a central role in the dim light vision. Exposure to light of wavelength centered around 500 nm on the rod cells induces isomerization of 11-cis-retinal (Fig. 18.14). This photo-isomerization switches rhodopsin to a series of unstable intermediates in the following sequence:

Opsin 11-Cis-Retinal: Rhodopsin, then Batho-rhodopsin then, then……..Meta-rhodopsin-II

The last one (meta-rhodopsin-II) is an activated" photo-excited conformation" referred to as **active rhodopsin (R*).** The Schiff-base between the all-trans-retinal and the active rhodopsin R* then hydrolyzes, and all-trans-retinal dissociates from the active rhodopsin R*.

(c) Regeneration of 11-cis-retinal:

Conversion of all-transretinal (released following dissociation of R*) back to 11-cis-retinal **is the final event of the Wald's visual cycle.**

This reaction can occur in the retina itself, or it may require participation of an enzyme system located in liver (Fig. 18.14).

A-In retinal cells: The trans- to cis-isomerization reaction is catalyzed in the dark by the enzyme **retinal isomerase.** The 11 cis-isomer then combines with opsin to regenerate rhodopsin, as discussed earlier.

B- In liver: The all-trans-retinal is released into blood circulation and transported to liver. Following uptake by hepatocytes, it is reduced to all-trans-retinol **by alcohol dehydrogenase,** an NADH-dependent, zinc containing, enzyme. The all-trans-retinol is isomerized to 11-cisretinol and carried to retina by blood where it is oxidized to 11-cis-retinal.

This completes the Wald's visual cycle.

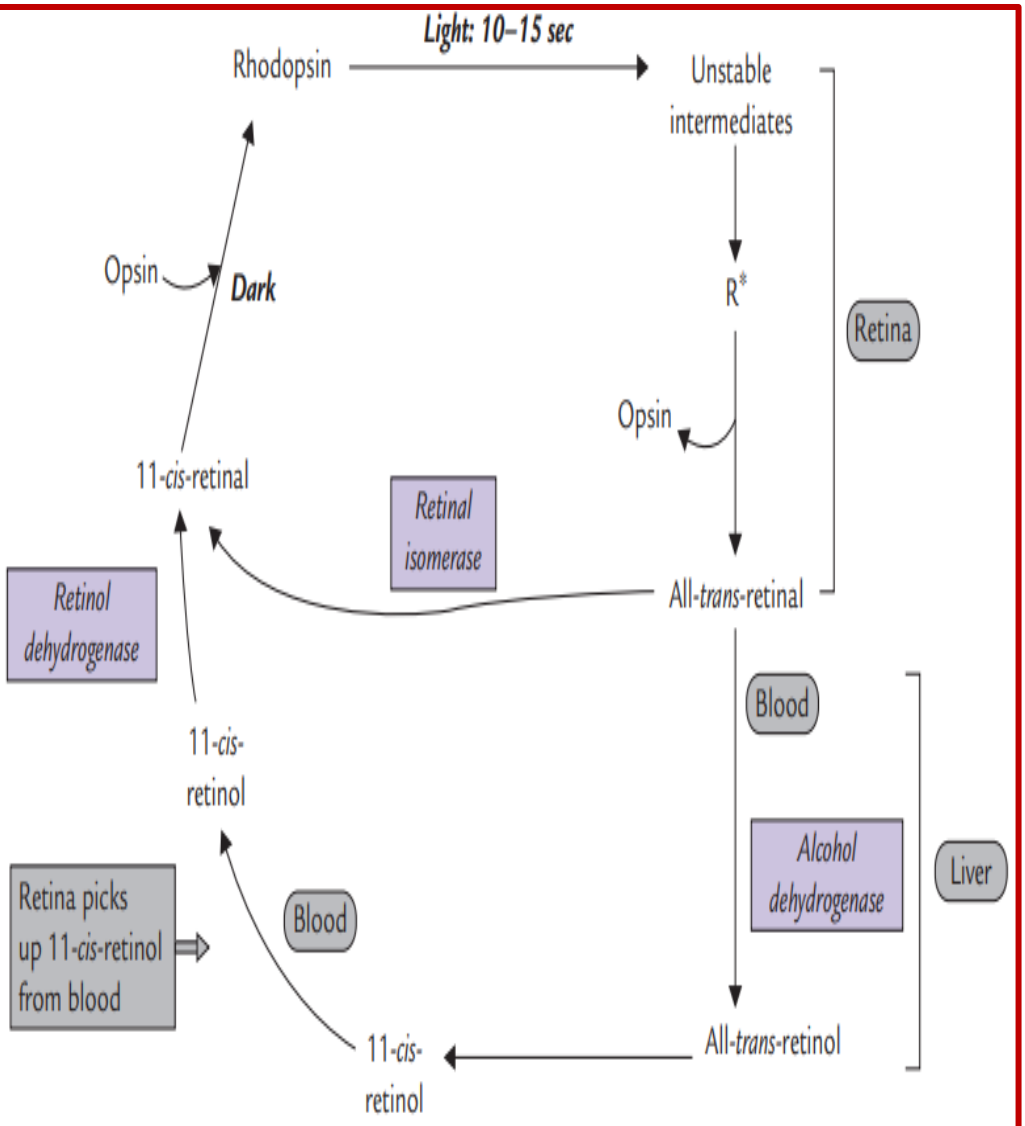


Fig. 18.14. Wald's visual cycle (rhodopsin cycle); R* activates transducin based cascade.

(d) Dark adaptation mechanism: The time taken for regeneration of rhodopsin (following light induced depletion of rhodopsin) is known as **dark adaptation time.** It is a common experience that when a person shifts from bright light to dark, there is difficulty in seeing (e.g. in cinema hall), and after a few minutes the vision improves. During these few minutes' rhodopsin is resynthesized, and the time taken is referred to as the dark adaptation time. It depends on vitamin A status of the person. It is prolonged in the vitamin A deficiency state.

Mechanism of Vision:

Rhodopsin is a transmembrane protein located in rod cells, bound to membranous structures in the outer segment.

Rod cell physiology: The rod cell consists of outer and inner segments connected to each other by a narrow cilium (Fig. 18.15). The outer segment has membranous disc structures while the inner section has mitochondria. Next comes cell bodies with axons to nerve endings and synapses.

About 1000 closed membranous 16 nm discs are placed within each outer segment. These discs contain light-sensitive rhodopsin. Sodium channels allow Na to enter the outer segment's rod plasma membrane.

Sodium is later pumped out of the cell by an ATPase located in the inner segment. Such movement of sodium ions is called the dark current because it takes place in dark (absence of light).

The sodium channels are kept open by the intracellular nucleotide, cGMP. When light strikes the rhodopsin, these sodium channels close, and the dark current ceases. This leads to polarization of the cell membrane and consequent generation of an electric current which results in a visual impulse.

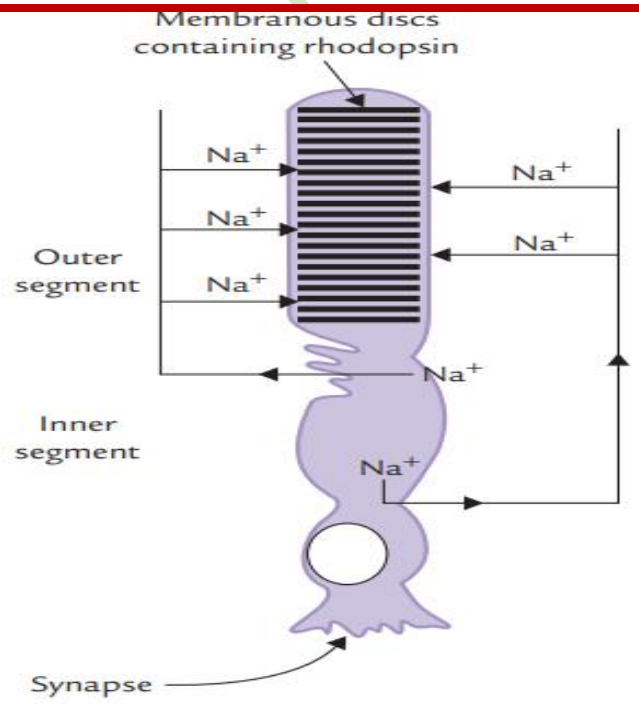


Fig. 18.15. A rod cell showing the dark current: the sodium ions enter the outer segment through cGMP-sensitive channels and are actively pumped out from the inner segment by an *ATPase*.

Other Functions of Vitamin A

2- Regulation of gene expression: Retinoic acid is an important regulator of gene expression. It is transported to the nucleus bound to intracellular proteins: cellular retinoic acid-binding protein (CRABP)-I and -II. In nucleus, retinoic acid binds to and activates two families of nuclear receptors, which regulates gene expression by binding to response elements on the DNA.

Note: Action of retinoic acid resembles that of steroid hormones, and the retinoic acid receptors (like the thyroid and steroid hormone receptors) belong to a large super-family of ligand-regulated transcription factors (Chapter 29).

3- Growth and differentiation: Because retinoic acid regulates gene expression, several processes associated with growth and differentiation depend on retinoic acid. These include maintenance of healthy epithelial cells, cell differentiation in spermatogenesis, and the differentiation of epithelial cells, among others. Severe vitamin A deficiency leads to production of abnormal epithelial tissues (keratinization).

4- Glycoprotein synthesis: Retinyl phosphates, obtained from retinol play a role in glycoprotein synthesis. Its hydrophilic portion serves as an anchor for growing oligosaccharide chains. This function appears analogous to that of dolichol phosphate (Chapter 5).

5- Role in reproduction: Retinol is required in reproduction. This function is mediated by control of expression of certain genes by retinol bound to cellular retinolbinding protein. Retinol, and to a lesser extent the retinal, support spermatogenesis in males and prevents fetal resorption in females.

6- Antioxidant role of vitamin A: The antioxidant properties of the carotenoids at low oxygen partial pressures in tissues have been reported. It is in this role that carotenoids are thought to prevent the development of diseases in which the action of free radicals is implicated, such as cancer and cardiovascular diseases.

7- Others: Retinoic acid exerts a number of metabolic effects on tissues, such as control of the biosynthesis of proteoglycans, particularly sulphation of the latter.

Requirements and Dietary Sources:

Vitamin A requirement is difficult to calculate because of the different forms in which it is present. It is usually expressed in terms of international units (IU: one IU is the activity present in 0.3 g of retinol, 0.344 g of retinol acetate or 0.6 g of -carotene).

Clinical Deficiency:

Vitamin A deficiency may be primary (dietary) or secondary.

Causes of secondary deficiency include:

1. Fat malabsorption.
2. Failure to synthesize chylomicrons into which vitamin A is normally incorporated after absorption (mostly due to inability to synthesize apoB48).
3. Failure to cleave -carotene because of an enzyme defect.
4. Impaired storage in hepatic cells in liver disease.
5. Failure to synthesize retinol-binding protein (RBP), thus impeding transport from liver to target tissues.

The deficiency of vitamin A leads to the following clinical manifestations (Symptoms)

1. **Vision impact:** Retinal is important for the pigment rhodopsin, which is responsible for low light vision. Thus, retinal insufficiency may impede of the 'dark adaptation'. Long-term deprivation kills visual cells permanently. Retinoic acid is essential for epithelium formation and maintenance, therefore chronic deficiency causes dryness of the conjunctival and corneal. Vitamin A (retinal) is necessary for vision mediated by the rod cells, so deficiency often presents as night blindness, dry eyes, and more serious consequences if untreated.therefore, Vitamin A supplementation is needed immediately to avoid this hazard.

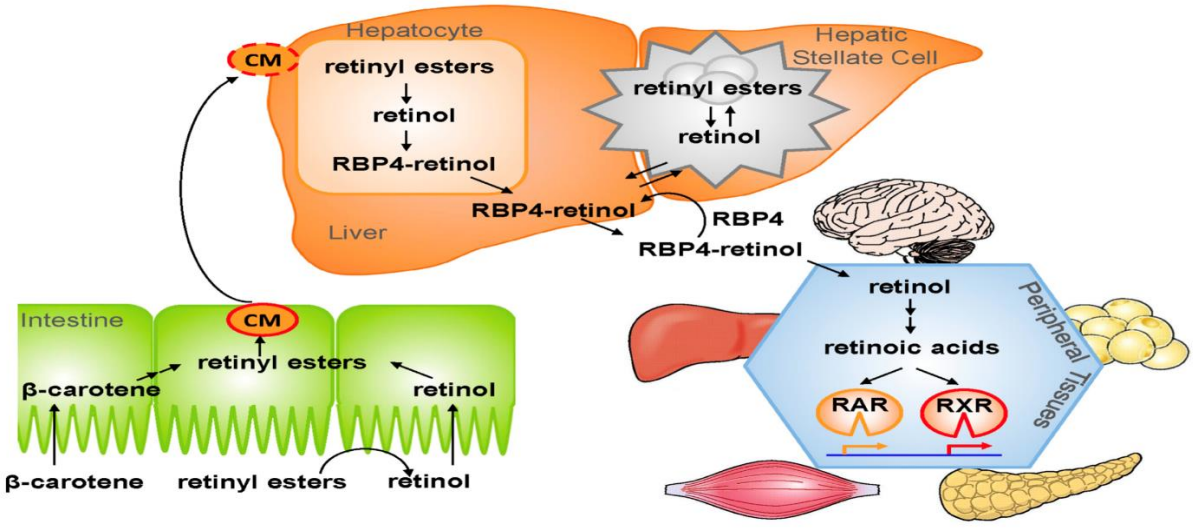
2. **Failure of bone remodeling** occurs leading to thick and solid bones in the skull with an increase in the cerebrospinal fluid pressure.
3. **Gonadal dysfunctions occur in deficiency of the vitamin A.** also deficiency of vitamin A lead to testicular degeneration in males and an increased incidence of miscarriage.
4. **Follicular hyperkeratosis (gooseflesh) :** is the transformation of columnar epithelium into heavily keratinized squamous epithelia. Follicular hyperkeratosis is an important sign, developing early stage of in the disease.
5. **Nerve lesions:** often occurring with bone lesions.
6. **Certain forms of skin diseases** are also common in vitamin A deficiency.

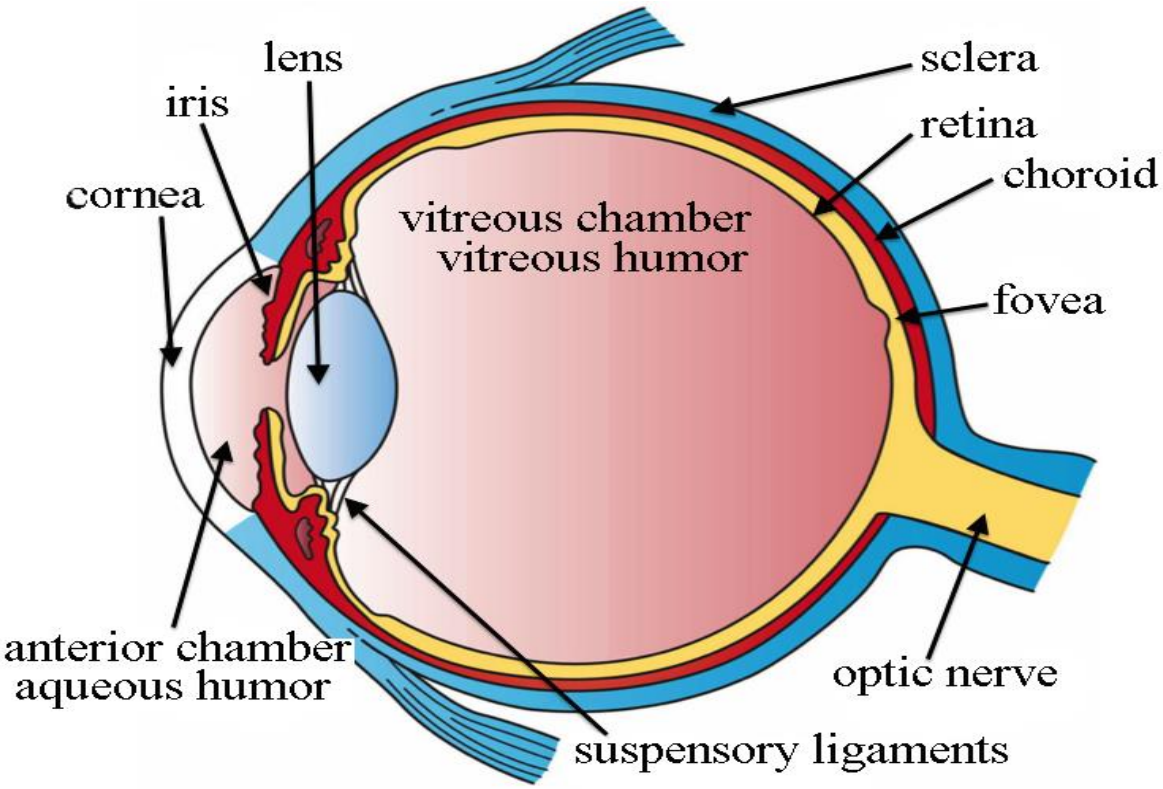
Vitamin A Toxicity (Hyper-vitamin A):

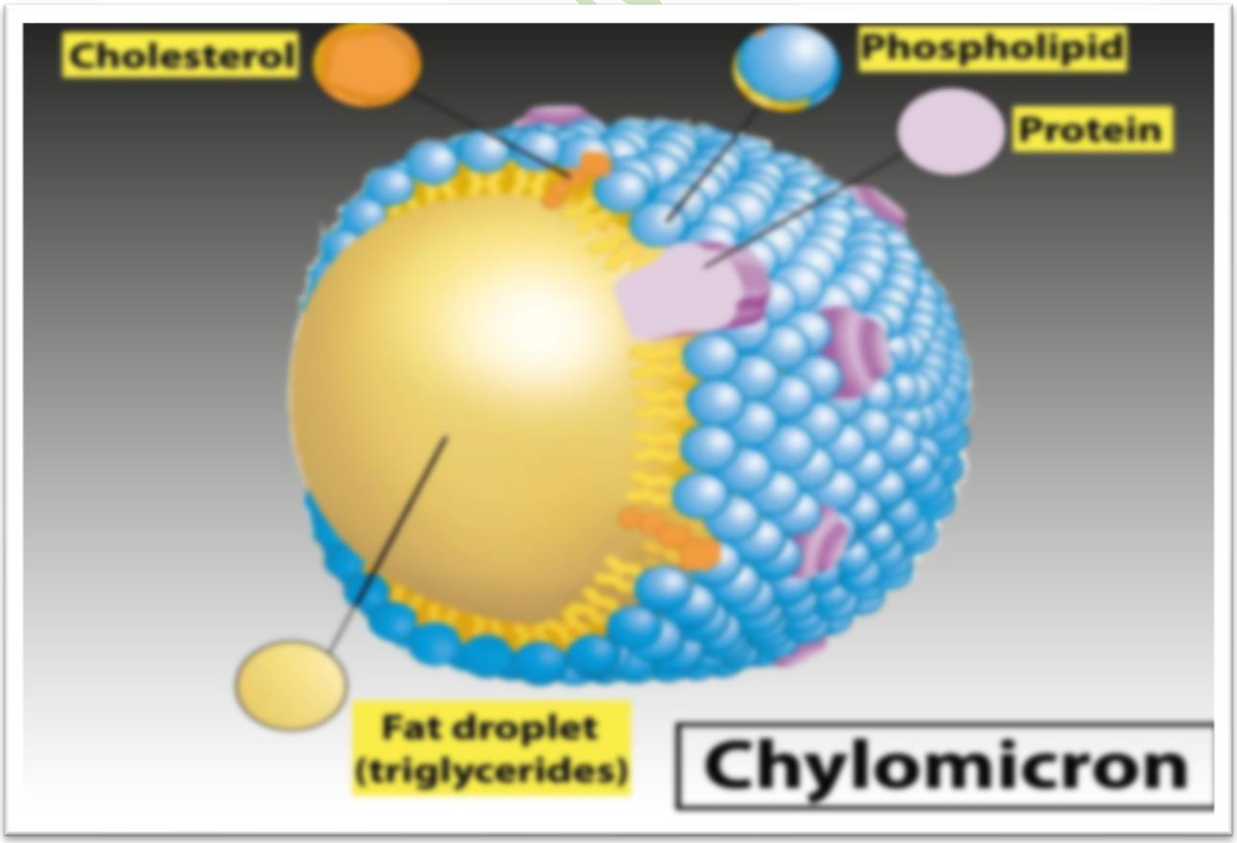
The earliest reports of vitamin A poisoning were among arctic explorers who devoured polar bear liver, which has high levels of the vitamin.

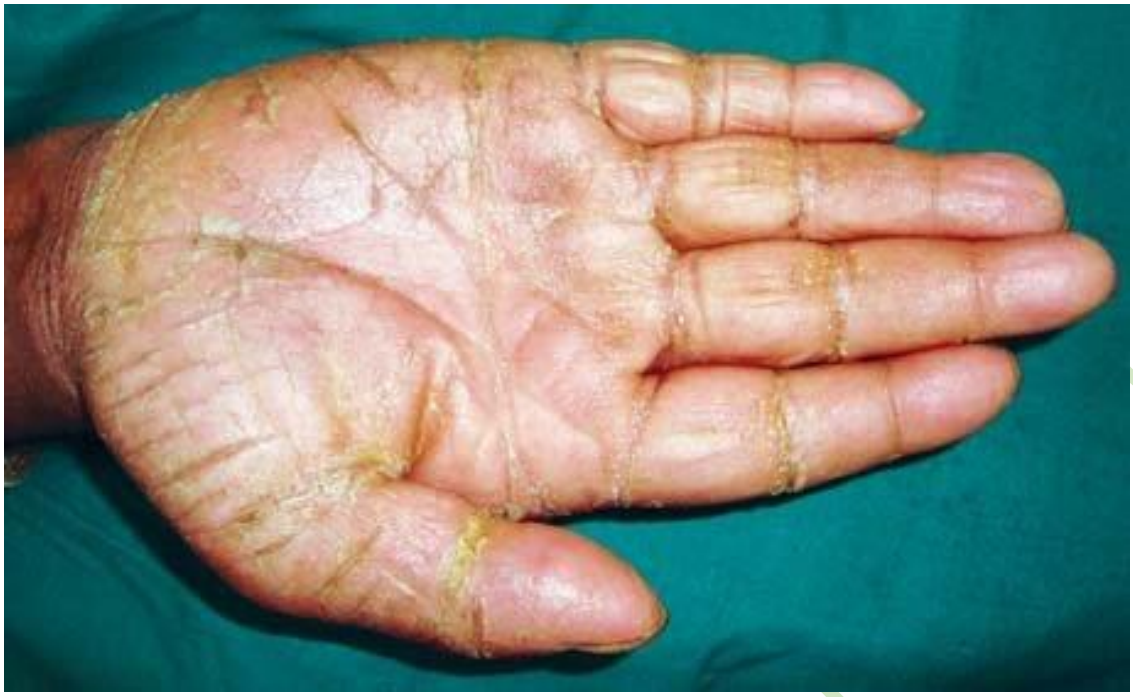
Vitamin A toxicity is rare with typical diets unless the patient self-medicates or takes pharmaceutical doses.

A single intake of more than 300 mg, or prolonged consumption of excessive quantities, causes hyper-vitaminosis, a hazardous disease. It causes dry, pruritic skin, hepatomegaly, and high intracranial pressure, which might simulate a brain tumor. Excessive consumption of vitamin A may cause congenital deformity. These dangers should be avoided by pregnant women.




lens
iris
cornea
sclera
retina
choroid
vitreous chamber
vitreous humor
fovea
anterior chamber
aqueous humor
optic nerve
suspensory ligaments


Cholesterol
Phospholipid
Protein
Fat droplet
(triglycerides)
Chylomicron



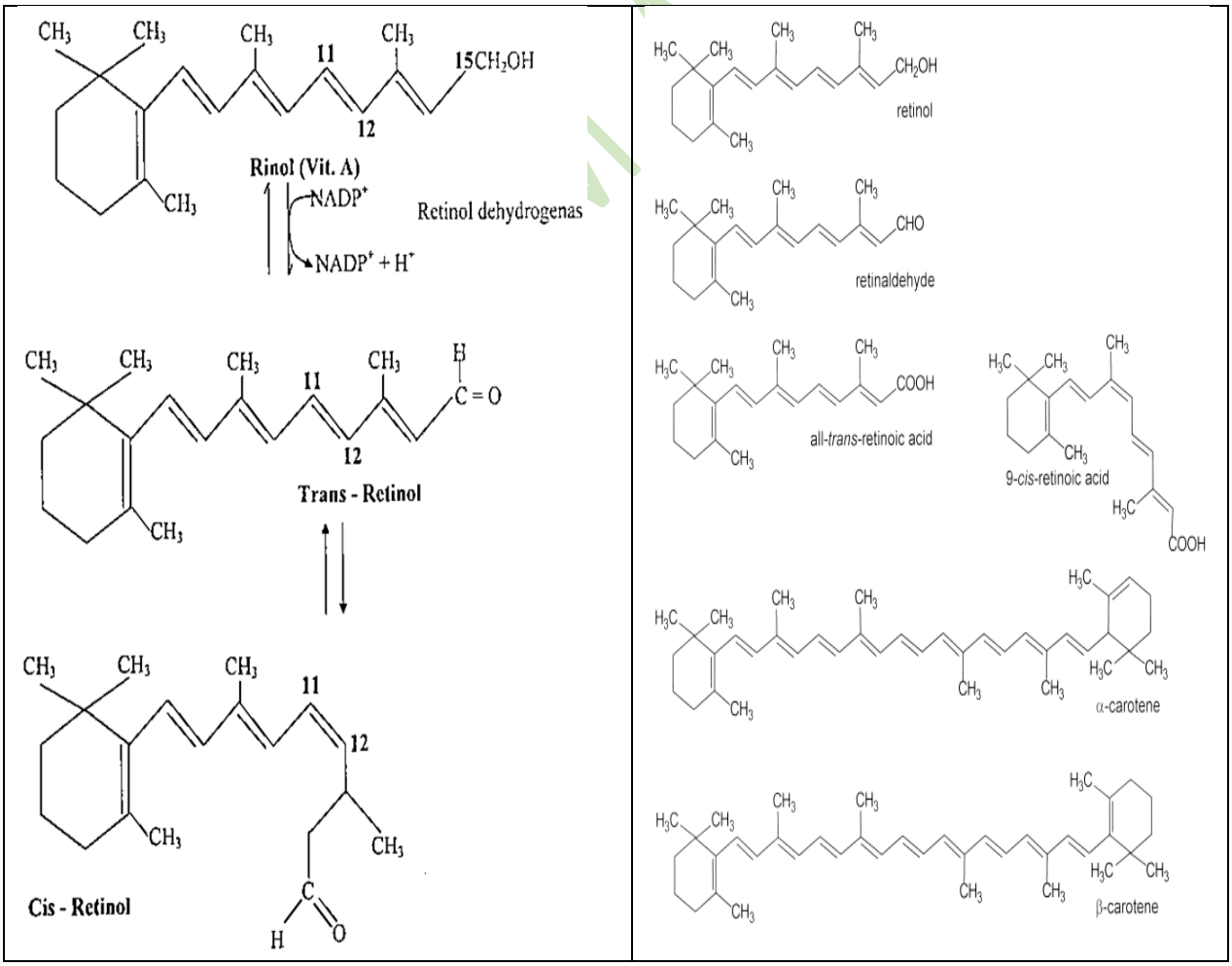


Figure 11.2 Vitamin A – retinoids and pro-vitamin A carotenoid

Vitamin D

Vitamin D is a group of sterol compounds that are required as accessory food factors in individuals not exposed to sunlight. It is the fourth vitamin to be discovered after A, B and C.

Rickets has been known throughout Europe, notably England, and may be treated by eating fish, especially fish oil, or exposing kids to sunshine. in the early 1900s, an essential factor was discovered that Rickets lacked vitamin D, the fourth vitamin identified after A, B, and C.

Note: Vitamin D is not usually required in the human diet.

Vitamers of vitamin D and international units

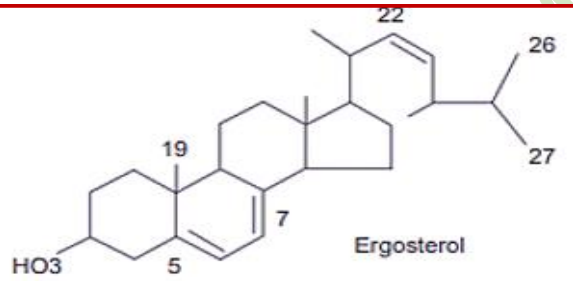
The normal dietary form of vitamin D is cholecalciferol (also known as calciol). This is also the compound that is formed in the skin by UV irradiation of 7-dehydrocholesterol. Some foods are enriched or fortified with the synthetic compound ergocalciferol, which is synthesized by UV irradiation of the steroid ergosterol. Ergocalciferol undergoes the same metabolism as cholecalciferol and has the same biological activity. Early studies assigned the name vitamin D_1 to an impure mixture of products derived from the irradiation of ergosterol; when ergocalciferol was identified it was called vitamin D_2, and when the physiological compound was identified as cholecalciferol it was called vitamin D_3. Like vitamin A, vitamin D was originally measured in international units of biological activity before the pure compound was isolated: 1 iu = 25 ng of cholecalciferol.

The nomenclature of the vitamin D metabolites is shown in Table 11.7.

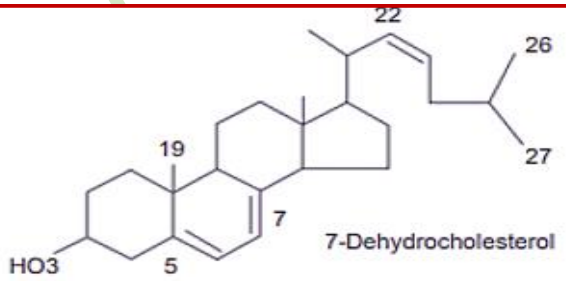
TABLE 11.7 *Nomenclature of vitamin D metabolites*

Trivial name	Recommended name	Abbreviation
Vitamin D_3		
Cholecalciferol	Calciol	–
25-Hydroxycholecalciferol	calcidiol	$25(OH)D_3$
1α-Hydroxycholecalciferol	1(*S*)-Hydroxycalciol	$1\alpha(OH)D_3$
24,25-Dihydroxycholecalciferol	24(*R*)-Hydroxycalcidiol	$24,25(OH)_2D_3$
1,25-Dihydroxycholecalciferol	Calcitriol	$1,25(OH)_2D_3$
1,24,25-Trihydroxycholecalciferol	Calcitetrol	$1,24,25(OH)_3D_3$
Vitamin D_2		
Ergocalciferol	Ercalciol	–
25-Hydroxyergocalciferol	Ercalcidiol	$25(OH)D_2$
24,25-Dihydroxyergocalciferol	24(*R*)-Hydroxyercalcidiol	$24,25(OH)_2D_2$
1,25-Dihydroxyergocalciferol	Ercalcitriol	$1,25(OH)_2D_2$
1,24,25-Trihydroxyergocalciferol	Ercalcitetrol	$1,24,25(OH)_3D_2$

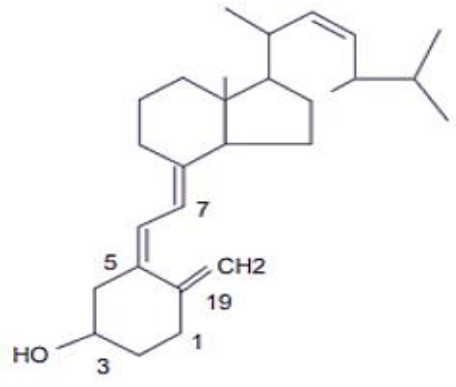
The abbreviations shown in column 3 are not recommended but are frequently used in the literature.



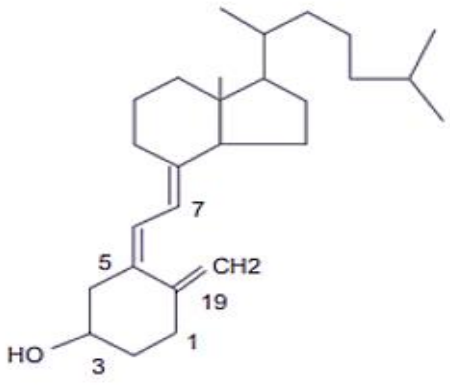
Ergosterol



7-Dehydrocholesterol



Vitamin D2 (ergocalciferol)



Vitamin D3 (cholecalciferol)

Absorption and metabolism of vitamin D

Like vitamin A, dietary vitamin D is absorbed in lipid micelles and incorporated into chylomicrons; therefore, people consuming a low-fat diet will absorb little of such dietary vitamin D as is available

1- Synthesis of vitamin D in the skin

As shown in Figure 11.6, the steroid 7-dehydrocholesterol (an intermediate in the synthesis of cholesterol that accumulates in the skin but not other tissues), undergoes a non-enzymatic reaction on exposure to UV light, yielding pre-vitamin D to form cholecalciferol, which is absorbed into the bloodstream. The 7-dehydrocholesterol is photo-chemically cleaved by radiation in the skin at the ultraviolet range (290–315 nm), with a relatively sharp peak at 296.5 nm, which causes scission of the B-ring of the steroid nucleus of 7-dehydrocholecalciferol. Thus cholecalciferol (Fig. 18.16). Thus 7-dehydrocholesterol **serves as a pro-vitamin.**

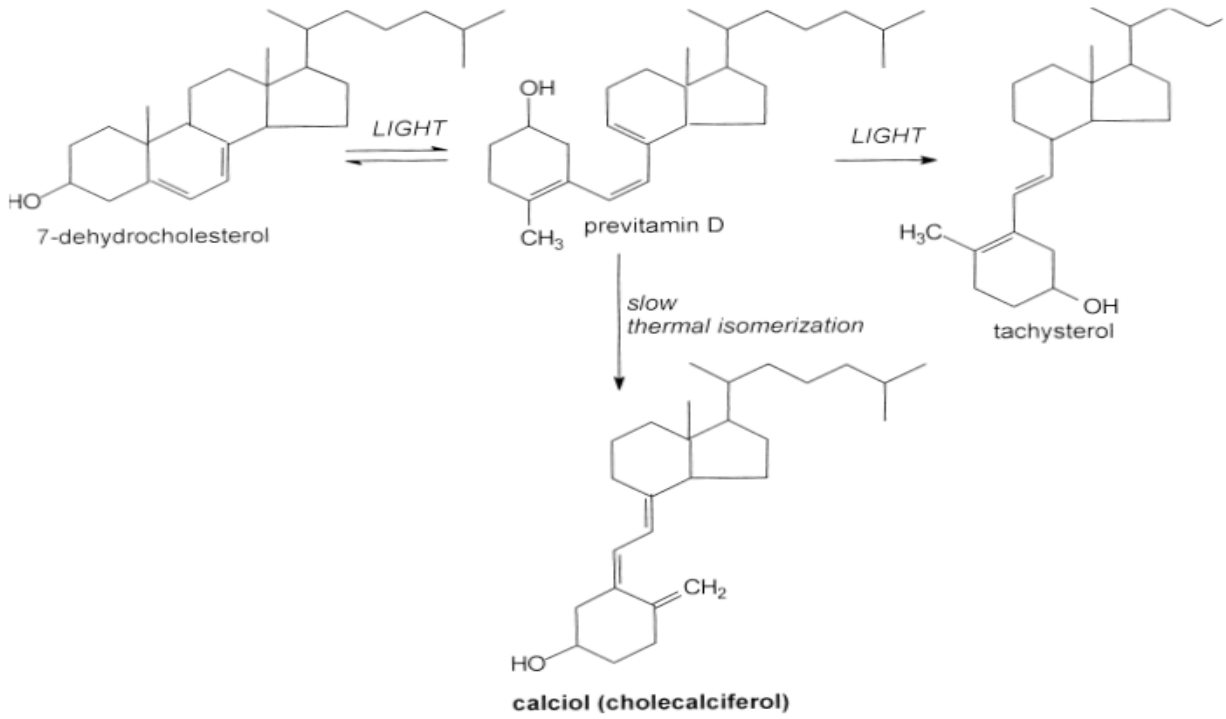


FIGURE 11.6 *The synthesis of vitamin D in the skin.*

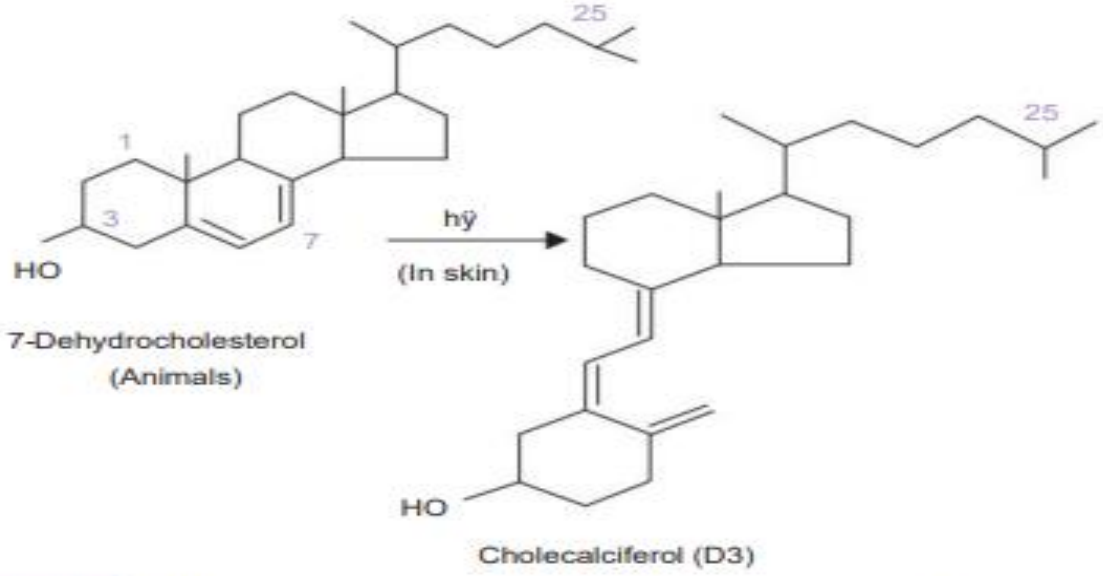


Fig. 18.16. Photochemical cleavage of B-ring of 7-dehydro-cholesterol by ultraviolet rays to form cholecalciferol.

2- Metabolism to the active metabolite, calcitriol

Cholecalciferol, either synthesized **in the skin** or taken in from foods, undergoes two hydroxylations to yield the active metabolite, **1,25-dihydroxyvitamin D** or **Calcitriol**, as shown in Figure 11.7.

Ergocalciferol from fortified foods undergoes similar hydroxylation to yield **Ercalcitriol.**

The first stage in vitamin D metabolism occurs **in the liver**, where it is hydroxylated to form the **25-hydroxy derivative**, **calcidiol.** This is released into the circulation bound to a vitamin D-binding globulin. There is no tissue storage of vitamin D; plasma calcidiol is the main storage form of the vitamin, and it is plasma calcidiol that shows the most significant seasonal variation in temperate climates.

The second stage of vitamin D metabolism occurs **in the kidney**, where calcidiol undergoes either 1-hydroxylation to yield the active metabolite 1,25-

dihydroxy vitamin D (calcitriol) or 24-hydroxylation to yield an inactive metabolite, 24,25- dihydroxy vitamin D (24-hydroxycalcidiol).

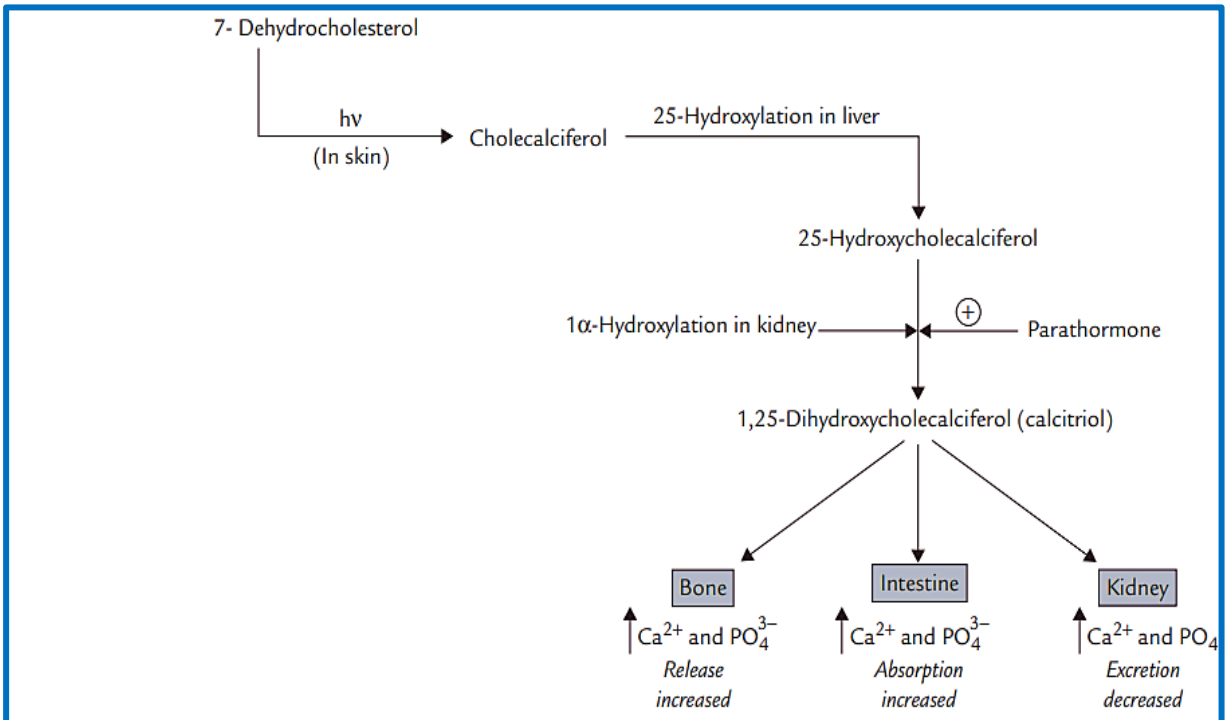


Fig. 18.17. Biosynthesis of biologically active vitamin D compound, calcitriol, from the pro-vitamin precursor.

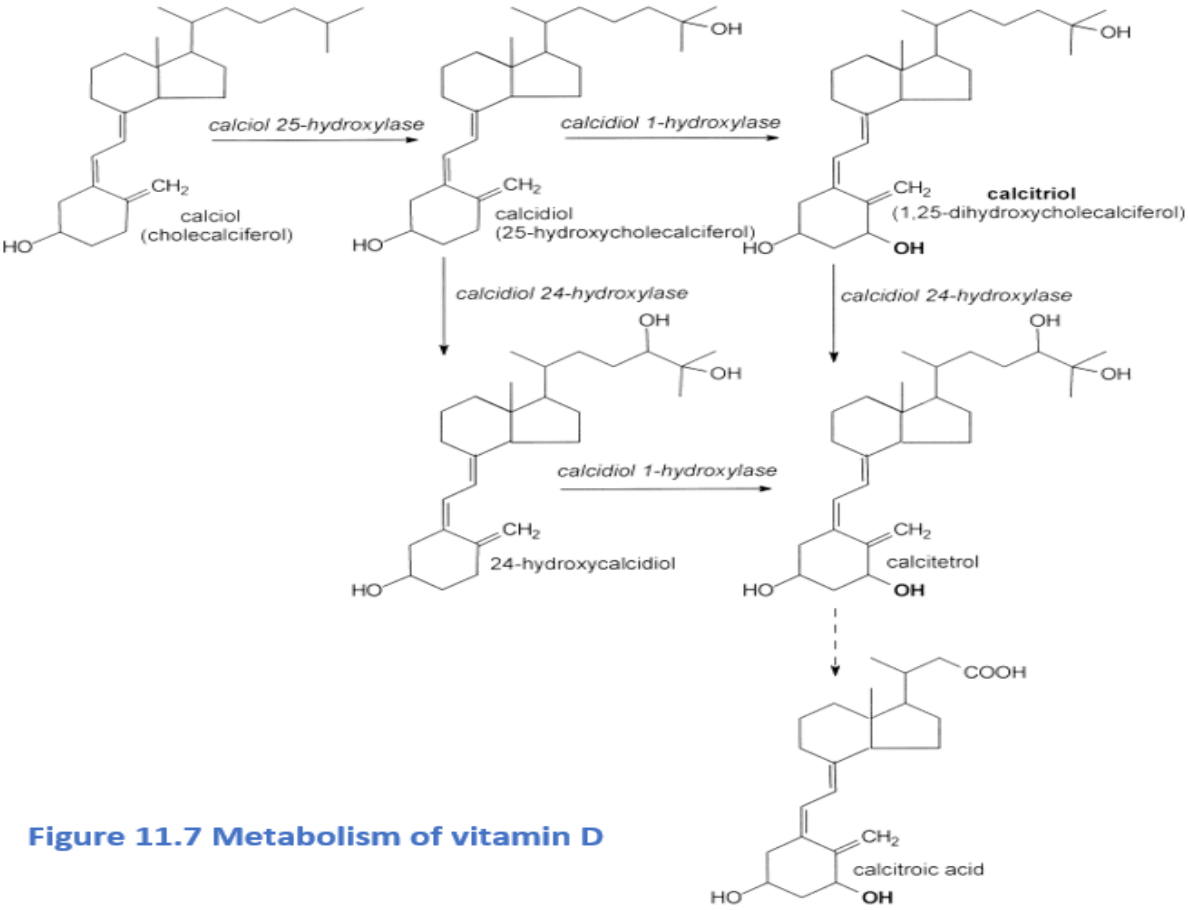


Figure 11.7 Metabolism of vitamin D

Regulate the metabolism of vitamin D :

The main function of vitamin D is in the control of calcium homeostasis, vitamin D metabolism is regulated, at the level of 1- or 24- hydroxylation, by factors that respond to plasma concentrations of calcium and phosphate:

1. Calcitriol acts to reduce its synthesis. It induces the 24-hydroxylase and represses the synthesis of 1-hydroxylase in the kidney, acting on gene expression by way of calcitriol receptors.
2. Parathyroid hormone is secreted in response to a fall in plasma calcium.
3. Calcium exerts its main effect on the synthesis and secretion of parathyroid hormone.

Biochemical Effects of vitamin D3 (calcitriol) :

The predominant target organs for calcitriol are the **intestine, bone, and kidney.**

It regulates serum calcium (normal 9–11mg/dL) and phosphate (2.5–4.5 mg/dL) concentrations by stimulating:

1- Absorption of calcium and phosphate from the intestine.
2- Reabsorption of calcium and phosphate from the renal tubules.
3- Mobilization of calcium and phosphate from bones.

Calcitriol is a Hormone:

Calcitriol is thought of as a hormone rather than a vitamin **because it has several hormone-like properties:**

1- It can be synthesized in the body, is released in the circulation, and has distinct target organs.
2- Further, its mechanism of action resembles some hormones. That it binds with a nuclear receptor, called **vitamin D receptor (VDR),** which is a ligand-regulated transcription factor belonging to the same family as the receptors for steroid hormones and thyroid hormones.

Metabolic functions of vitamin D

The principal function of vitamin D is to maintain the plasma concentration of calcium. Calcitriol achieves this in three ways:

1. increased intestinal absorption of calcium;
2. reduced excretion of calcium (by stimulating resorption in the distal renal tubules);
3. mobilization of bone minerals.

Calcitriol also has several regulatory effects. It is a necessary, but not sufficient, factor, such as:

1. insulin secretion;
2. synthesis and secretion of parathyroid and thyroid hormones;
3. inhibition of the production of interleukin ;

In all of these actions, the role of calcitriol seems to be the induction or maintenance of synthesis of calcium-binding proteins, and the effects are secondary to increased calcium uptake into the target cells.

The role of Calcitriol (vitamin D_3) in bone metabolism

The maintenance of bone structure is due to the balanced activity of osteoclasts,

Mineralization of the organic matrix is largely controlled by the availability of calcium and phosphate.

Calcitriol (vitamin D_3) raises plasma calcium by activating osteoclasts to stimulate the mobilization of calcium from bone. It acts later to stimulate the laying down of new bone to replace the loss, by stimulating the differentiation and recruitment of osteoblast cells.

Deficiency of Vitamin D: rickets and osteomalacia

Inadequate exposure to sunlight among people living in congested slums may result in vitamin D deficiency (i.e. rickets) in children. and in the adult rickets is called osteomalacia. The classical features of rickets is bone deformities such as bow legs and pain of the knee. In osteomalacia, the patients suffer from bone pains and are likely to suffer fractures. Also, now, there is evidence that with advancing age (elderly), the capacity of the skin for the synthesis of cholecalciferol decreases, resulting in age-related disorders of calcium metabolism.

Causes of deficiency of Vitamin D because of:

1- Inadequate dietary intake

2- Decreased intestinal absorption, and

a. Impaired activation: it occurs in liver and kidney disorders, since in these conditions the disease is termed **hepatic rickets** and **renal rickets**, respectively.

The effect of the deficiency of vitamin D is a decrease of the plasma calcium concentration, which is promptly restored by parathormone (PTH) at the expense of bones (PTH mobilizes calcium from bones), resulting in soft and poorly mineralized under stress (especially in weight-bearing areas).

Abnormalities of biochemical parameters in both conditions **rickets and osteomalacia** are low serum calcium and phosphate, and elevated serum alkaline phosphatase (bone isoenzyme) activity. Serum calcitriol level is low.

Vitamin D toxicity (Hyper-vitamin D):

Prolonged intake of high doses of vitamin D (above 155 units/day) may result in toxic symptoms such as polyuria, thirst, confusion, difficulty in speaking, and weight loss. **Biochemical alterations** are hypercalcemia, hypokalaemia, and metabolic alkalosis. Furthermore, prolonged exposure of pre-vitamin D to UV light results in further reactions to yield biologically inactive compounds.

Vitamin E (α-Tocopherol)

Vitamin E is an important antioxidant. It was isolated from wheat germ oil, and its structure was determined in 1936 by Paul Karrer (Nobel Prize 1937).

Vitamin E was earlier referred to as **an anti-sterility vitamin. The word tocopherol is derived from the Greek words tokos, meaning childbirth,** and pherin, to bear, because it was believed that tocopherol is required for fertility. However, such an **anti-sterility** effect has been observed only in some animals, not humans.

Vitamers and units of activity Vitamin E

Vitamin E is a general term for two families. Of compounds, the **Tocopherols,** and the **Tocotrienols** (Figure 11.8). The different vitamers have different biological potency, as shown in Table 11.8. The most active is α-tocopherol, and it is usual to express vitamin E intake in terms of milligrams of α-tocopherol equivalents.

The obsolete international unit of vitamin E activity is sometimes used: 1 iu = 0.67 mg α-tocopherol equivalent; 1 mg α-tocopherol = 1.49 iu.

TABLE 11.8 *Relative biological activity of the vitamin E vitamers*

	iu/mg	Relative activity
D-α-Tocopherol (*RRR*)	1.49	1.00
D-β-Tocopherol (*RRR*)	0.75	0.49
D-γ-Tocopherol (*RRR*)	0.15	0.10
D-δ-Tocopherol (*RRR*)	0.05	0.03
D-α-Tocotrienol	0.45	0.29
D-β-Tocotrienol	0.08	0.05
D-γ-Tocotrienol	–	–
D-δ-Tocotrienol	–	–
L-α-Tocopherol (SRR)	0.46	0.31
RRS-α-Tocopherol	1.34	0.90
SRS-α-Tocopherol	0.55	0.37
RSS-α-Tocopherol	1.09	0.73
SSR-α-Tocopherol	0.31	0.21
RSR-α-Tocopherol	0.85	0.57
SSS-α-Tocopherol	1.10	0.74

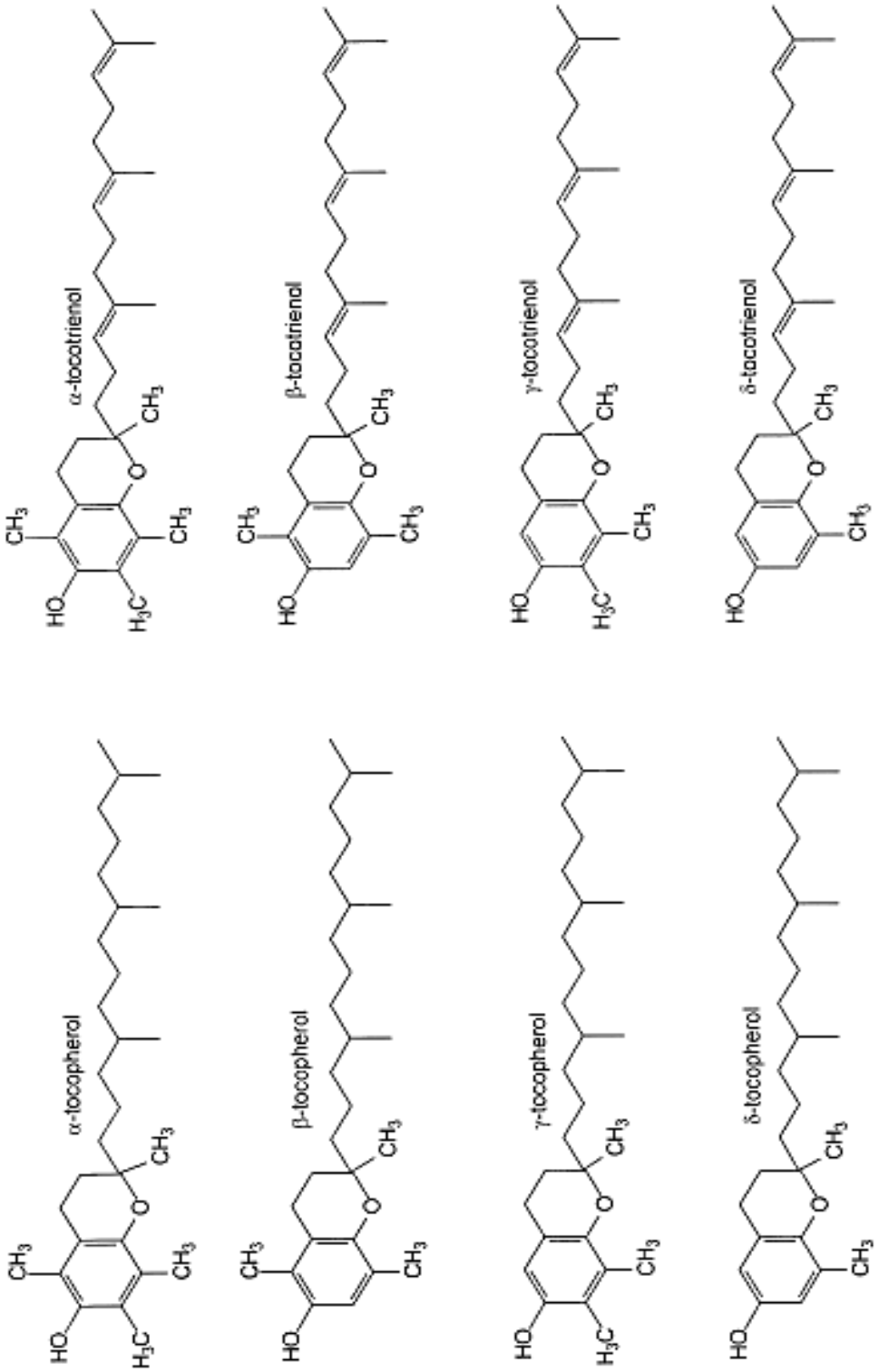


FIGURE 11.8 *Vitamin E vitamers.*

Chemical Nature

At least eight naturally occurring plant compounds with vitamin E activity are presently known, the most important being **α, β,γ, and δ-tocopherol.** All are viscous, light yellow oils that are heat stable but readily degraded by oxygen or ultraviolet light (UV). They have a substituted chromane nucleus with a polyisoprenoid side chain of variable length (Figure 11.3). Of these vitamers, the most abundant and potent is **α-tocopherol.**

Absorption, Transport, and Metabolism of vitamin E

The richest naturally occurring vitamin E sources are vegetable oils and nuts (Table 18.1).

Vitamin E is absorbed as free tocopherol along with other lipid components. The absorption requires bile salts: in their presence the vitamin is absorbed with effi ciency of 20–40%.

Vitamin E has no specific binding proteins and is found in circulation in association with plasma lipoproteins and erythrocytes (plasma concentration is 0.5–1 mg/dL).

Vitamin E is abundant in all lipid-containing tissues, with the significantly highest concentrations observed in n-fat depots of adipose tissue.

Vitamin E is mainly excreted in feces via the hepato-biliary route after the chromane ring is oxidized, followed by its conjugation with glucuronic acid.

Metabolic function of vitamin E

1. **Antioxidant role:** Vitamin E is accepted as nature's most potent and abundant biological antioxidant, a membrane antioxidant.

In cellular and subcellular membranes, it acts as a first line of defense against free radicals by acting as a chain-breaking antioxidant (Fig. 18.19).

As such, it is associated with the membrane lipid structure and can promptly protect the membranes from attack by endogenous and exogenous free radicals.

Vitamin E may be located near enzyme complexes that produce free radicals, such as NADPH-dependent oxidase systems, where it scavenges free radicals formed during lipid peroxidation, interrupting free radical chain reactions.

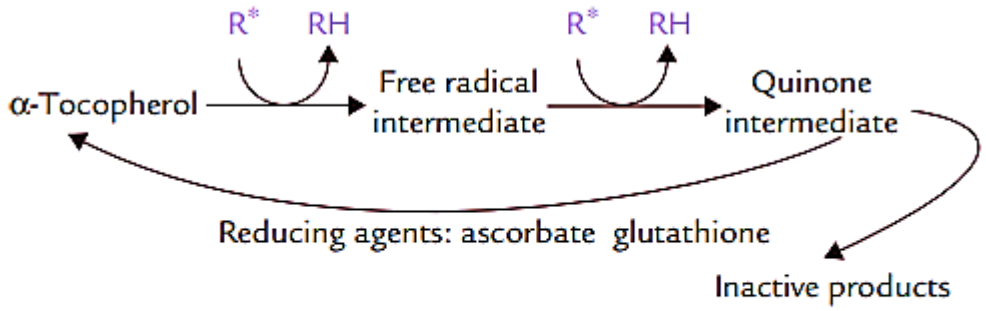


Fig. 18.19. The action of α-tocopherol as a scavenger of free radicals (R* = free radical).

2- Antiatherogenic role: Several epidemiological studies suggest an inverse relationship between vitamin E intake and the incidence of morbidity and mortality from coronary artery disease. Supplements of 400 IU of vitamin E for about two years result in a 40% reduction in the incidence of heart attack (1 mg of α-tocopherol is equivalent to one IU of vitamin E).

The following properties of vitamin E may contribute toward its antiatherogenic role:

(a) Vitamin E retards the oxidation of LDL, thereby decreasing the production of the pro-atherogenic oxidized LDL.

(b) Vitamin E impedes various cellular signalling pathways (e.g., protein kinase-C initiated pathways), inhibiting the proliferation of smooth muscle cells, platelet adhesion and aggregation, and function of adhesion molecules.

(c) Vitamin E may also decrease the synthesis of leukotrienes and increase the synthesis of prostacyclin by upregulating phospholipase A2 and cyclooxygenase.

3. The correlation between vitamin E and selenium metabolism:

Selenium contributes to reducing certain symptoms of vitamin E deficiency by behaving as a cofactor for glutathione peroxidase. Glutathione peroxidase is an essential enzyme that catalyzes the oxidation process and removal of free radicals. Also, Vitamin E decreases the requirement for selenium and, inversely, indicates a beneficial connection between them.

4. Others: Vitamin E enhances immunological response, protects RBC from hemolysis, maintains cell structure and function, slows aging, and prevents Alzheimer's disease.

Actions of Tocotrienols (Vitamin E) on the Hypo-cholesterolaemic

Tocotrienols (Vitamin E) have lower biological activity than tocopherols, and indeed, it is conventional to consider only γ-tocotrienol as a significant part of vitamin E intake. However, the tocotrienols have a hypocholesterolemic action not shared by the tocopherols.

In plants, tocotrienols are synthesized from hydroxymethylglutaryl CoA (HMG CoA), the precursor for cholesterol synthesis (see Figure 7.21). High levels of tocotrienols reduce the activity of HMG CoA reductase, which is the rate-limiting enzyme in the pathway for synthesizing both cholesterol and tocotrienols, by repressing the synthesis of the enzyme.

Deficiency of Vitamin E

Vitamin E insufficiency is rare in premature babies because breastfeeding is a high supply. In addition, vitamin E deficiency is rare in adults due to its widespread distribution in food and the body's vitamin stores, which can satisfy the requirement for several months.

A daily intake of (10–30) mg is considered adequate and is provided by most diets.

Defective lipid absorption or transport may sometimes cause the deficiency. Vitamin E is often called a "**vitamin in search of disease**"; human deficiency sometimes occurs. It is seen in infants born with low tissue stores and with poor intestinal absorption for several weeks after birth.

Toxicity of Vitamin E (Hypervitaminosis E):

High doses of vitamin E (greater than 400 IU/day) reduce coagulation. Patients with bleeding disorders, as well as those using warfarin, should avoid using it. In addition, vitamin E is the least toxic of all fat-soluble vitamins. Even 50 times the recommended intake has been reported as non-toxic.

Indicators of the Vitamin E status

Erythrocytes (RBC) are incapable of newly generated lipid synthesis. Therefore, peroxidative damage caused by oxygen stress significantly decreases red cell life, possibly causing hemolytic anemia in vitamin E deficiency. This can be used to determine the status (however, unrelated factors influence the results) by measuring the hemolysis of RBC (red cells) caused by dilute hydrogen peroxide.

An alternative method of assessing functional antioxidant status, again one that is affected by both vitamin E and other antioxidants, is by measuring the respiration of pentane from the metabolism of ω-6 polyunsaturated fatty acids

peroxides or ethane from ω-3 polyunsaturated fatty acid peroxides is another way to assess functional antioxidant status, which is influenced by vitamin E and other antioxidants as well.

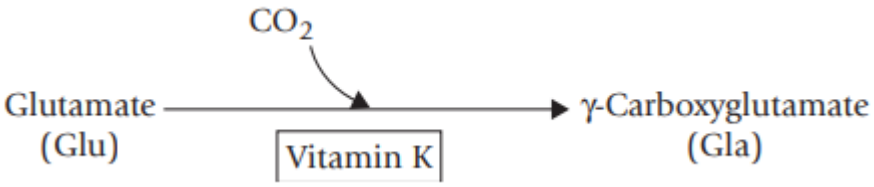
Vitamin E requirements

It is difficult to determine the requirements of vitamin E, mainly because deficiency is mostly unknown. The requirement is also dependent on polyunsaturated fatty acid intake. Based on limited experimental evidence, the recommended vitamin E intake is 0.4 mg α-tocopherol equivalent per gram of dietary polyunsaturated fatty acid. This is not an issue because polyunsaturated fatty acid-rich plant oils are also rich in vitamin E.

Vitamin K

Vitamin K was initially recognized as an anti-haemorrhagic dietary factor. It is the only fat-soluble vitamin that acts as a coenzyme. Its major function is to synthesize γ-carboxyglutamate by incorporating carbon dioxide (CO2) into specific glutamyl residues of certain proteins such as prothrombin, osteocalcin, and certain clotting factors.

The designation" K" derived from the initial description of the vitamin as the "koagulation vitamin".



Chemistry (Structure) of vitamin K

Vitamin K activity is present in a group of structurally related compounds. All have a 2-methyl-1,4-naphthoquinone nucleus but vary in the number of isoprenoid units in its side chain (Fig. 18.20).

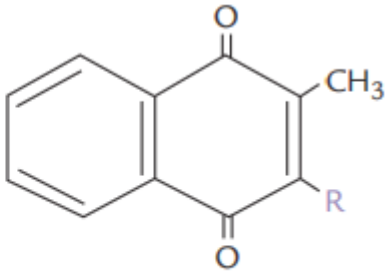


Fig. 18.20. Structure of vitamin K.

Vitamers of vitamin K

Three compounds have the biological activity of vitamin K (Figure 11.10):

Two of them are naturally included: **Menaquinone (K2)** and **phylloquinone (K1),**

1- **Phylloquinone,** present in plants, found in green leafy vegetables;
2- **Menaquinones**, present in animals synthesized by intestinal bacteria, with differing lengths of side-chain;
3- **Menadione and Menadiol diacetate are** synthetic compounds that can be metabolized to phylloquinone.

R = H in Menadione

R = 20 C in Phylloquinone

R = 30 C in Menaquinone

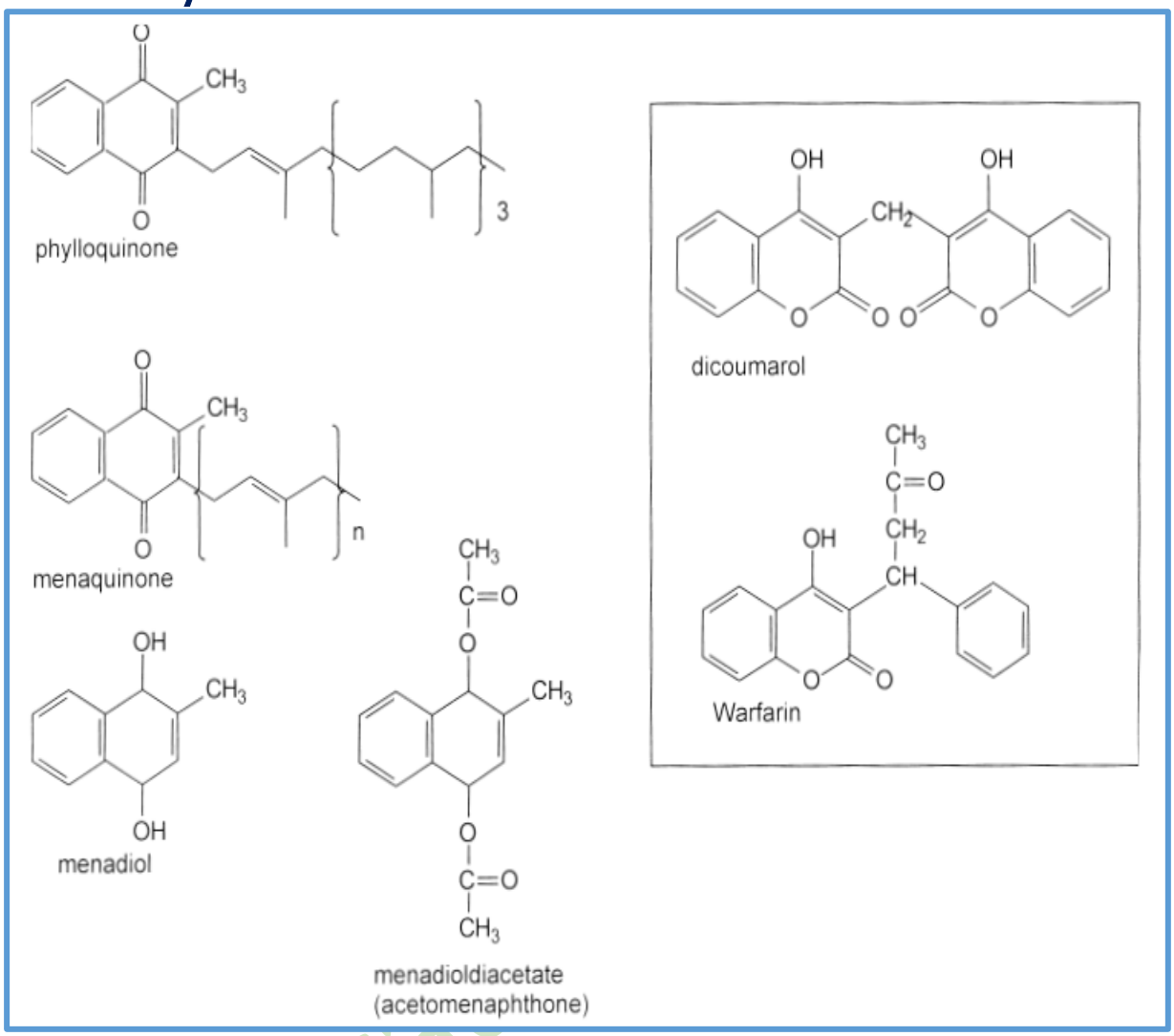


Figure 11.10 Vitamin K vitamers; the vitamin K antagonists dicoumarol and warfarin are shown in the box. Menadione and menadiol diacetate are synthetic compounds that are converted to menaquinone in the liver and have vitamin activity.

Metabolic Function of vitamin K:

Vitamin K plays an important role in blood coagulation for it is required for the post-translational processing of several proteins required in the coagulation cascade (e.g. factor-II, -VII, -IX and -X) in the ER of liver cells.

All these protein clotting factors are initially synthesized as inactive precursors in the liver.

vitamin K is the cofactor for the carboxylation of glutamate residues in the post synthetic modification of proteins to form the unusual amino acid γ-carboxyglutamate, abbreviated to **Gla** (Figure 11.11).

Formation of mature clotting factors requires that the glutamyl residues of the precursor proteins be converted to γ-carboxyglutamate (Gla) residues by the addition of a carboxylate group. This reaction depends on vitamin K, which serves as a coenzyme (Fig. 18.21).

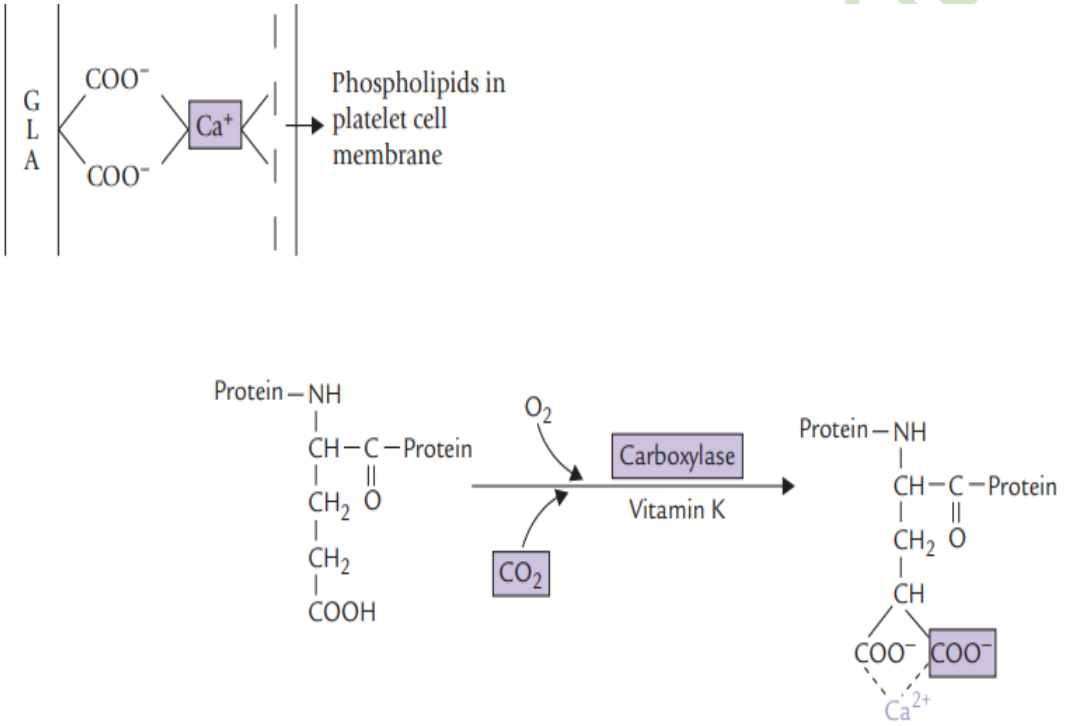


Fig. 18.21. Vitamin K as cofactor in synthesis of Gla residue.

1- Role of γ- carboxyglutamate (Gla) in clotting:

The γ-carboxyglutamate (Gla) residues so formed serve as binding sites for calcium ions; each Gla contains two negative charges which chelate the positive calcium ion. In prothrombin molecule, for instance, there are 10 such carboxylated Gla residues and all of these are required for this protein's specific chelation of calcium ions.

The calcium then binds with the negatively charged phospholipids present on the platelet cell membrane. In this way, bridging of the phospholipids to the Gla residue of prothrombin occurs via calcium ion.

Thus, prothrombin becomes based on (anchored) to the surface of the activated platelets. This provides a high local concentration of the prothrombin, which accelerates its activation by about 50- to 100-fold, an essential requirement for blood clotting.

2- Bone vitamin K-dependent proteins

Two proteins in the bone matrix contain γ-carboxyglutamate:

1- Osteocalcin: osteocalcin constitutes 1–2% of total bone protein.
2- and a less well-characterized protein known simply as bone matrix Gla protein.

Osteocalcin is interesting in that, as well as γ-carboxyglutamate, it also contains hydroxyproline, so its synthesis depends on vitamins K and C. In addition, its synthesis is induced by vitamin D, and the release into the circulation of osteocalcin provides a sensitive index of vitamin D action

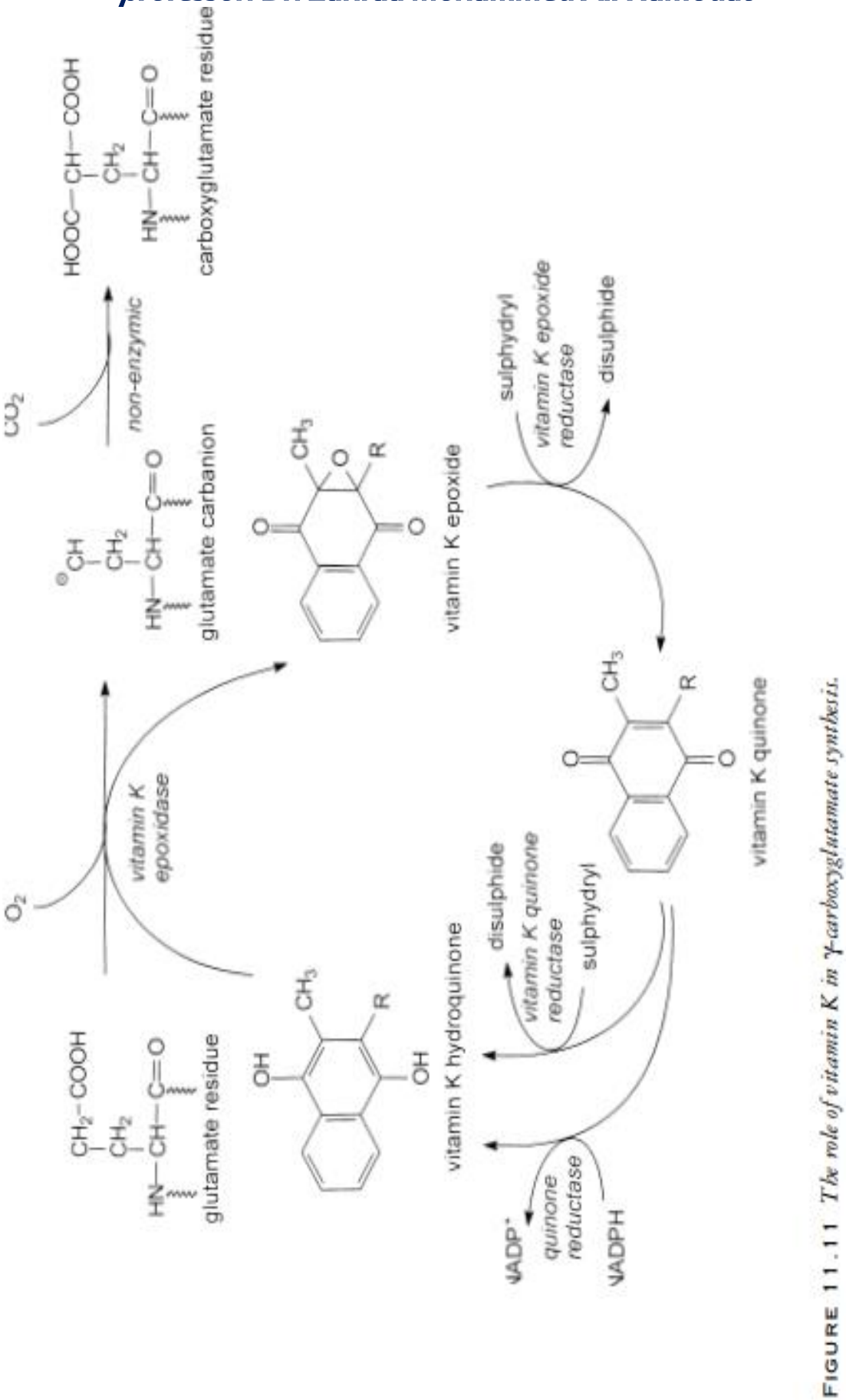


FIGURE 11.11 *The role of vitamin K in γ-carboxyglutamate synthesis.*

Absorption and Storage of vitamin K:

The intestinal absorption of vitamin K is dependent on appropriate fat absorption, and it requires bile salts. It may be obtained from diet or intestinal bacterial synthesis. But colonic bacteria do not significantly contribute because vitamin K is absorbed only in the small intestine. Chylomicrons carry the absorbed vitamin to the liver, where it is stored. From the liver, it is released into blood circulation, where it is transported in association with B-lipoproteins. Unlike the other fat-soluble vitamins, the body stores of vitamin K are insignificant (50–100g), **so vitamin K is the first fat-soluble vitamin deficient in acute fat malabsorption.**

Clinical Deficiency and Requirements of Vitamin K:

Deficiency of vitamin K is rare. A deficiency of vitamin K may be induced in the following ways:

1- Due to treatment with antibiotics.
2- In fat malabsorption syndromes,
3- In liver diseases, and
4- By vitamin K antagonists, such as dicoumarin or warfarin. They act competitively, inhibiting the gamma-carboxylation system due to structural similarity with vitamin K.

Vitamin K deficiency increases the time for blood coagulation, so bleeding tendency is the prominent deficiency manifestation. Even a minor cut may cause prolonged bleeding.

The only important deficiency sign is an increase in **Prothrombin time (PT),** the most important laboratory test for evaluating vitamin K status. Note that PT may also rise in liver damage due to the inability of the liver to synthesize prothrombin. Therefore, vitamin K is administered parenterally (injection) to

distinguish between these two conditions. PT remains unaffected in vitamin K deficiency but returns to normal in liver diseases.

Newborn infants with inadequate vitamin K stores may suffer from the hemorrhagic disease of the newborn. It is the most common nutritional deficiency in newborns.

Based on the determination of clotting time of **Prothrombin time (PT)** and direct measurement of prothrombin and preprothrombin, an intake of 1 µg per kg body weight per day is considered adequate; this forms the basis of reference intakes of between 65 and 80 µg/day for adults. A small number of newborn infants have very low reserves of vitamin K and are at risk of potentially fatal hemorrhagic disease

Vitamin K Toxicity:

Vitamin K is non-toxic, even in large amounts. However, some special cases merit attention. Large doses (5 mg) of menadione and its water-soluble derivatives are known to cause hemolytic anemia and kernicterus in infants; premature infants are more at risk. Hemolytic reactions may also occur in adults having glucose 6-phosphate dehydrogenase deficiency.

Note: *kernicterus is a nuclear jaundice, a type of brain damage seen in newborns caused by a high percentage of red blood cells. When red blood cells break down, bilirubin is produced, and its level in the blood rises. Kernicterus in infant children has higher bilirubin levels than in female children.*

Vitamin C (ascorbic acid)

Ascorbic Acid (Vitamin C)

King and Waugh in 1933, isolated from orange, an antiscurvy substance having strong reducing nature. Its structure was established in 1938 by Howarth and it was named as ascorbic acid. Its reducing nature was due to its strong tendency to donate reducing equivalents.

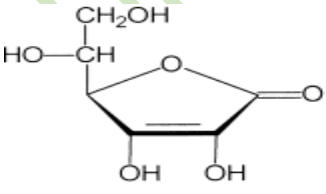
Absorption and Storage of Vitamin C

Vitamin C is absorbed from the small intestine by a carriermediated process at the luminal surface that requires a sodium gradient. The transport resembles the sodium-dependent transport of sugars and amino acids (Chapter 7). The effi - ciency of absorption is high (80–90%). Following absorption, the vitamin circulates in plasma, red cells and leukocytes. It is found in highest concentrations in the adrenals, the pituitary and the retina, in that order.

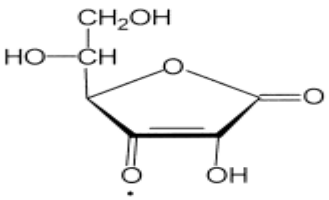
Structure and Synthesis of Vitamin C

The structure of ascorbic acid resembles monosaccharides (hexoses) and it can exist as L- and D-isomers. Only the L form possesses the vitamin activity.

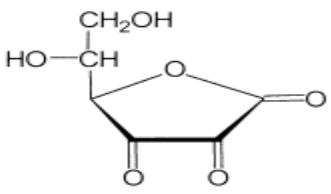
Vitamin C is synthesized by most of the plant and the animal kingdom in uronic acid pathway (Chapter 10). Only humans, higher primates, guinea pigs, and fruit-eating bats have lost the ability to synthesize the vitamin because of lack of the enzyme L-gluconolactone oxidase, that converts gluconolactone to ascorbic acid. Therefore, vitamin C is an essential nutrient in these animals.



ascorbate



monodehydroascorbate
(semidehydroascorbate)



dehydroascorbate

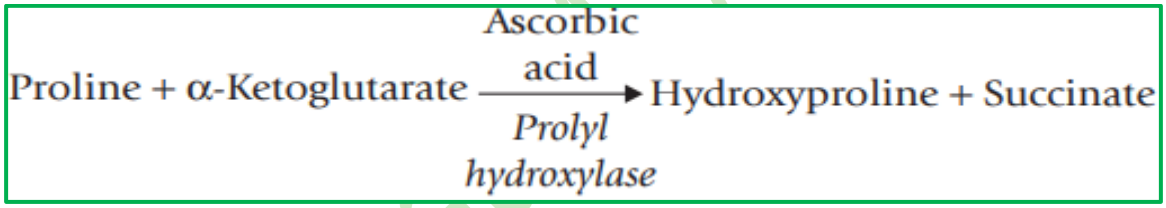
Functions of Vitamin C (Ascorbic acid)

Vitamin C (Ascorbic acid) functions as a reducing agent and a scavenger of free radicals (antioxidant).

1- As a reducing agent: Ascorbic acid is promptly oxidized to its biological equivalent dehydroascorbic acid, which can be readily reduced to re-form ascorbic acid. Mechanism of action of ascorbic acid relative to its many activities is explained by its ability to undergo such reversible oxidation and reduction reactions. In a large proportion of reactions, the prime function of this vitamin is to maintain metal co-factors in their lower valence state, e.g. Fe2and Cu.

Some of the important ascorbate-dependent reactions are as below:

(a) During collagen biosynthesis, the hydroxylases causing post-translational hydroxylation of prolyl and lysyl residues require ascorbate.



Thus, vitamin C plays a role in the formation of matrix of bones, cartilages, and connective tissue. In absence of vitamin C, newly synthesized collagen cannot form fi bres properly, which accounts for the prominent connective tissue abnormalities of scurvy.

(b) Synthesis of norepinephrine from dopamine by the enzyme dopamine b-monooxygenase depends on vitamin C (Fig. 13.23).

(c) Carnitine synthesis requires two Fe2-containing ascorbate-dependent dioxygenases. Carnitine deficiency decreases mitochondrial fatty acid oxidation and thereby contributes to the fatigue, characteristic of scurvy.

(d) During bile acid synthesis in liver mitochondria, the 7-a-hydroxylase reaction requires ascorbic acid.

(e) Absorption of iron is aided by vitamin C by converting ferric to ferrous ions (Fig. 19.2).

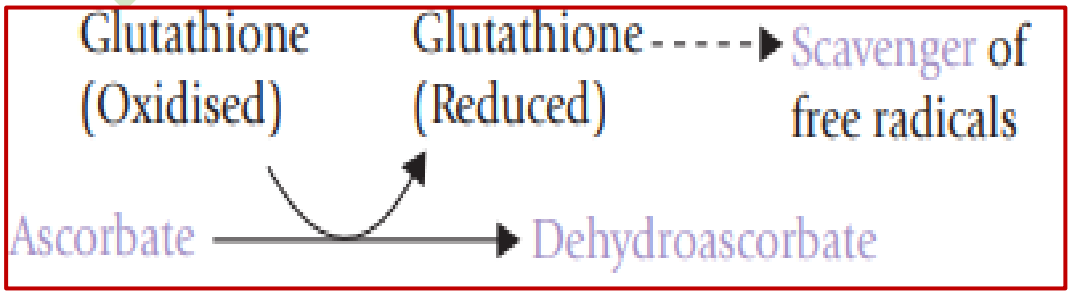
(f) During steroidogenesis, ascorbic acid is thought to participate in several oxidation-reduction reactions. This may explain highest tissue concentrations of ascorbate in the adrenal cortex.

(g) Ascorbate participates in tyrosine catabolism by serving as coenzyme for 4-hydroxyphenylpyruvate dioxygenase.

(h) Vitamin C also participates in bone mineral metabolism.

NOTE: Vitamin C deficiency (scurvy) results in connective tissue problems because of impaired collagen synthesis.

2. As an antioxidant: Ascorbic acid is not only one of the strongest naturally occurring reducing agents known, but it can also serve as an antioxidant in several nonenzymatic reactions. It decreases oxidation of DNA and arrests protein damage, reduces lipid peroxidation and oxidation of low-density lipoproteins, and decreases production of extracellular oxidants from neutrophils. Because of these actions it provides several health benefi ts, especially in prevention of atherosclerosis and coronary heart disease.



An important area associated with its antioxidant properties is in the prevention and treatment of cancer. For example, it may suppress the formation of potentially carcinogenic nitrosamines from dietary nitrite and nitrate in the stomach, which may explain its protective effect in cancer. Epidemiological studies suggest that vitamin C exerts a synergistic effect with other dietary antioxidants, vitamin E and carotenoids, and this may have a signifi cant role in the prevention of cancer, cardiovascular disease and cataract formation. The quantitative contributions of these components to the overall effect are not known.

NOTE: Vitamin C (water-soluble antioxidant), acts together with membrane antioxidants (vitamins A and E) to limit the extent of free radical-mediated oxidative reactions. They prevent lipid peroxidation, and may have antimutagenic properties

Clinical Deficiency of Vitamin C (Ascorbic acid), Scurvy,

Scurvy, the vitamin C deficiency disease, first became prominent during the 15th century among sailors on long voyages whose diets were devoid of fresh foods. The introduction of limes to the diet of the British navy alleviated scurvy and led to the nickname ”limey” for the British sailors.

The disease is characterized by reduced crosslinking of collagen fibres, resulting in fragile blood vessels and haemorrhagic diathesis, which manifests in various forms. There is tendency to bleeding, especially in joints and under the skin.

Gums become soft and spongy, teeth become loose and there is poor wound healing. Bones become weakened and anaemia and infections develop. If untreated, these infections may prove fatal.

Vitamin C requirements

Vitamin C illustrates extremely well how different criteria of adequacy, and different interpretations of experimental evidence (section 11.1), can lead to different estimates 404 Vitamins and minerals of requirements, and to reference intakes ranging between 30 and 90 mg/day for adults. The requirement for vitamin C to prevent clinical scurvy is less than 10 mg/day.

Note: Though RDA for vitamin C is 45 mg/day, a daily intake of 10 mg is sufficient to prevent scurvy.

Toxicity of vitamin C

There is little evidence of any significant toxicity from these high intakes. Once the plasma concentration of ascorbate reaches the renal threshold, it is excreted more or less quantitatively with increasing intake, and there is no evidence that higher intakes increase the body pool above about 1500 mg per kg body weight.

Folic acid

Folic Acid Folic acid is obtained from green leafy vegetables and its name refl ects this—foliage, derived from the Latin word folium, meaning leaf. It serves as a carrier of one-carbon (C1) units during several biosynthetic processes. Two other co-factors are also known to be involved in the addition of C1 unit to a metabolic precursor, biotin in carboxylation reactions, and S-adenosylmethionine (SAM) as a methylating agent. However, folic acid is more versatile than either of these two because it can transfer the C1 units in several oxidation states.

Structure

Folic acid consists of three components: a pteridine ring linked in sequence to para-aminobenzoic acid (PABA) and a glutamate residue (Table 18.2). Up to fi ve additional glutamate residues are linked to the fi rst glutamate via isopeptide bonds

(between terminal carboxylate group and the amino group of the next glutamate residue) to form a polyglutamyl tail.

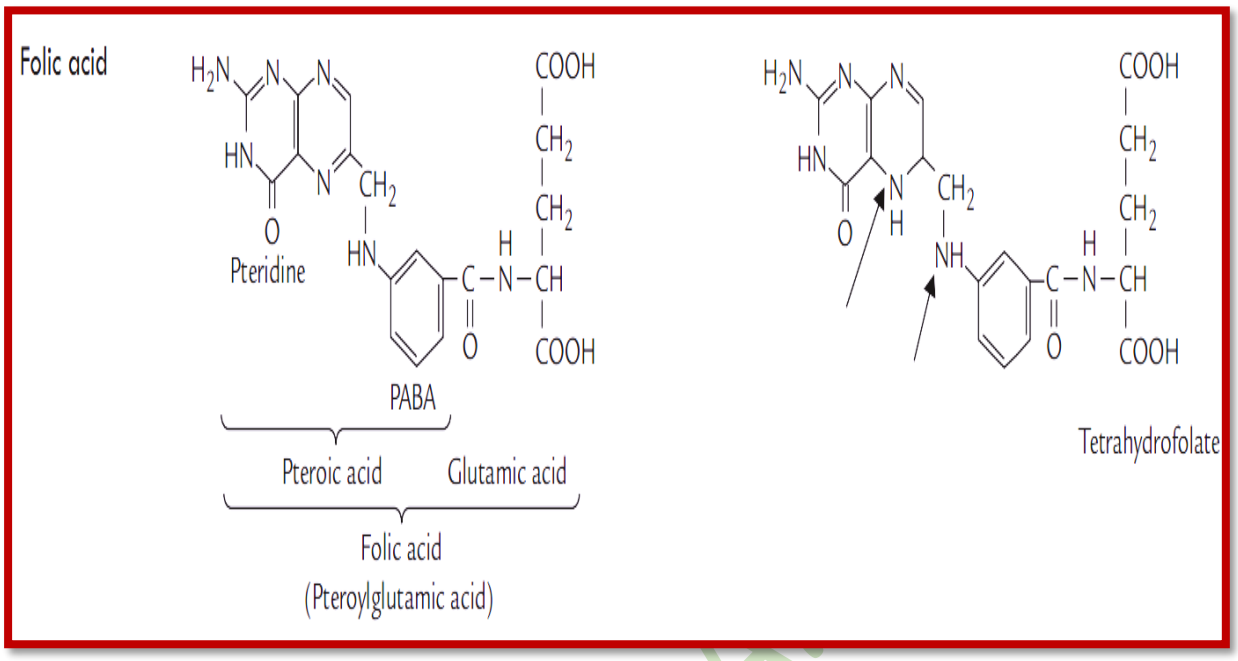


Table 18.2

Synthesis of folic acid

Pteridine and para-aminobenzoic acid are linked covalently by pteroyl synthetase to form pteroic acid. The latter is attached to a glutamate residue to form folic acid (Fig. 18.8). A polyglutamyl tail is built by addition of more glutamyl residues, which imparts multiple negative charges to the molecule and so it cannot traverse biological membranes by passive diffusion. Thus, polyglutamylation serves to sequester folate in the cells in which it is required.

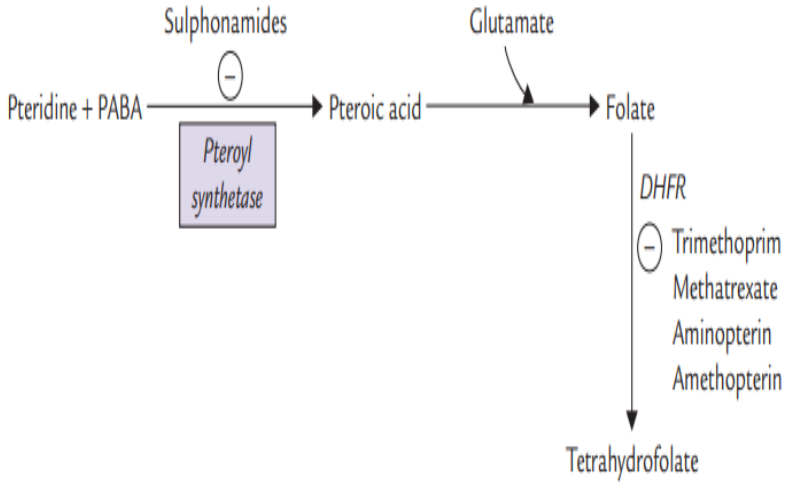


Fig. 18.8. Tetrahydrofolate synthesis and action of antifolates. Sulphonamides and trimethoprim are pharmacological inhibitors in bacteria. Methatrexate, aminopterin and amethopterin inhibit the *DHFR* reaction (PABA = para-amino benzoic acid).

Activation of folic acid

Folate in the human organism must be doubly reduced to become an active coenzyme tetrahydrofolate (THF). The reduction reaction is a stepwise one: folate to dihydrofolate and then to THF. A single NADPH-dependent enzyme dihydrofolate reductase (DHFR) catalyzes both the steps.

$$\text{Folate} \xrightarrow{DHFR} \text{Dihydrofolate} \xrightarrow{DHFR} \text{Tetrahydrofolate}$$

Folate antagonists:

The DHFR reaction is inhibited by the antitumour agents (e.g. methotrexate, amithopterin, and amenopterin), which competitively inhibit the DHFR. This blocks the synthesis of tetrahydrofolate.

Because THF is required for DNA biosynthesis and tumour cells have a very high level of DNA biosynthetic activity, even modest decrease in THF availability will inhibit tumour growth.

Mammals cannot synthesize folic acid, so it must be provided in the diet or by intestinal microorganisms. Many microorganisms can synthesize their own folate as long as PABA is present in the medium. Therefore, the PABA analogues (e.g. sulphonamides) can inhibit formation of folate (Fig. 18.8).

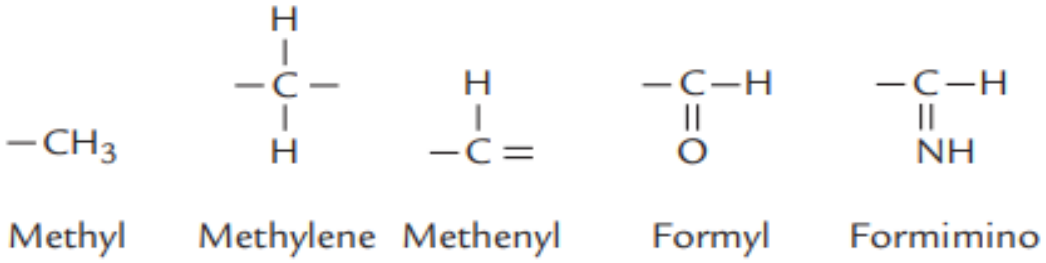


Fig. 18.9. The one-carbon groups transferred by THF.

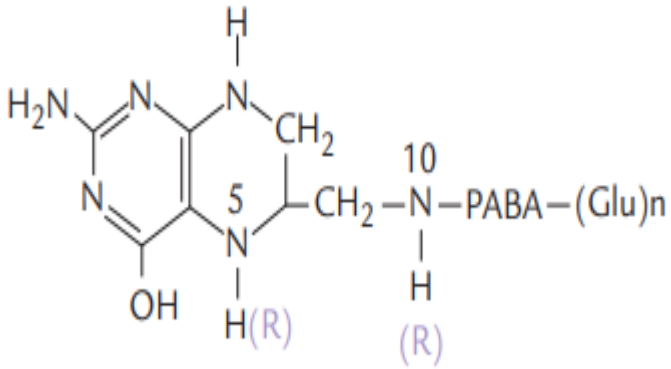


Fig. 18.10. The one-carbon units bound two N^5 and N^{10} of tetrahydrofolate.

Absorption and Distribution of folic acid

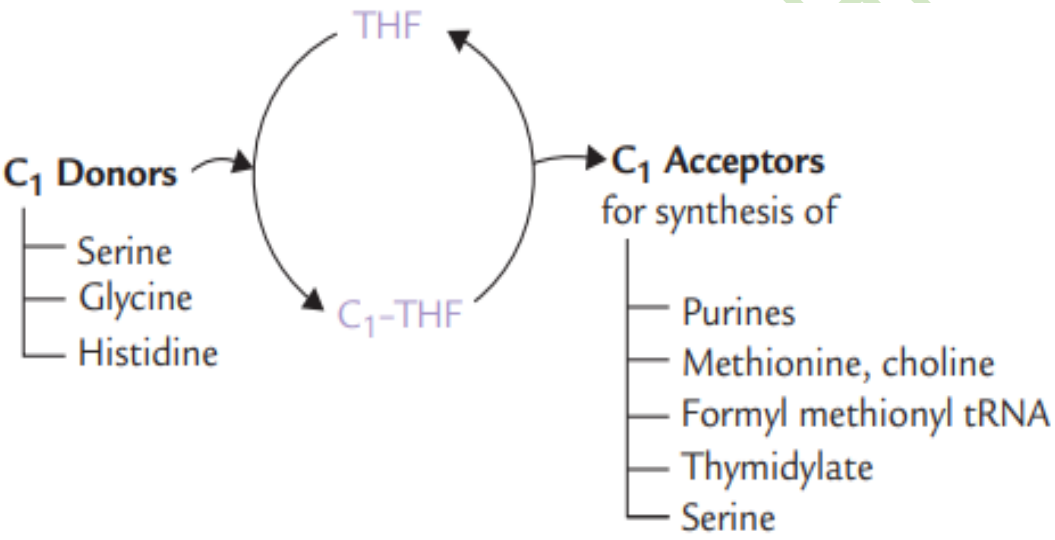
Folic acid is absorbed in the jejunum. In intestinal lumen, all but one of the glutamyl residues are removed by hydrolysis prior to absorption. This is achieved by two -glutamyl hydrolases. Following absorption, folic acid is transported in blood by two B-globulins.

The major circulating form is methyltetrahydrofolate and the normal concentration range is 5–15 ng/mL. Once it arrives in the liver, the methyl derivatives are taken up

by hepatocytes where various coenzyme forms are produced. Folic acid is not stored in tissues.

Coenzyme Functions

Tetrahydrofolate serves as carrier of one-carbon units at different oxidation levels (Fig. 18.9). These C1 units are bound to one or both of the two nitrogens in the molecule, N5 and N10 (Fig. 18.10). THF receives the C1 units from various donor molecules during catabolic reactions and can transfers them to specific acceptors for the synthesis of various compounds. Role of THF is thus vital in those reactions that require either addition or removal of C1 units of various oxidation states.



1- Donors of C1 units:

THF acquires C1 units from various donors during the following reactions:

Once bound to THF, the C1 unit can be oxidized or reduced enzymatically. Thus, various oxidation states are interconvertible (Fig. 18.11)

(a) Serine to glycine conversion by serine hydroxymethyltranferas.

$$\text{THF} + \text{Serine} \rightleftharpoons N^5\ N^{10}\ \text{Methylene THF} + \text{Glycine} + H_2O$$

(b) Glycine breakdown by the glycine-cleavage enzyme

$$THF + glycine + NAD^+ \rightarrow N^5\,N^{10}\,Methylene\,THF + NADH + CO_2 + NH_4^+$$

(c) Histidine breakdown, where a C1 unit from FIGLU is transferred to THF (Fig. 13.13).

$$FIGLU + THF \rightarrow N^5\text{-Formimino THF} + glutamate$$

2- **Acceptors of C1 units:** Some important one-carbon addition reactions in which C1 unit is transferred to an acceptor are as here.

(a) Synthesis of purine nucleotides:

(b) Conversion of homocysteine to methionine: The methyl group required for the synthesis of methionine from homocysteine is provided by methyl THF ***(Fig. 18.7).***

(c) Synthesis of formylmethionyl-tRNA: This is required for initiation of protein synthesis in prokaryotes and in mitochondria (Chapter 22).

(d) Methylation of deoxy-uridylate to thymidylate: Methylene THF donates a carbon unit (C1 unit) for the formation of deoxy-thymidylate (dTMP) (a nucleotide unit present in DNA) from deoxy-uridylate (dUMP).

$$\text{2′-Deoxyuridylate} + \text{Methylene THF} \xrightarrow{\textit{Thymidylate synthase}} \text{2′-Deoxythymidylate} + \text{Dihydrofolate}$$

This reaction is commonly referred to as thymidylate synthase reaction. It is unique as the tetrahydrofolate changes to dihydrofolate in this reaction.

This is the only 1-C transfer reaction in which the redox state of methylene (the transferred 1-C unit) changes as the methyl group is added to dUMP.

(e) Synthesis of serine: The hydroxymethyl group required for glycine to serine conversion is provided by N5 , N10 Methylene THF.

(f) Synthesis of choline: Serine to choline conversion requires methyl group from N5 , N10 methylene THF.

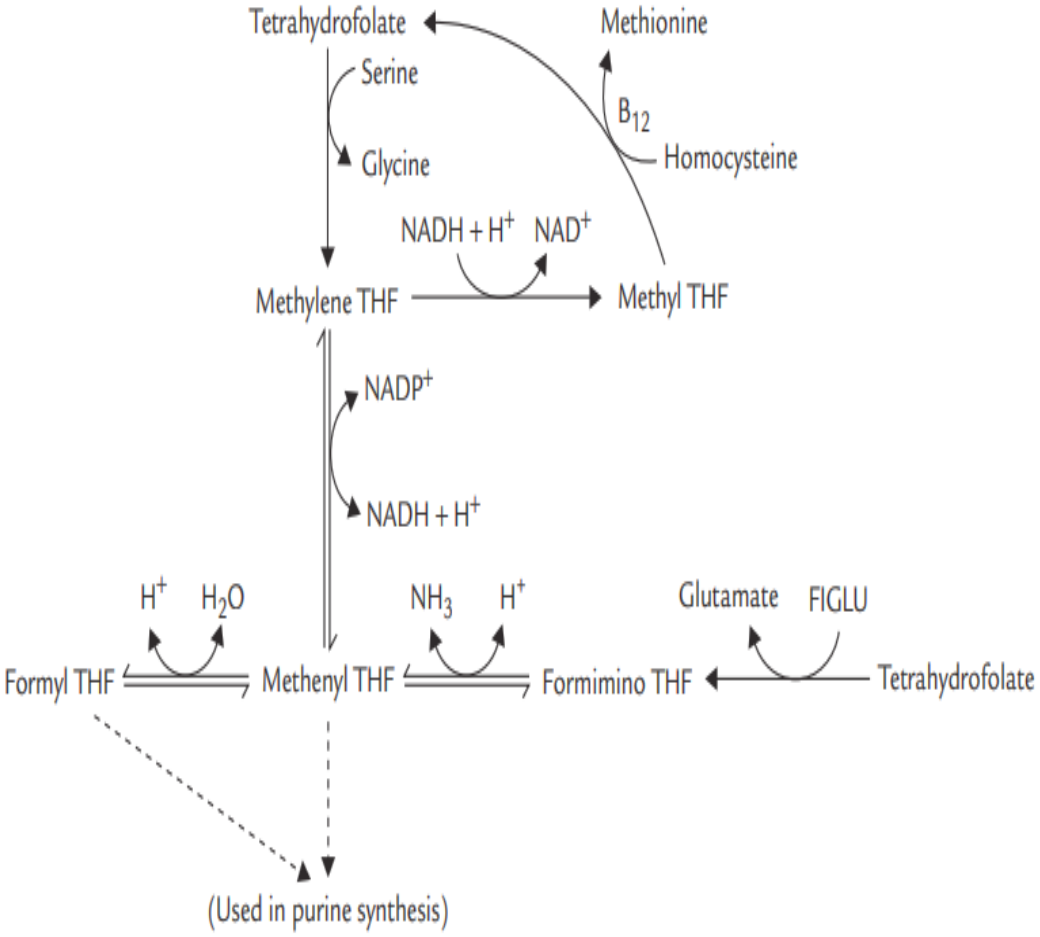


Fig. 18.11. Tetrahydrofolate as carrier of one-carbon units (FIGLU = formiminoglutamate).

NOTE: Folic acid is involved in addition of one-carbon units to several metabolic precursors. It is more versatile than biotin (in carboxylation reactions) and S-adenoxylmethionine (methylating agent) as a carrier of one-carbon units.

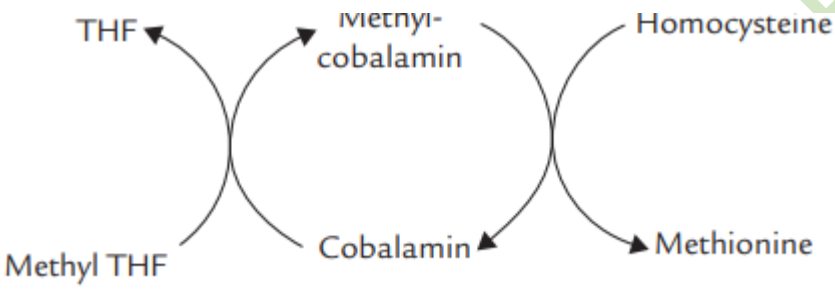
Causes and Effects of Folate Deficiency

Deficiency of this vitamin is very common in India, particularly during pregnancy when the requirement is increased. Common causes are dietary defi ciency and defective intestinal absorption. Chronic alcoholism is also a known cause.

DNA synthesis indirectly requires folic acid because of its role in the synthesis of purines and in thymidylate synthesis. Hence, folic acid is needed in DNA replication and cell division.

Folate Trap Hypothesis

Deficiency of cobalamin leads to functional folate deficiency by the following mechanism. During the metabolism of one-carbon units, a small amount of methylene tetrahydrofolate is reduced irreversibly to methyltetrahydrofolate (Fig. 18.11). Since it cannot be used for the synthesis of purines or thymine, methyl-THF has to be converted back to one of the other coenzyme forms. The only reaction of methyl-THF is the methylation of homocysteine to methionine, which regenerates free THF. This reaction requires cobalamin, and therefore, methyl THF tends to accumulate in cobalamin defi ciency.



Accumulation of methyl THF leads to depletion of the other coenzyme forms that are needed for nucleotide synthesis. Thus, folate trap hypothesis explains the anaemia of cobalamin defi ciency but it cannot account for the neurological manifestations of pernicious anaemia.

Assessment of folate status

Measurement of the serum or red blood cell concentration of folate is the method of choice, and a number of simple and reliable radio ligand-binding assays have been developed. There are a number of problems involved in radio ligand-binding assays for folate.

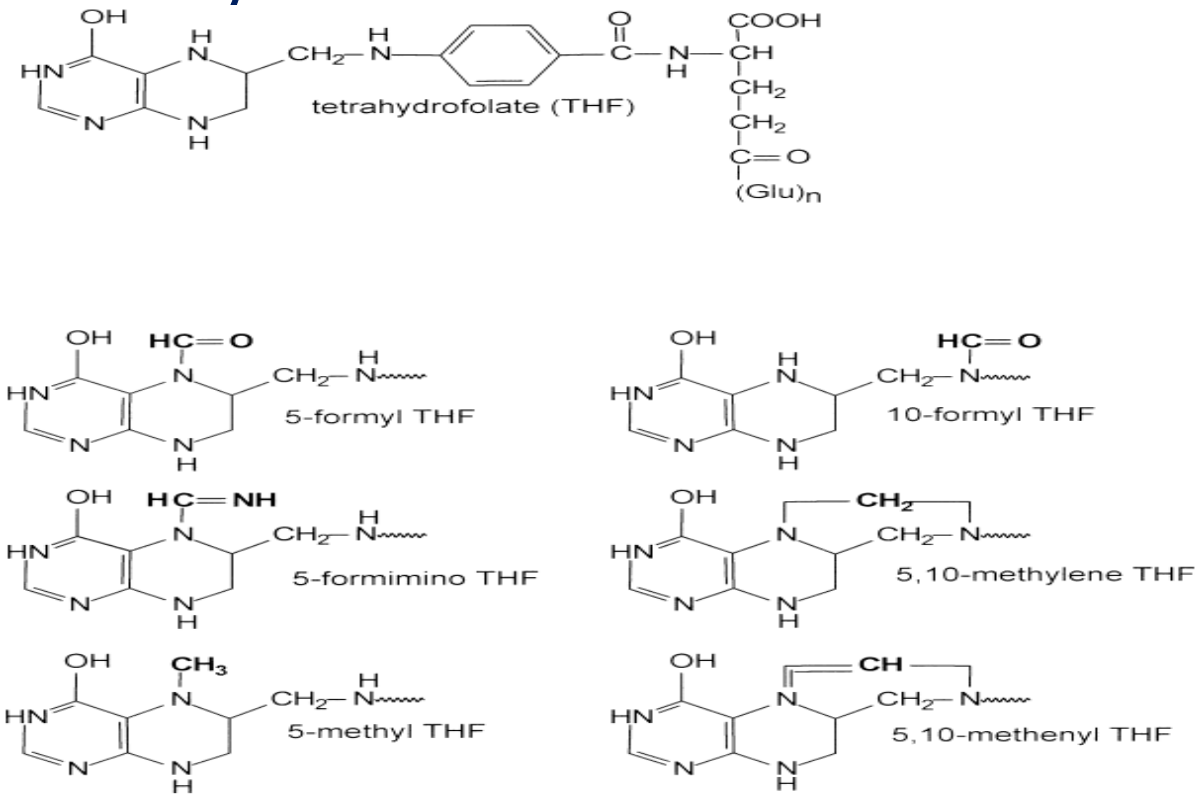


Figure 11.19 Folic acid and the various one-carbon substituted folates