NUTRITIONAL BIOCHEMISTRY

Vitamins

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Introduction

Vitamins are heterogeneous group of compounds i.e., they do not have any chemical or structural similarities. They take part in diverse biochemical reactions, thus perform many different metabolic functions in body. The commonalities which are the very essence of bringing them together as a group of food compounds are: (a) they all are *organic substances* and (b) unlike other organic components of food e.g., carbohydrates, protein and lipids, *the requirement of vitamins is very less* for our body like many inorganic elements (trace and ultra-trace elements). Thus they are micronutrients. They are *present in minute quantity in natural foodstuffs.* (c) Hardly any vitamins (except vitamin D) are synthesized in human cells. Hence, we are dependent *mostly on exogenous sources* to fulfill our vitamin requirements. However, our gut flora can synthesize some vitamins (e.g., vitamin K) and supplements our total need. (d) Deficiency of vitamin(s) leads to ill health.

Depending upon extent of deficiency, the deficiency features of many vitamins are manifested in different stages, a spectrum from sub-clinical/minor to severe deficiency state. Antagonists to vitamin, known as *anti-vitamins* are present in some foodstuff and the intake of large amount of these foods may lead to a vitamin deficiency state. An experimental vitamin deficient animal model may be produced in laboratory either by providing vitamin deficient food to animals or by giving anti-vitamins. These models are important to study the mechanism of action and effects of deficiency state in various pathological and patho-physiological states.
Based on their physical property, vitamins are classified as: (a) Fat soluble vitamins e.g., vitamins A, D, E and K and (b) Water soluble vitamins e.g., vitamin B complexes and vitamin C. The term vitamine (now termed as vitamin) which means ‘vital amine’ was coined by Funk in 1911.

FAT-SOLUBLE VITAMINS

Vitamin A

Chemistry

Vitamin A refers to the derivatives of *beta-ionone having biological activity of all-trans retinol*. Retinol, retinal and retinoic acid are the most commonly used vitamin A (Chemical structures shown in Figure 1). Three other terms used frequently with this vitamin are retinoids, carotenoids and pro-vitamin A. *Retinoids* are wide variety of natural and synthetic compounds structurally related to retinal having been used therapeutically. *Carotenoids* are compounds that *contain at least one β-ionone ring and are not hydroxylated*, hence *per se* do not show vitamin activity. Many of them are precursors of vitamin A and are called *pro-vitamin A*. Among them, *β-carotene* (Figure 1 shows the chemical structure) is the most abundant in natural food and has two β-ionone rings. It has alternate double bond containing aliphatic structure joining two β-ionone rings.

Vitamin A is fat-soluble. The tetraene side chain is composed of two isoprene units. Hence, vitamin A belongs to polyprenoid (isoprenoid/terpin) group of lipids. This side chain may have a *cis* or *trans* configuration at C-9, 11 and 13 positions. *Trans* form is straight but *cis* forms have bent configuration. In retinol, C-15 contains a primary alcohol group which is esterified with

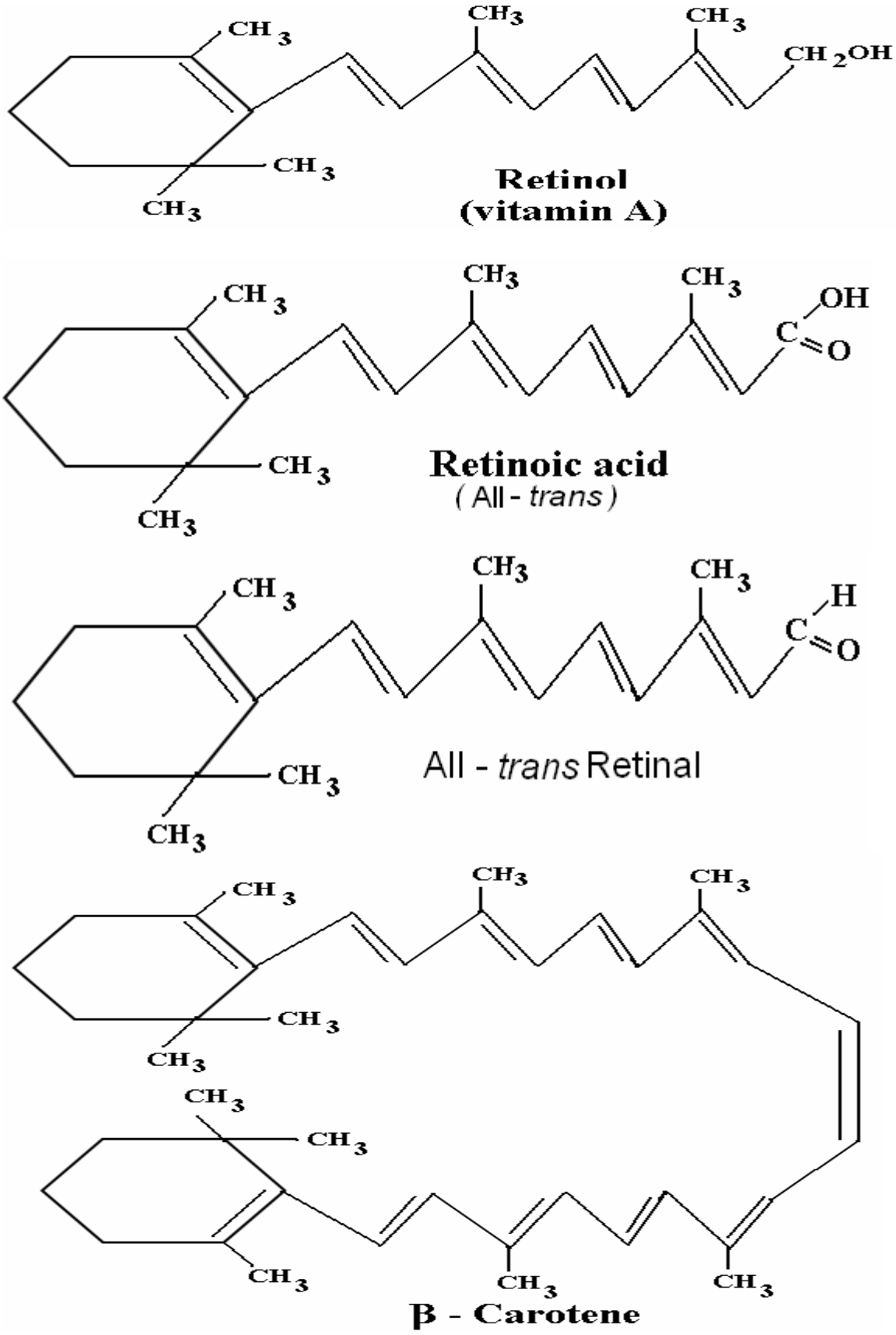


Figure 1: Structure of retinol, retinal, retinoic acid and β-carotene

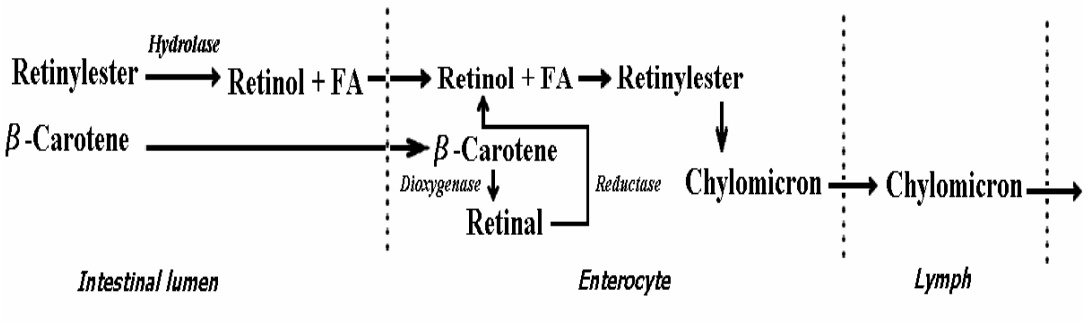
long-chain fatty acid e.g., palmitic or stearic acid in nature. Esterification is done with short-chain fatty acid (e.g., acetate) for making pharmaceutical preparations.

Sources

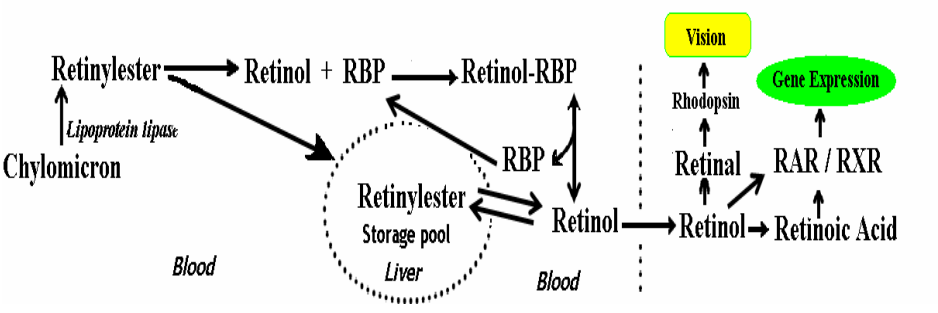
Plants synthesize carotenoids. The animal kingdom acquires it from plants and produces vitamin A from these precursors. Thus vitamin A enters into food chain and the amount becomes very high in animals (e.g., polar bear, halibut, sharks, seal, cod) belonging to terminal end of the food chain of vitamin A. So for human being, both animal and plant foods can serve as source of vitamin A. As vitamin A is stored in liver, the liver of animals are very good source of it. Other rich sources from animal foods are milk (cow: ~38 Retinol Equivalent (RE)/100gm) and dairy products (butter: ~825 RE/100gm; cheese: ~350 RE/100gm), kidney, heart, egg (~140 RE/100gm) and many fishes (~40 RE/gm). Rich plant sources are carrot (~1167 RE/100gm), tomato (~84 RE/100gm), green-leafy vegetables (spinach: ~607 RE/100gm) and fruits like mango (~313 RE/100gm), papaya (~118 RE/100gm), oranges (~25 RE/100g). Cereals particularly polished and milled ones are poor source of it.

Metabolism

The metabolism of vitamin is depicted schematically in Figure 2.



(a)



(b)

Figure 2: Metabolism of vitamin A (a⟶ b)

Digestion and absorption

The most abundant dietary forms of vitamin A are retinyl ester and β-carotene. Retinyl ester is hydrolysed by a pancreatic and an intestinal retiny ester hydrolase to retinol and fatty acid. Retinol being emulsified with bile salts and other lipids is absorbed. β-Carotene is taken up by enterocytes and then cleaved by a dioxygenase to retinal which is converted to retinol by a reductase enzyme. Retinol is re-esterified with fatty acid in enterocytes and gets incorporated in chylomicrons which is secreted to lymphatic channels. The activity of dioxygenase in intestine is low. Hence, the conversion of β-carotene to retinol in intestine is limited and a substantial amount of ingested β-carotene *per se* appears in circulation without being digested.

Transport and storage

Being acted upon by lipoprotein lipase, chylomicron forms chylomicron remnant. This remnant along with vitamin A is taken up by liver where it is stored in the form of retinyl ester or binds to 'retinol binding protein (RBP)' and released into the circulation. From there, it is taken up by target cells where it performs its various functions.

Excretion

Along with retinol binding protein, some amount of vitamin A may be lost. Retinoic acid is acted upon by cytochrome P450, the side chain is shortened, then glucoronidated and excreted.

Functions

β-Carotene

Besides being a pro-vitamin, β-carotene has become a molecule of interest because of its anti-oxidant property, been evaluated for its anti-cancer activity and is a part of many antioxidant pharmaceutical preparations. As it reacts with a free radical, it can form a resonance stabilized carbon centered radical at its aliphatic part with alternate double bonds. Because of this, it acts as an anti-oxidant. β-Carotene is a weaker antioxidant but more lipophilic than α-tocopherol. Hence, β-carotene is presumed to scavenge radicals within lipophilic compartment more effectively than α-tocopherol. A co-operative interaction between vitamin E and β-carotene in this regard is possible.

Retinal

Retinal is responsible for vision. Retinal is a component of rhodopsin, present in rod cells which are responsible for dim light vision and other visual pigments of cones are responsible for bright light and color vision.

Rhodopsin contains protein opsin that forms a protonated schiff base with 11-*cis*-retinal. As light falls on rhodopsin, the energy of light isomerizes 11-*cis*-retinal to all-*trans*- retinal. This isomerization leads to straightening of side chain of retinal pulling the part where retinal binds to opsin to 10 anstrom apart from it original position. This conformational change occurs in rhodopsin that leads to the production of metarhodopsin II through some very unstable intermediates (Figure 3). Metarhodopsin II activates a G-protein known as transducin that activates a phosphodiasterase enzyme. This phosphodiasterase uses cGMP as substrate and converts it to 5'GMP. Thus it brings down the concentration of cGMP in rod cell. High levels of cGMP keep the Na^+ channels on rod cell membrane in open state to allow Na^+ to enter into the cells maintaining a depolarized state. As cGMP levels fall due to activation of phosphodiasterase

(following activation of rhodopsin to metarhodopsin II by light rays), Na^+ channels close leading to hyperpolarization of rod cell membrane. In hyperpolarized state, rod cells release neurotransmitter that finally leads to activation of optic nerve.

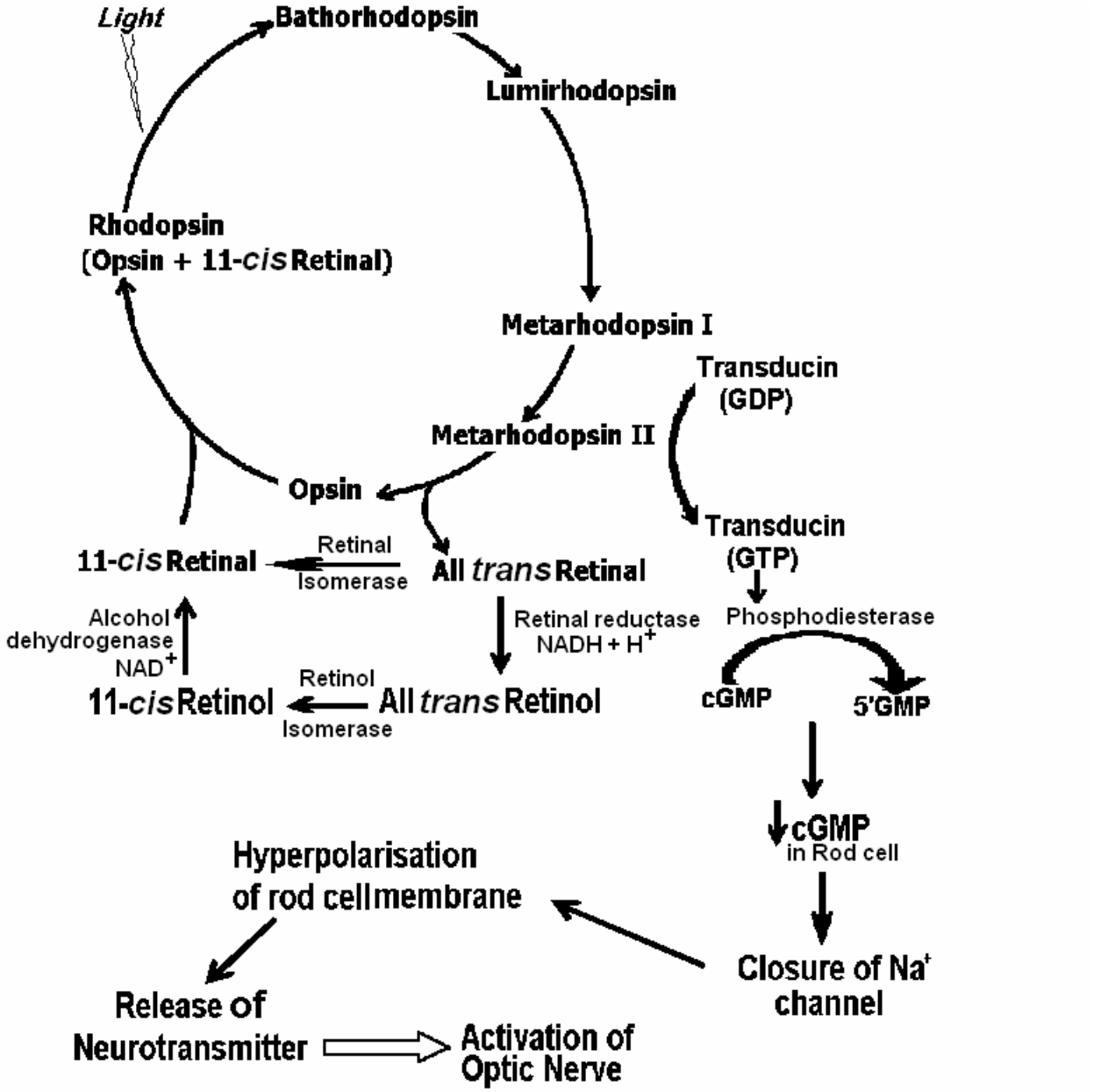


Figure 3: Visual (Wald's) cycle with intermediates of rhodopsins and hyperpolarization of rod cells.

Metarhodopsin II is also very unstable and breaks into opsin and all-*trans* retinal. All-*trans* retinal is converted to 11-*cis*-retinal by an isomerase enzyme. Then 11-*cis* retinal combines with opsin to regenerate rhodpsin. The breaking of rhodopsin on activation of light and re-synthesis of

rhodopsin to replenish it are cyclical events, known as Wald's or visual cycle. 11-*cis* retinal may be synthesized by another pathway through retinol (Figure 3). Rhodopsin is coloured pigment. As light falls on it, it breaks leading to bleaching of colour of retina.

Retinol
It helps in maintenance of epithelium and is of prime importance in reproduction and healing of lesions in respiratory and gastrointestinal system. It contributes to some stage of growth and differentiation along with retinoic acid.

Retinoic acid
Retinoic acid acts like a steroid hormone. It binds to nuclear retinoic acid receptors (RAR or RXR). Retinoic acid–receptor complex then binds to DNA and modulates expression of some genes which plays crucial role in growth and differentiation of tissues, limbs and formation of lung surfactant. It has anticancer activity and therapeutically used in some cancers. It plays a role in glycosylation of protein, thereby in the formation of glycoproteins. The glycan(carbohydrate) content in glycoproteins is decreased in vitamin A deficiency state.

Requirement
Different units are used to depict the requirement of vitamin A. Retinol equivalent (RE) is the most commonly used for expressing concentration in food or recommended dietary allowance. Pharmaceutical preparations use mostly international unit (I.U). For expressing concentration in tissues SI unit (μmol/L) are often used. The conversions of different units are shown bellow:

1 RE = 1 μg of retinol = 3.33 I.U. of vitamin A = 1.83 μg of retinol palmitate = 6μg of beta-carotene.
1 μmol retinol/L =286.46μg of retinol/L ≈ 524μg /L of retinyl palmitate.

Recommended dietary allowances are shown bellow:

Category (age in yrs)	**RDA in RE**
Infants (0-1)	375
Children (1-10)	400-700
Male adults	1000
Female adults	800
Pregnant	800
Lactating (1^{st} 6months)	+500
Lactating (2^{nd} 6months)	+400

Deficiency disorders
Protein-energy malnutrition is often associated with Vitamin A deficiency. Measles, recurrent diarrhea and respiratory infections and other infections further worsen the deficiency state or are the high risk group for development of vitamin A deficiency. Eyes are the most frequently affected organ in vitamin A deficiency, although extra-ocular parts are also involved. The salient features of *ocular manifestations* at different stages (WHO staging) of deficiency are as below:

Stage	Characteristic feature(s)
X_N	Night blindness (nictalopia, the earliest feature)
X_{1a}	Night blindness and conjunctival xerosis (dryness and corogation)
X_{1b}	Bitot's spot (triangular white spot on conjunctiva on either side of cornea)
X_2	Corneal xerosis/dryness
X_{3a}	Corneal Ulcer/ Keratomalacia (liquefaction of cornea) involving less than $1/3^{rd}$ of cornea.
X_{3b}	Corneal Ulcer/ Keratomalacia (involving more than $1/3^{rd}$ of cornea)
X_S	Corneal scar
X_F	Xerophthalmic fundus

Extra-ocular features
Anorexia, growth retardation, hyperkeratosis, infections etc are some extra-ocular manifestations of vitamin A deficiency.

Management
Last two stages are medical emergency, as total or partial blindness is the consequences. Besides care of both eyes, deficiency is usually treated with oral Vitamin A 200,000 I.U. for two days.

Prevention
To prevent such consequences, as a part of blindness control program in India, oral vitamin A (100,000 I.U.) in oil is recommended to infants at six months of age and then 200,000 I.U. every six months till six years of age.

Toxicity or Hypervitaminosis
Vitamin A absorption through gut is unregulated at very high doses of vitamin. Being fat soluble vitamin, it can accumulate in body if given over doses during therapy. Toxicity is seen when taken ten times above RDA that supersedes the binding capacity of RBP in plasma. Persons consuming excess liver of polar bear, seal, or other animals (food faddism) or excess intake (self medication) of cod liver oil (contains ~18000 RE/100gm) or halibut liver oil (contains ~900,000 RE/100gm) may show the features of toxicity. Patients present with features of anorexia, nausea, vomiting, pseudo-tumor cerebri (increased CSF pressure and papilloedema), headache, liver damage and sleep disorders. Membrane destabilization and inappropriate expression of genes regulated by nuclear retinoid receptors might be responsible for the toxicity. Very high dose may have teratogenic effects. Management of toxicity is symptomatic and the removal of cause.

Vitamin D
Chemistry
Among a few compounds (designated as vitamin D_1, D_2 etc), cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) are the most common and widely known vitamin D (Figure 4). Both of them are derived from steroid. Steroid nucleus in the B ring is open between C_9 and C_{10} in vitamin D. Cholecalciferol is obtained from animals and ergocalciferol from plants. They are equally potent vitamin D. They differ in the side-chain; ergocalciferol has one extra methyl group attached to C_{24} and a double bond between C_{22} and C_{23}. Vitamin D being derived from sterol is lipid soluble. They are relatively resistant to heat, oxidation, acidic and alkaline medium. Hence, they are not easily destroyed by cooking or food preservation. McCollum in 1922

discovered this vitamin by showing that cod liver oil on heating and oxygenation lost its xerophthalmia curing capacity but retained its capability to cure rickets. Thus he told that xerophthalmia and rickets curing property of cod liver oil are due to different compound. The anti-ricketic factor was named as vitamin D.

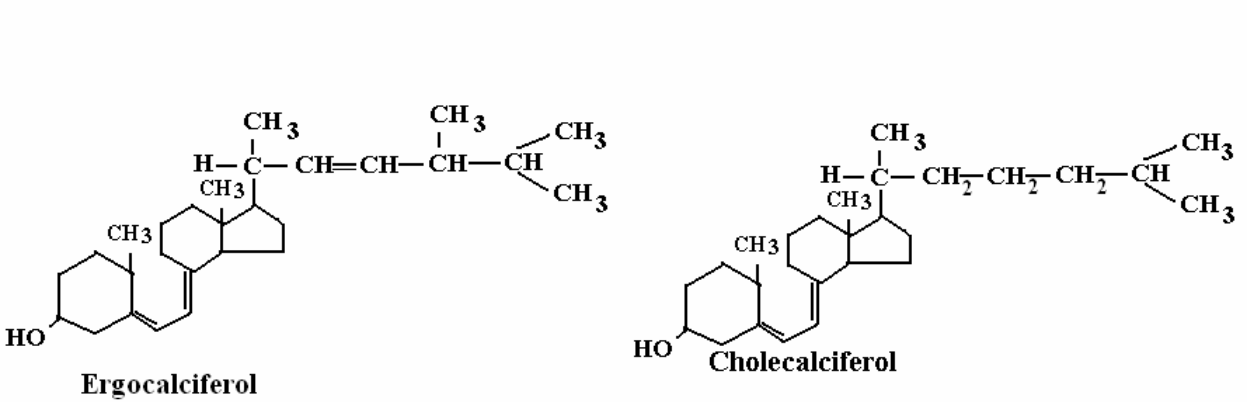


Figure 4: Structure of cholecalciferol and ergocalciferol

Sources

Vitamin D is obtained from animal food and sparingly from plant food source. Ergosterol of plants and 7-dehydrocholesterol of animals are the pro-vitamins and are converted to ergocalciferol and cholecalciferol as B ring of steroid nucleus opens up on exposure to UV rays. On exposure to sunlight, 7-dehydrocholesterol of skin is converted to vitamin D (Figure 5) and thus UV rays present in sunlight prevents vitamin D deficiency. Vitamin D content is increased in cod liver oil and other foods when they are irradiated and this method was employed as vitamin D fortification method in many places to prevent vitamin D deficiency state. Sardine, salmon, herring, liver and liver oils of fish, liver of mammals and other sea animals, milk and milk products, mutton and egg are rich sources of vitamin D. Plant foods are very poor source of vitamin D. Fruits and nuts contain no vitamin D. Vegetables and vegetable oils contain negligible amount. So, major source of vitamin D for human is animal food and milk. As sources of vitamin D are scanty in nature, fortification of food with vitamin D was a common practice in many countries where rickets was highly prevalent public health problem.

Metabolism

Absorption

Along with lipids, vitamin D is absorbed predominantly in the proximal GIT. Absorbed vitamin D enters circulation through chylomichrons.

Metabolism, transport and storage

Cholecalciferol is stored in adipose tissue and subsequently channeled to liver where it is hydroxylated at C_{25} to form 25-hydroxycholecalciferol by a Cyt P450 dependant monooxygenage system (Figure 5). Then it comes in circulation and it is the major form of vitamin D in blood. It is transported by vitamin D-binding protein. 25-Cholecalciferol is further hydroxylated to 1,25-dihydroxycholecalciferol (calcitriol), the most active form of vitamin D in

kidney which acts on its target tissues. Placenta and some other tissues also contain this hydroxylase enzyme. Placental enzyme probably helps in regulation of fetal calcium metabolism. 25-Hydroxycholecalciferol may be hydroxylated at C_{23}, C_{24} and C_{26} positions and 1,25 dihydroxycholecalciferol is also further hydoxylated at C_{24} position. These products are inactive and further metabolized to water soluble calcitrionic acid or may even be oxidized at A ring and excreted.

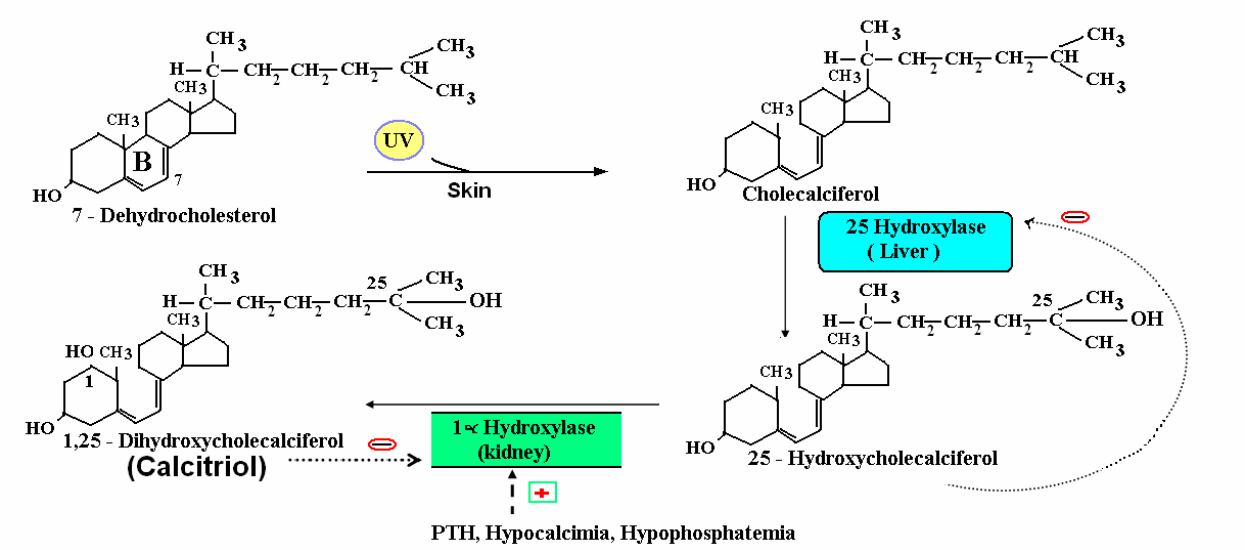


Figure 5: Synthesis of vitamin D in skin and subsequent activation

Regulation of vitamin D metabolism

Metabolism of vitamin D is regulated at many levels. The conversion of 7-dehydrocholesterol to cholecalciferol is dependant on exposure to sunlight. 25-Hydroxylase in liver is controlled by its feed-back inhibition by 25-hyroxycholecalciferol. Hydroxylation at C_1 position is more tightly regulated. Depending upon the need, 25-hydroxycholecalciferol is differentially hydroxylated at C_1 or C_{24} position. 1α- Hydroxylase in kidney is regulated by various factors. Hypocalcimia, hypophosphatemia and PTH stimulate this enzyme. Estrogen and some other hormones also help in regulation of its activity.

Functions

The functions of vitamin D are depicted in the Figure 6.

Action on intestine

Vitamin D acts as a steroid hormone. It enhances intestinal calcium absorption. Enterocytes have cytosolic receptors for calcitriol. Calcitriol-bound receptor forms heterodimer with RXR (retinoid receptor) and binds to DNA at 'vitamin D responsive element' that stimulates synthesis of mRNA of calcium-binding protein. This calcium-binding protein (calbindin) is responsible for intestinal calcium absorption. Thus, vitamin D enhances calcium absorption and helps in maintaining calcium level in blood.

Action on bones

Vitamin D acts on bones also. As such vitamin D promotes formation of osteoclast cells from macrophages, thereby helps in bone resorption and maintains serum calcium. On the other hand, osteoblasts also have vitamin D receptors and vitamin D enhances expression of osteocalcin, esteopondin and alkaline phosphatase in this cells. The mechanism how vitamin D helps in bone mineralization is not clear. Probably, it maintains calcium and phosphorus level at supersaturated state that favors mineralization of bone.

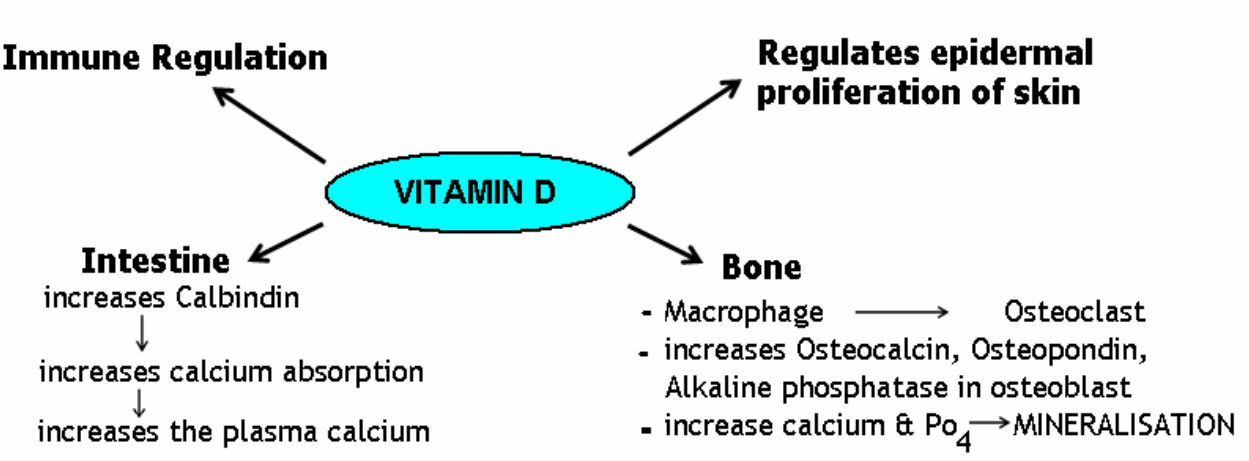


Figure 6: Functions of vitamin D

Action on other tissues

Many other tissues have vitamin D receptors. The exact functions of vitamin D in these tissues are not clearly defined. In studies with cancer cell lines, calcitriol was found to promote differentiation and to inhibit proliferation. Vitamin D is found to be effective in psoriasis, a hyperproliferative disorder of epidermal cells. Vitamin D helps in immune regulation and in pharmacologic doses, it is found to be effective in animal models for some autoimmune diseases.

Requirement

The reference dietary intake for vitamin D (cholecalciferol) is 5 μg/day (200 IU/day). Even in pregnancy and lactation, it is 5 μg/day. As age advances, the requirement increases (to check senile osteoporesis). It is 10 μg/day (400 IU/day) for persons with age between 51- 70yrs and 15 μg/day (600 IU/day) for persons with age above 71. The requirement for the subjects who are sparingly exposed to sunlight is more. The recommendation for them has not yet been defined clearly.

1μg cholecalciferiol= 40 IU of vitamin D.

Deficiency disorders

There are many factors that influence vitamin D level in blood. (a) Intestinal diseases leading to fat mal-absorption can lead to vitamin D deficiency. Site of action of vitamin D also being intestine, intestinal diseases can influence vitamin D function. (b) In hepatobiliary diseases, absorption of fat-soluble vitamins may be affected because of inadequate bile in gut. Liver is the

site for 25 hydroxylation of vitamin D that is crucial for its activation. Thus, liver diseases can lead to vitamin D deficiency state. (c) Renal disorders may also affect 1α-hydroxylation and thus affect activation of vitamin D leading to a deficiency state. Vitamin D deficiency is considered as one of the cause of renal osteodystrophy. (e) In hypoparathyroidism, calcitriol level in blood is reduced. Plasma calcitriol level is elevated in hyperparathyroidism. (f) Certain drugs like phenobarbitone, phenetoin, oral contraceptives, alcohol can influence vitamin D level. (g) Dietary deficiency may be a cause of vitamin D deficiency. (h) But level of exposure to sunlight is more important determinant. Hence, prevalence of rickets in 1930s was much more in European countries (having foggy weather) than in developing countries like India despite high prevalence of malnutrition. (i) One form of Vitamin D resistant rickets follows X-linked dominant mode of genetic transmission. (j) Age is another determinant of vitamin D requirement. The requirement of vitamin D increases as the age advances leading to a relative deficiency state.

Deficiency of Vitamin D leads to rickets in pediatric age and osteomalacia in adults. The important clinical features of rickets are widening of wrists, rarification of bones, rickety rosary, pigeon chest, bowing of legs, knocked knees and frontal bossing. Serum alkaline phosphatase is invariably increased but serum calcium level is decreased only in some cases.

Vitamin D and calcium supplementation are recommended form of treatment. In some form of resistant rickets high dose of vitamin D is given. Where activation of vitamin D is defective, calcitriol, the active form of the vitamin is administered for therapy and acts better than its dihydroxy form.

Toxicity

There is hardly any chance of development of toxicity of vitamin D by intake of natural food. Toxicity develops only when vitamin D is given at very high doses for vitamin D resistant rickets, hypoparathyroidism, renal osteodystrophy, osteoporesis etc or when food fortified with excessive vitamin D is taken regularly. Vitamin D fortification of milk and other food eradicated rickets in some country, but severe vitamin D intoxication were reported due to indiscriminate fortification of milk with excess vitamin D. In Europe, vitamin D fortification of milk is banned now for this reason.

The minimum toxic dose is 50,000 IU for adults and 1000-2000 IU for infants. The features of vitamin D intoxication are anorexia, nausea, vomiting, hypercalcimia, hypercalciurea, thirst, polyuria, muscular weakness, nephrocalcinosis, calcification of other soft tissues like heart, lungs etc. ECG changes of hypercalcimia are seen. In vitamin D intoxication, the blood level of 25-hydroxycholecalciferol is seen to be more than 15 fold of normal but 1,25-dihydroxycholecalciferol level does not rise. Hence, hypercalcimia and related features are thought to be related to calcitriol-mimicking action of 25-hydroxycalciferol at high concentrations.

Treatment is withdrawal of vitamin D and reduction of dietary calcium. In severe cases, glucocorticoids and calcitonin are thought to be useful because glucocorticoid antagonizes the action of vitamin D and calcitonin reduces blood calcium level.

Vitamin E

Chemistry

The compounds that have the activity of α-tocopherol (Figure 7) are collectively known as vitamin E. β-, γ- and δ-Tocopherols are other few vitamin E. α-Tocopherol is having tocol ring (6-hydroxychromanes) with one polyisoprenoid substitute and 3 methyl groups. So, vitamin E also belongs to isoprenoid or terpin group. β-, γ- and δ-Tocopherols differ from α-Tocopherol in number and position of methyl group substitution in tocol ring. Among them, *RRR*α-tocopherol (one stereoisomer present in *all-rec-α-tocopherol)* is the most potent and widely distributed in nature. They are fat-soluble, degradable by freezing, cooking at high temperature and food processing.

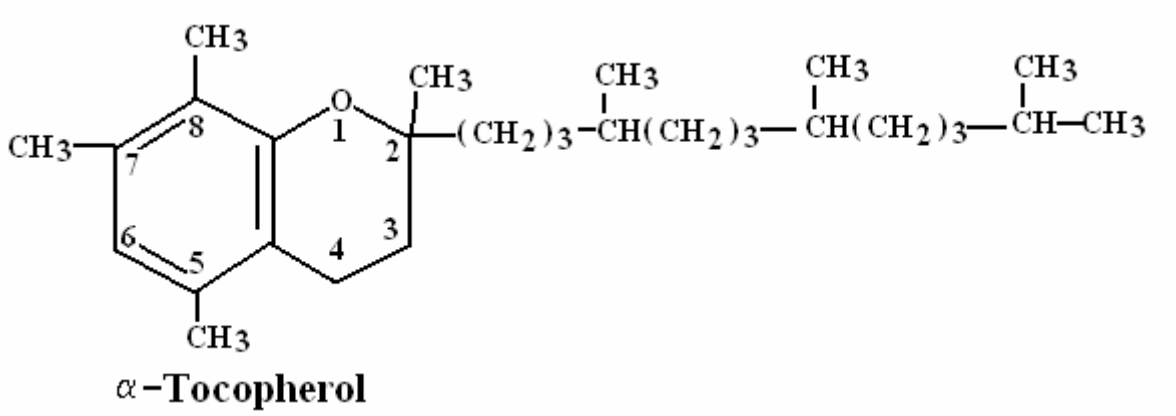


Figure 7: α-Tocopherol structure

Sources

Vitamin E is widely distributed in foodstuffs obtained from plants and animals. However, vegetable oils e.g., wheat germ oil, sunflower oil, safflower oil, corn oil, palmolin oil are very rich sources, but not olive oil. Animal fat, eggs, milk, butter and fish oil contain good amount of vitamin E. Fruits and vegetables contain less amount.

Metabolism

Digestion and absorption

The ester form of vitamin E is cleaved. Pancreatic esterase performs this function. Bile and pancreatic juice are crucial for assimilation of vitamin E into system. The exact mechanism how vitamin E enters into enterocytes is not known. No separate transporter is yet detected on enterocyte membrane. Vitamin E is absorbed in gut along with lipids. It is incorporated into chylomichron there.

Transport

From gut, it comes to circulation by being packaged into chylomicron. As chylomicron is acted upon by lipoprotein lipase, vitamin E along with lipids is delivered to the tissues. No separate protein is detected to receive vitamin E in tissues except in heart. Vitamin E in chylomicron remnant is taken by liver and comes back to circulation being incorporated in VLDL. VLDL also

delivers vitamin E to tissues when it is acted upon by lipoprotein lipase. Part of vitamin E goes to HDL from VLDL and chylomicron and from HDL, it is distributed to other lipoproteins. Probably, phospholipids transfer protein (PLTP) helps in this process. Part of the vitamin E content goes to LDL as VLDL is converted to LDL. As LDL is taken up by LDL receptors, vitamin E is also taken up along with by the tissues.

Storage

There is no storage site for vitamin E from where vitamin E is released at the time of demand. However, it is interesting to note that 90% of vitamin E is distributed in adipose tissue.

Excretion

Some vitamin E molecules cannot enter into recycling process and form inactive oxidation product (e.g., quinine derivative of vitamin E) which is glucoronated by conjugation reaction and excreted in bile. These products can be further degraded and these degraded products are excreted in urine.

Functions

Vitamin E acts as an anti-oxidant. It is a chain-breaking anti-oxidant that work in lipid phase, thereby protects membrane from lipid peroxidation by free radicals. Tocopherol reacts with peroxyl radical ($ROO^{\bullet}$) and neutralize it to ROOH. Tocopherol itself gets converted to tocophryl radical. Tocopheryl radical either interacts with water soluble reactants (e.g., vitamin C, glutathione) to regenerate tocopherol to take part in scavenging another $ROO^{\bullet}$ (Vitamin E recycle) or reacts with another tocopheryl radical to form an inert product (Figure 8). Organs rich in unsaturated fats (e.g., brain) are most vulnerable to damage due to vitamin E deficiency. In spite of many studies, if vitamin E can prevent coronary artery disease has remained controversial.

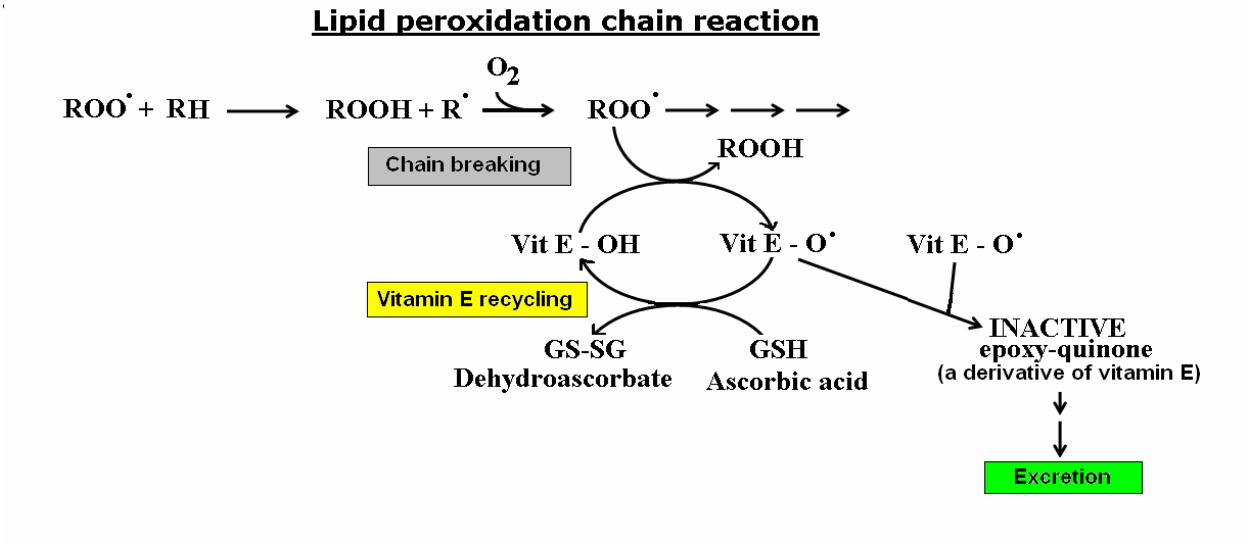


Figure 8: Vitamin E recycling

Requirement

The RDA for adult men is 10mg of α-tocopherol equivalent (α-TE) and for women, it is 8mg of α-TE. In pregnancy, the RDA is 10mg of α-TE and during lactation, RDA is 11-12 mg of α-TE. The requirement for infants is 3-4mg of α-TE and for children, it is 6-7mg of α-TE.

Deficiency disorders

The deficiency of vitamin E affects estrous cycle in animals. But such alteration in reproductive function by the deficiency of vitamin E is not observed in human. The vitamin E deficiency is rare because it is ubiquitous in nature and food. Hence, dietary deficiency is not usually a cause of vitamin E deficiency. Vitamin E deficiency occurs in fat malabsorption, total parenteral nutrition and failure of transportation of vitamin E due to genetic defect in lipoprotein synthesis or α-tocopherol transport protein. Neurological disorder (see table) is the most important clinical manifestations in vitamin E deficiency and neonates may present with anemia due to rapid RBC destruction (hemolytic anemia) and decreased hemoglobin production.

Some neurological manifestations of vitamin E deficiency
Axonal degeneration and dystrophy
Hypo- or areflexia
Delay in sensory conduction
Spinocerebellar ataxia
Neuropathy
Myopathy
Ophthalmoplegia

Toxicity

Any obvious toxicity of vitamin E is not known. However, long term and high doses are to be avoided.

Vitamin K

Chemistry

Vitamin K was discovered by Henrik Dam in 1929. Phyloquinone (Vitamin K_1), menaquinone (vitamin K_2) and menadione (vitamin K_3) have vitamin K activity (Figure 9). All of them have 2-methyl-1,4 napthaquinone ring. Menaquinone has a polyisoprene unit (number of isoprene units varies in different specis) attached at position C_3, hence it is an isoprenoid (terpin). Vitamin K_1 also has a side chain. Vitamin K_1 and K_2 are fat soluble. Menadione does not have any other group attached to it. It is the only vitamin K which is soluble in water and used therapeutically.

Sources

Phyloquinone is of plant origin and menaquinone is obtained from animals. Menadione is not found is nature but when given therapeutically is converted to menaquinone. Hence, it is considered as a pro-vitamin. Vitamin K is widely distributed in foods of plant and animal origin. Excellent plant sources are alfalfa, kale, turnip greens, spinach, cabbage, soy bean oil, etc and good animal sources are butter, eggs, liver, etc. Gut flora also produce menaquinone and supplement the need.

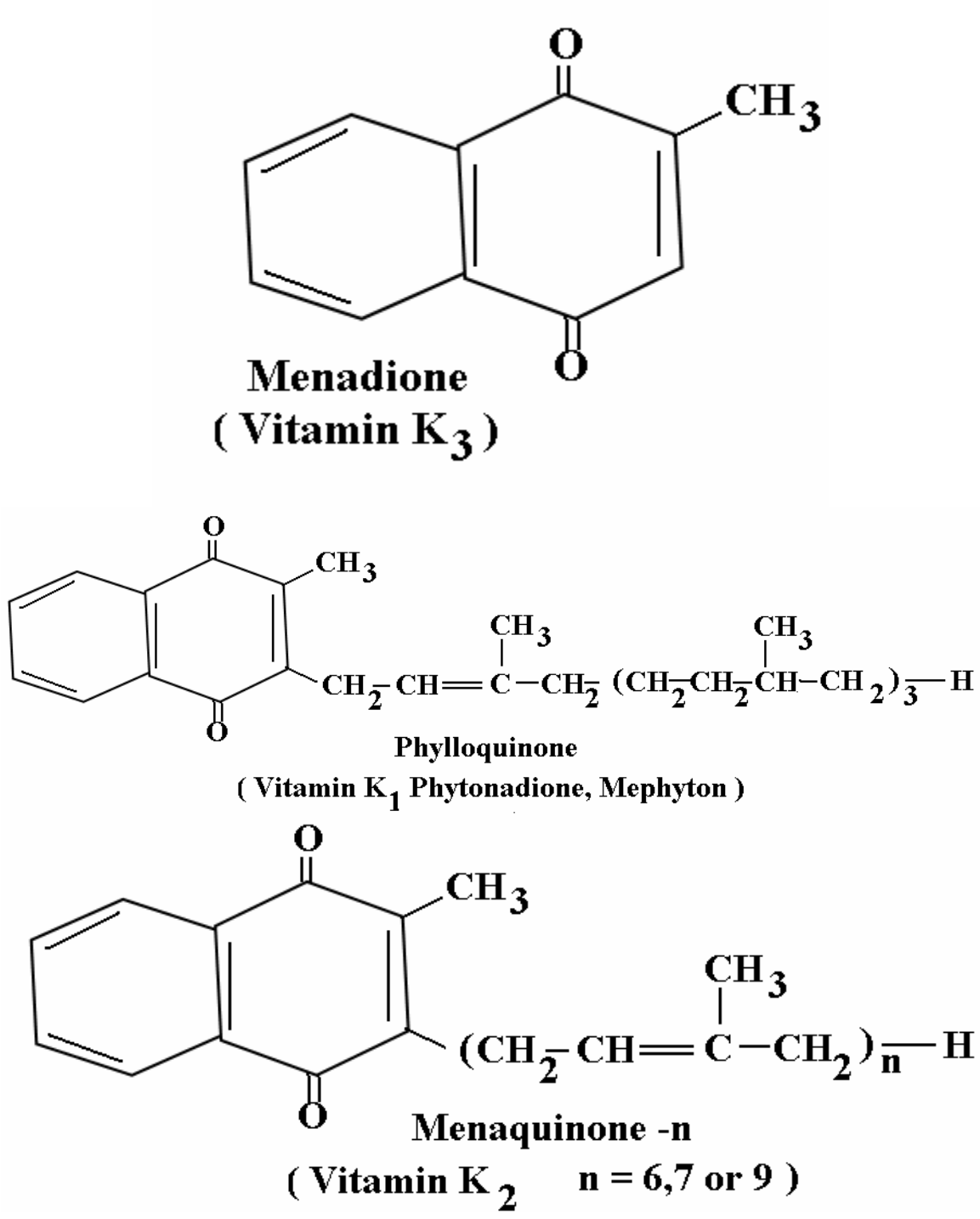


Figure 9: Structure of different forms of vitamin K

Metabolism

Vitamin K of diet is absorbed in small intestine in presence of bile and pancreatic fluid. Like vitamin A, it is also incorporated in chylomicron and stored in small amount in liver. From liver, it comes out in VLDL which is converted to LDL and from LDL, it gets distributed to other lipoproteins and tissues. It is metabolized to γ-lactone which is excreted as glucuronide.

Menadione is converted to menaquinone by addition of a side chain at C_3 by enzymatic alkylation. Thus, menadione is converted to active vitamin K.

Functions

It plays most important role in coagulation; hence it is called vitamin K (K for *Koagulation*). It causes γ-carboxylation of some glutamate residues in vitamin K dependant coagulation factors e.g., factor II (prothrombin), VII, IX and X. γ-Carboxylated glutamate is known as gla residue on proteins. It is a post-translational modification of the coagulation factors. Addition of this extra carboxylate group (a negatively charged group) makes the coagulation factor suitable for binding of Ca^{+2} which is crucial step in activation of coagulation cascade. Hence, vitamin K deficiency manifests as increased prothrombin time and bleeding as prothrombin can not be γ-carboxylated. During the process of γ-carboxylation, vitamin K itself gets converted to epoxide form and is regenerated to its active hydroxyquinone form by two steps of reduction (Figure 10). This process is known as vitamin K cycle.

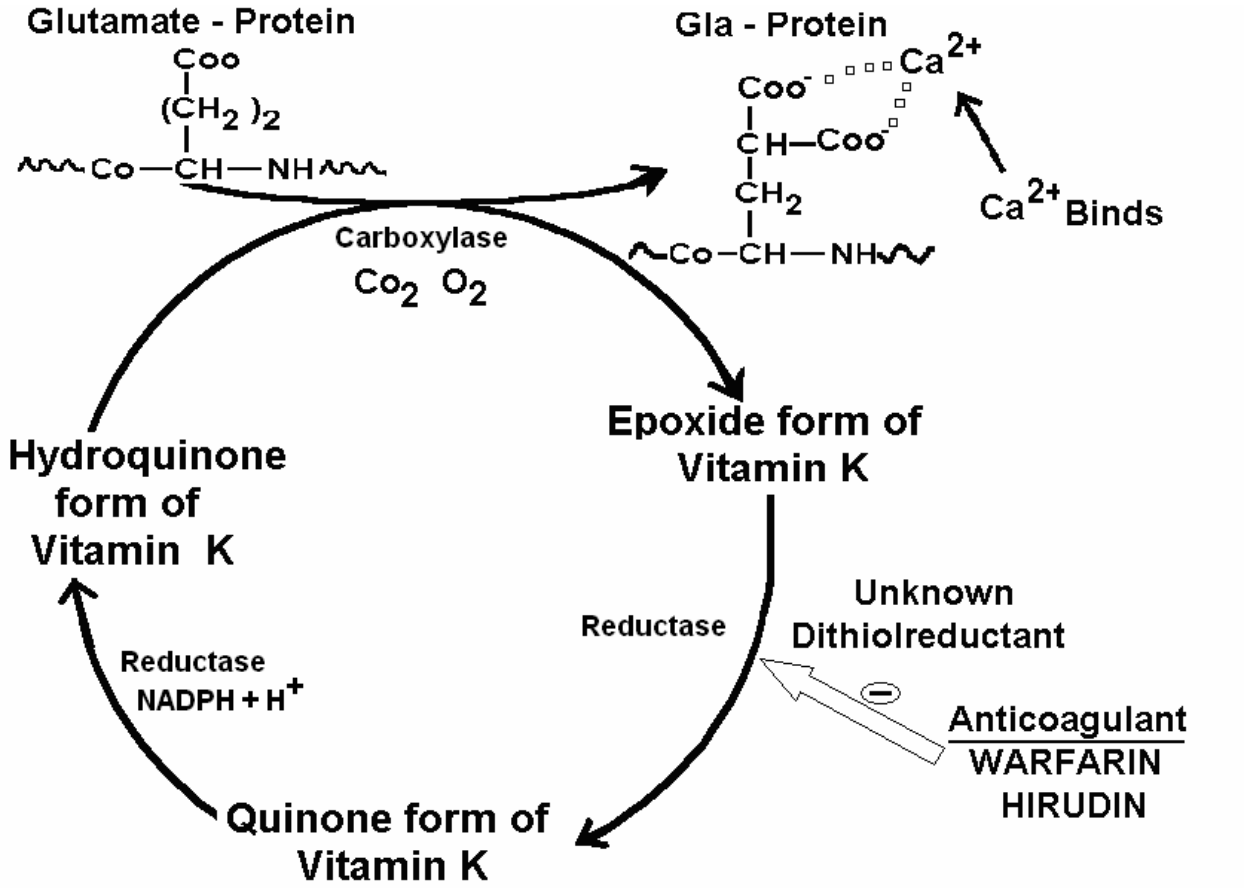


Figure 10: Vitamin K cycle

Besides coagulation factors, some other proteins also undergo γ-carboxylation by vitamin K. These proteins also belongs to "gla proteins" and are present in kidney, bones (osteocalcin or bone gla protein) and cartilages (matrix gla protein). The role of these proteins is yet not very clear.

Requirement
RDA for vitamin K varies with age, sex and body weight.

Category	**RDA in μg**
Infants	5-10
Children	15-30
Adults	45-80
Pregnancy and lactation	65

Neonates, especially premature neonates are recommended to give a dose of 1-2 mg of vitamin K orally. Despite of an inference drawn from an epidemiological study that vitamin K given to neonates can cause leukemia; it has been disproved by well-designed studies.

Deficiency disorders
Vitamin K deficiency in neonate, especially in pre-maturity often leads to hemorrhagic disease of new born (HDN). Human milk is a poor source of vitamin K (0.2 μg/100gm) and neonatal gut is mostly sterile initially. Hence, supply of vitamin K from gut flora is negligible and vitamin K level comes to its lowest level (nadir) on 2-3 days of birth. Incidence of HDN is also high on 2nd and 3rd day of birth. Prothrombin time becomes normal within few hours of vitamin K therapy for HDN. Hence, prothrombin time cannot be used for diagnosis of HDN once vitamin K therapy is started. Some other gla-proteins (not prothrombin) which take longer time (in days) to be increased in the blood following vitamin K therapy can be assayed for retrospective diagnosis of HDN within a few days. HDN manifests with bleeding at various site including brain. Intra-cranial hemorrhage usually has serious consequences.

Deficiency manifestations of vitamin K in adults are rare. But some factors (alone or in combination) can decrease vitamin K level or its activity in body e.g., anticoagulant, salicylates and antibiotic therapy, hepatobiliary diseases, malabsorption syndrome, total parenteral nutrition, protein-energy malnutrition etc. Prothrombin time or INR is frequently used to monitor anticoagulant therapy. If a single therapeutic dose of vitamin K can not improve prothrombin time in a hepatic disorder, it indicates a severe liver failure, not a vitamin K deficiency which is also possible in hepatobiliary disease due to fat malabsorption. Thus, the result of single injection of vitamin K can differentiate hemorrhagic manifestation due to inability of liver to synthesize prothrombim from vitamin K deficiency due to fat malabsorption in liver disease.

Toxicity
Phyloquinone does not have any known toxicity even at very high doses. Menadione competes with bilirubin for binding with albumin, displaces bilirubin to make it free and can thus precipitate karnicterus in neonates. It can aggravate neonatal jaundice by causing excessive hemolysis by binding to sulfhydril groups on RBC membrane.

WATER SOLUBLE VITAMINS

Vitamin C

Chemistry

Ascorbic acid and dehydroascorbic acid (Figure 11) together constitute vitamin C. Cevitamic acid, hexuronic acid, L-xyloascorbic acid and 2,3-didehydro- L-threohexano 1,4-lactone are few other names used in past to describe ascorbic acid. It is a water soluble vitamin. Cooking and heating destroy vitamin C, whereas acidification, reducing agents and metal chelators stabilize vitamin C.

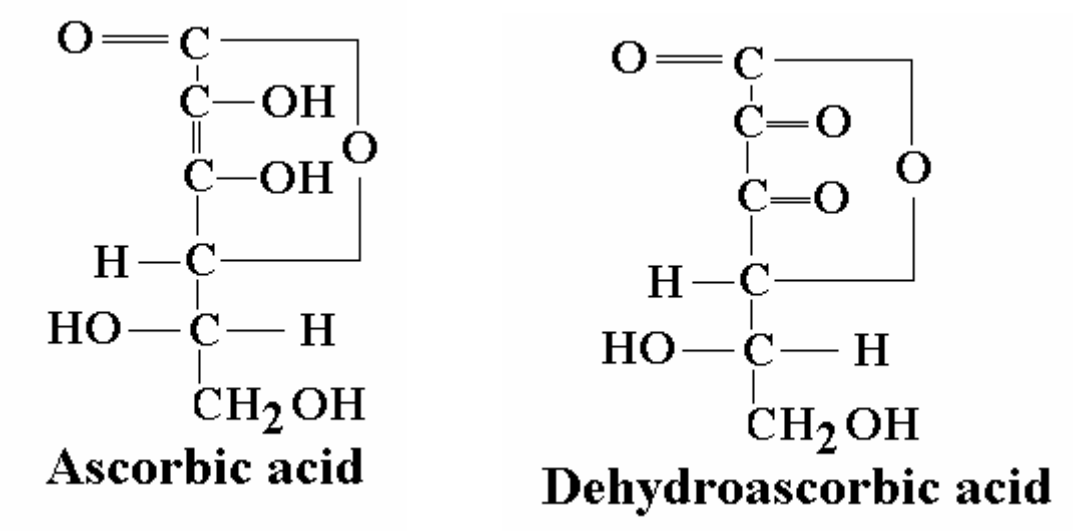


Figure11: Structure of ascorbic acid and dehydroascorbic acid

Sources

Fruits and vegetables are good source of vitamin C. The content of vitamin C in Amla (Indian gooseberry, 600mg/100gm), Guava (212mg/100gm), Cabbage (124mg/100gm), Lime (63mg/100gm), Cauliflower (56mg/100gm), Orange (30mg/100gm), etc. is very high. Amla (Indian gooseberry) and hypophae berries are some richest source of the vitamin. Milk (2mg/100gm) is a poor source of vitamin C. Potato contains 10mg/100gm of vitamin C. But as per-capita potato consumption is high, it acts as a significant source of vitamin C for human.

Some animals can synthesize vitamin C from glucose by uronic acid pathway (Figure12). But gluconolactone oxidase that is crucial for vitamin C biosynthesis is absent in human. Hence, human can not synthesize it.

Metabolism

Absorption

L-Ascorbic acid is absorbed by active transport which is saturable. Dehydroascorbic acid is absorbed at a faster rate than ascorbic acid. At very high dose, the rate of absorbtion and post-

absorptive degradation in gut is increased. Despite claims, it is not proved that bioavailability in natural form of vitamin C is more than synthetic forms.

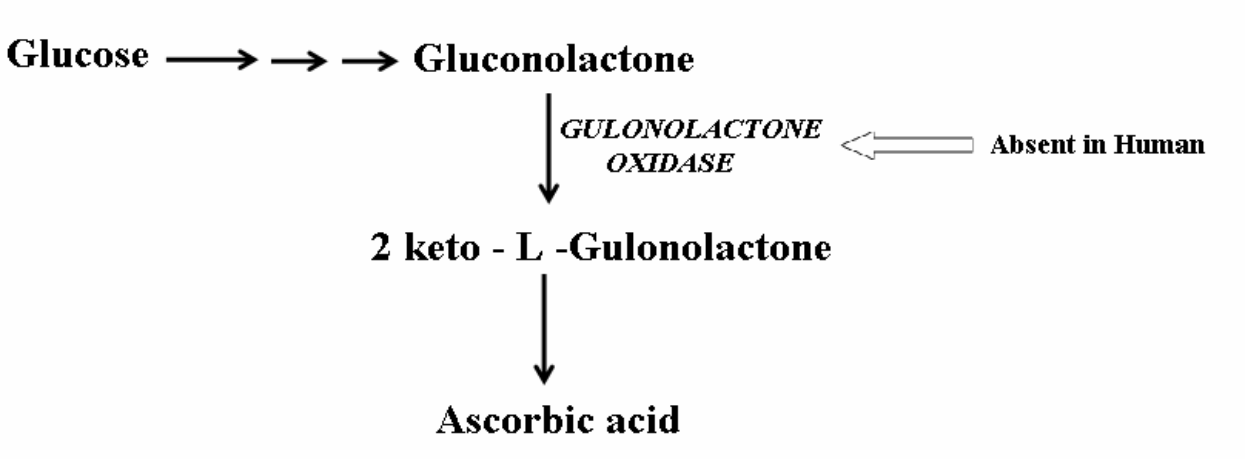


Figure 12: Uronic acid pathway for vitamin C biosynthesis

Distribution

The concentration of vitamin C varies from organ to organ in human body. The highest level of vitamin C is found in pituitary, adrenal, leukocytes, lens of eyes and brain. The concentration is lowest in plasma and saliva.

Metabolism

Ascorbic acid is oxidized to dehydroascorbic acid which may be reduced by reductant e.g., reduced glutathione. Dehydroascorbic acid, if not reduced, may be converted to diketogulonic acid which is further converted to oxalic acid, threonic acid or other oxidized products and are excreted in this form. It is not stored; hence lack of vitamin C in diet produces deficiency symptoms within a short period.

Functions

The followings are a few important functions of vitamin C:

(a) Collagen synthesis: Immature collagen contains many proline moieties. Proline is hydroxylated to hydroxyproline by the enzyme, prolyl hydroxylase. Prolyl hydroxylase needs vitamin C, Fe^{+2} and α-ketoglutarate. Similarly, hydroxylation of lysine also require vitamin C.

$$\text{Proline} \xrightarrow[\text{Vitamin C, Fe+2, } \alpha\text{-ketoglutarate}]{\text{Prolylhydroxylase}} \text{Hydroxyproline}$$

$$\text{Lysine} \xrightarrow{\text{Vitamin C}} \text{Hydroxylysine}$$

These hydroxyl groups are crucial for maturation of collagen. These hydroxyl groups form the cross-links with amino groups to form schiff's link between protein chains in the triple helical structure of collagen and bring stability and strength to it (Figure 13). Without cross-

links the collagen structure is loose and lacks tensile strength to perform it function. Vitamin C is shown to play a role in gene expression, mRNA processing and secretion of collagen.

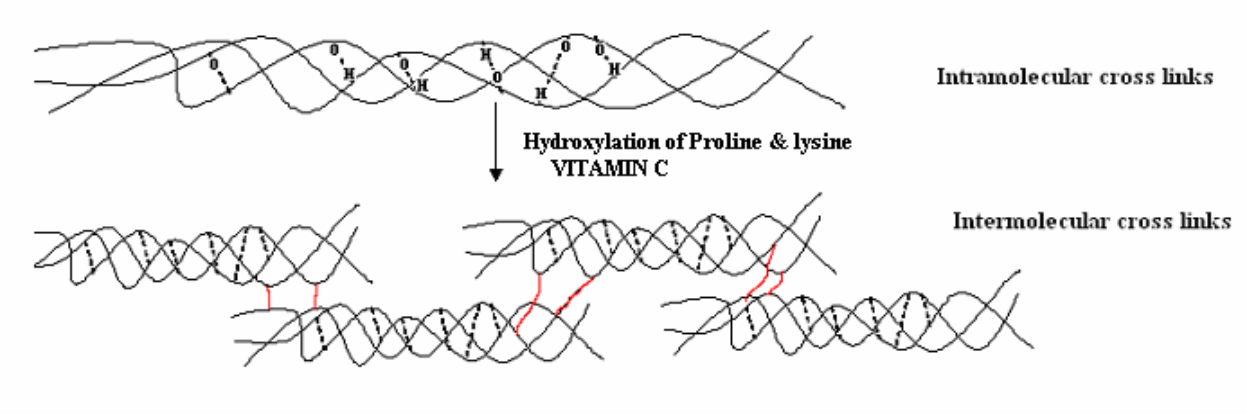
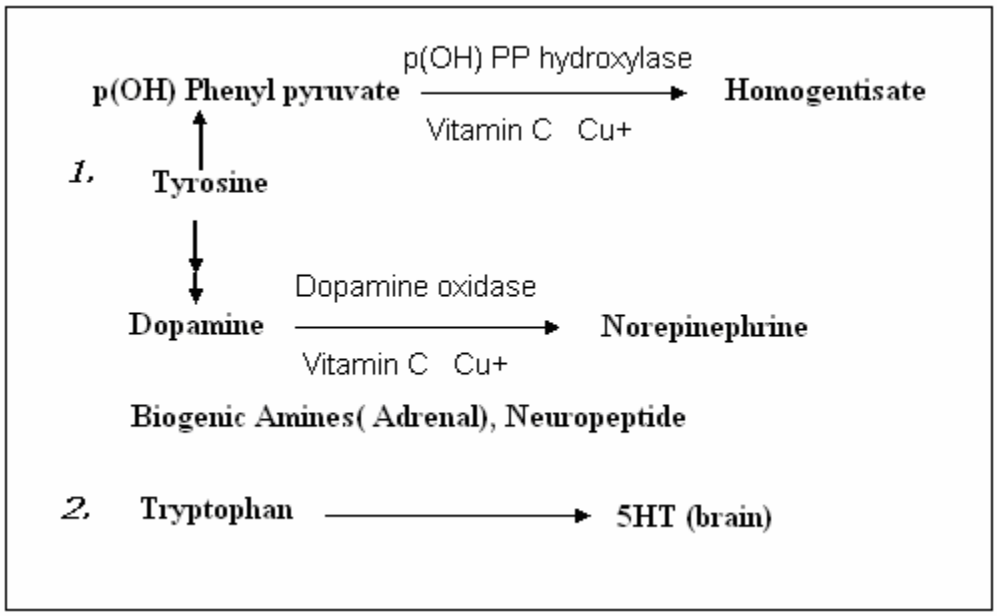


Figure 13: Collagen structure with inter-chain cross-links

(b) Tyrosine and other amino acid metabolism require vitamin C (Box 1). Parahydroxyphenylpyruvate produced from tyrosine is catabolized to homogentiste by parahydroxyphenylpyruvate hydroxylase. This hydroxylation reaction also needs vitamin C. Dopamine, a metabolic product of tyrosine is converted to norepinephrine by dopamine oxydase which needs vitamin C and Cu+. For the synthesis of 5-hydroxytryptamine from tryptophan in adrenal gland also vitamin C is essential. Synthesis of neuropeptides and biogenic amines also needs vitamin C. Vitamin C helps in carnitin biosyntheis.



Box 1: Reactions of amino acid metabolism requiring vitamin C

(c) Bile acid synthesis and steroidogenesis: The rate-limiting enzyme of bile acid biosynthesis is 7α-hydroxylase that catalyze the conversion of cholesterol to 7α-hydroxycholesterol (Figure 14). This enzyme requires vitamin C, molecular oxygen and $NADPH_2$. Steroid hormones are synthesized in adrenal and require reduction and cyt P450 mediated reactions. They require vitamin C. The concentration of vitamin C is high in adrenal gland. On stimulation by ACTH as adrenal gland synthesizes steroid hormones, vitamin C is depleted from the gland. This indicates that steroid hormone biosynthesis needs vitamin C. For Cyt P450 mediated xenobiotic metabolism also, vitamin C is needed.

(d) Anti-oxidant: Vitamin C is an anti-oxidant that prevents free radical mediated damages (Figure15). Unlike vitamin E, this works in polar medium (aqueous phage). Vitamin C can regenerate tochopherol from tochopheryl radical. On the contrary, in high dose vitamin C is pro-oxidant and enhance free radical generation by Fenton's reaction involving transition metals e.g., Fe+2 (Figure 15). Because of this property, a mix of ascorbic acid and ferrous sulfate is used often as free radical generating system for *in-vivo* and *in-vitro* experiments.

(e) Iron absorption: Vitamin C enhances iron absorption from gut by maintaining iron in Fe^{+2} state.

(f) Immune functions: Neutrophil chemotaxis, machophage and NK cell functions, serum complement levels are improved by vitamin C. The levels of histamines and prostanoids that modulate immune function are influenced by vitamin C levels.

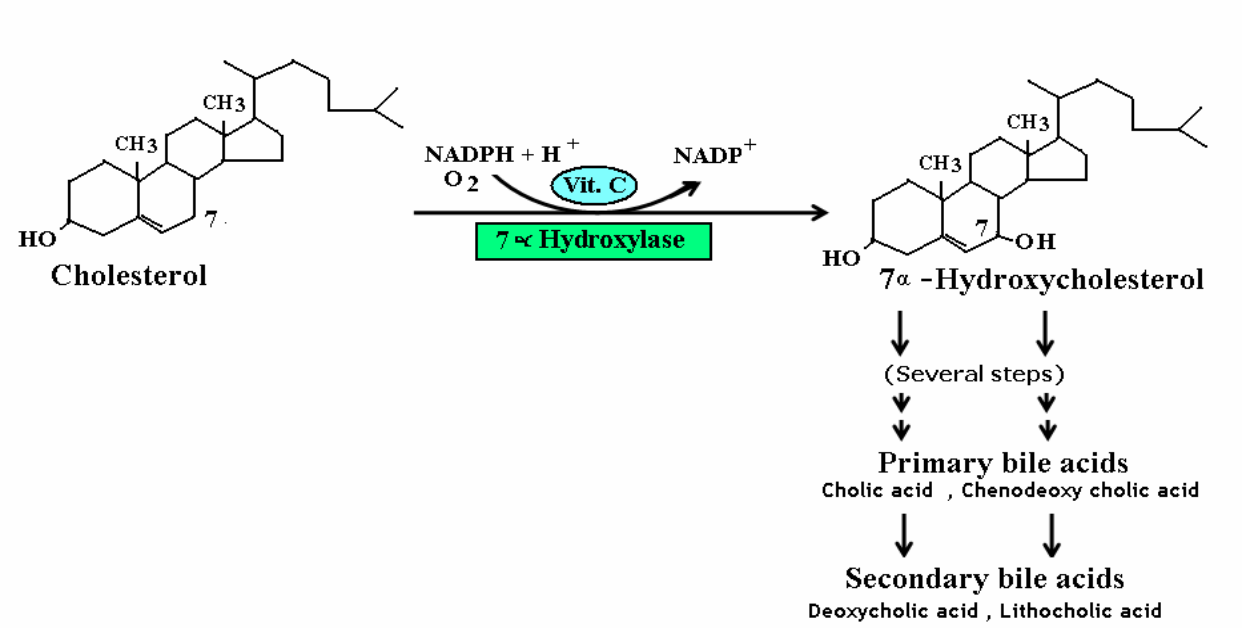


Figure 14: Biosynthesis of bile acid

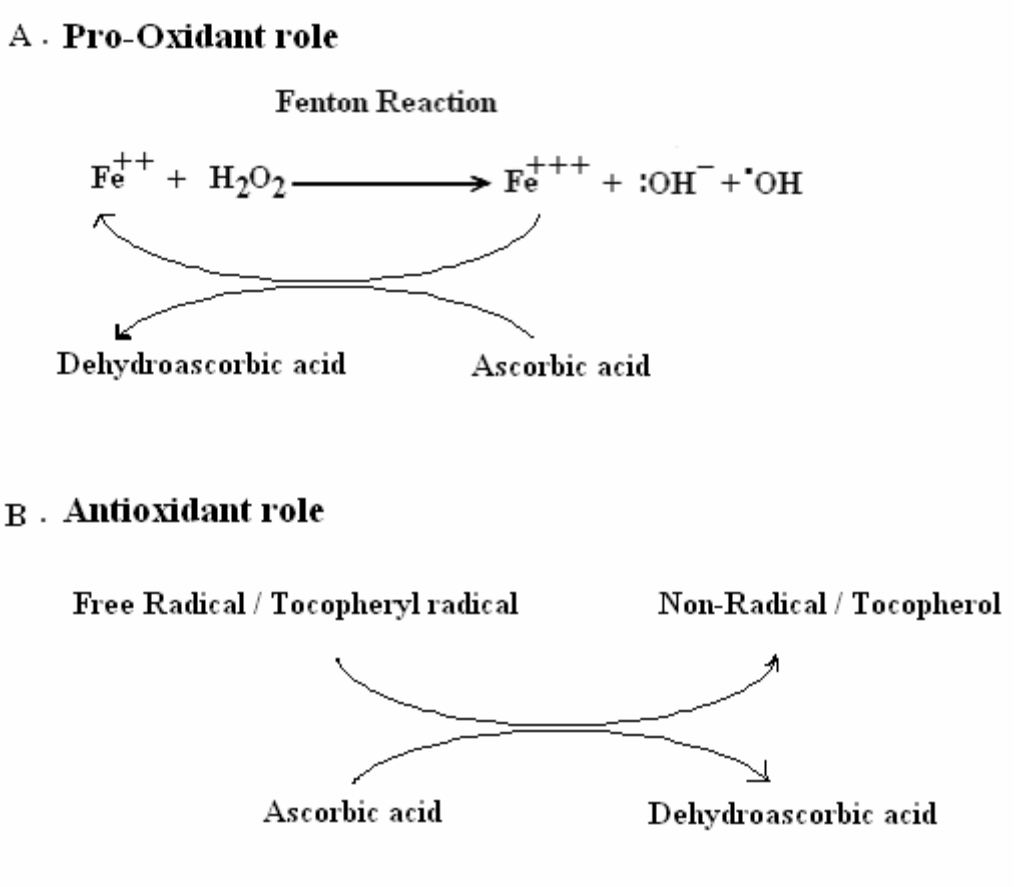


Figure 15: Antioxidant and pro-oxidant role of vitamin C

(g) Vitamin C is reported to be beneficial in upper respiratory infection, in glycemic control and in reducing glycation of haemoglobin in diabetics. The requirement of vitamin C is high among the smokers. It is used beneficially in Parkinson'd disease, osteogenesis imperfecta, Chidiac Hagasi syndrome and in prevention of cancers (particularly cancer of stomach and esophagus), stroke, ischemic heart disease and cataract. However, prospective interventional studies could not prove many of these effects seen in epidemiological studies.

Requirement

Daily requirement for adults are 40-60mg per day. In pregnancy, the requirement is the same. But in lactation it is increased to 80mg/day. For infants, the requirement is 20mg/day. Average percapita intake of vitamin C is 83-107 mg/day in USA. But percapita intake is low in India.

Deficiency disorders

The deficiency of vitamin C leads to scurvy. The earliest feature is inflamed and bleeding gum. The teeth become loose and bleeding occurs in other sites. Important features of scurvy are listed in the following table:

System affected	Features
Mesenchymal system	Inflammation and bleeding of gum, petechiae, echymosis, coiled hairs, perifollicular hemorrhage, hyperkeratosis, joint effusion, joint pain, edema, impaired wound healing and fracture (these effects are mostly due to defective collagen).
Neurological	Depression, hysteria, hypochondria, vasomotor instability (due to defect in metabolism of neurotransmitters, neuropeptide and biogenic amines)
Systemic	Weakness, fatigue, lassitude (carnitin deficiency thereby impaired fatty acid oxidation may be the cause)

Status assessment

Vitamin C status is frequently assessed from plasma and leukocyte vitamin C level. Plasma levels in different vitamin C deficiency states are indicated in following table:

Status	Plasma level of vitamin C in mg/dl
Normal range	0.4-1.5
Adequate	More than 0.4
Low	0.2-0.4
Deficient	Less than 0.2

Toxicity

Being water soluble, it is excreted very easily. There is no known hypervitaminosis state or toxicity of vitamin C. High dose of oral intake may produce abdominal pain, nausea and osmotic diarrhea. It is reported to raise uric acid, aminotransferase and lactate dehydrogenase activity in serum. Regular intake of high dose may lead to renal oxalic acid stone formation. High dose may precipitate hemolysis in G6PD deficiency and exacerbate iron overload state.

Vitamin B Complex

These are a group of compounds which mostly act as coenzyme and thereby take part in different biochemical reactions. Thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid and cobalamin belong to vitamin B complex.

Thiamin

Chemistry

The structure of thiamin is shown in Figure 16. It contains a pyrimidin and thiazolium ring. Chemically it is 3-(-4-amino-2-methylpyrimidin-5-methyl)-5-(2-hydroxyethyl)-4-methylthiazolium. Few other names used for it are vitamin B_1, vitamin F and aneurine. The coenzyme form, also known as active form, is thiamin pyrophosphate or TPP (figure 16). It is water soluble and is destroyed by heat, alkali and irradiation. Hence, food preservation and cooking can be the cause of loss of it from food.

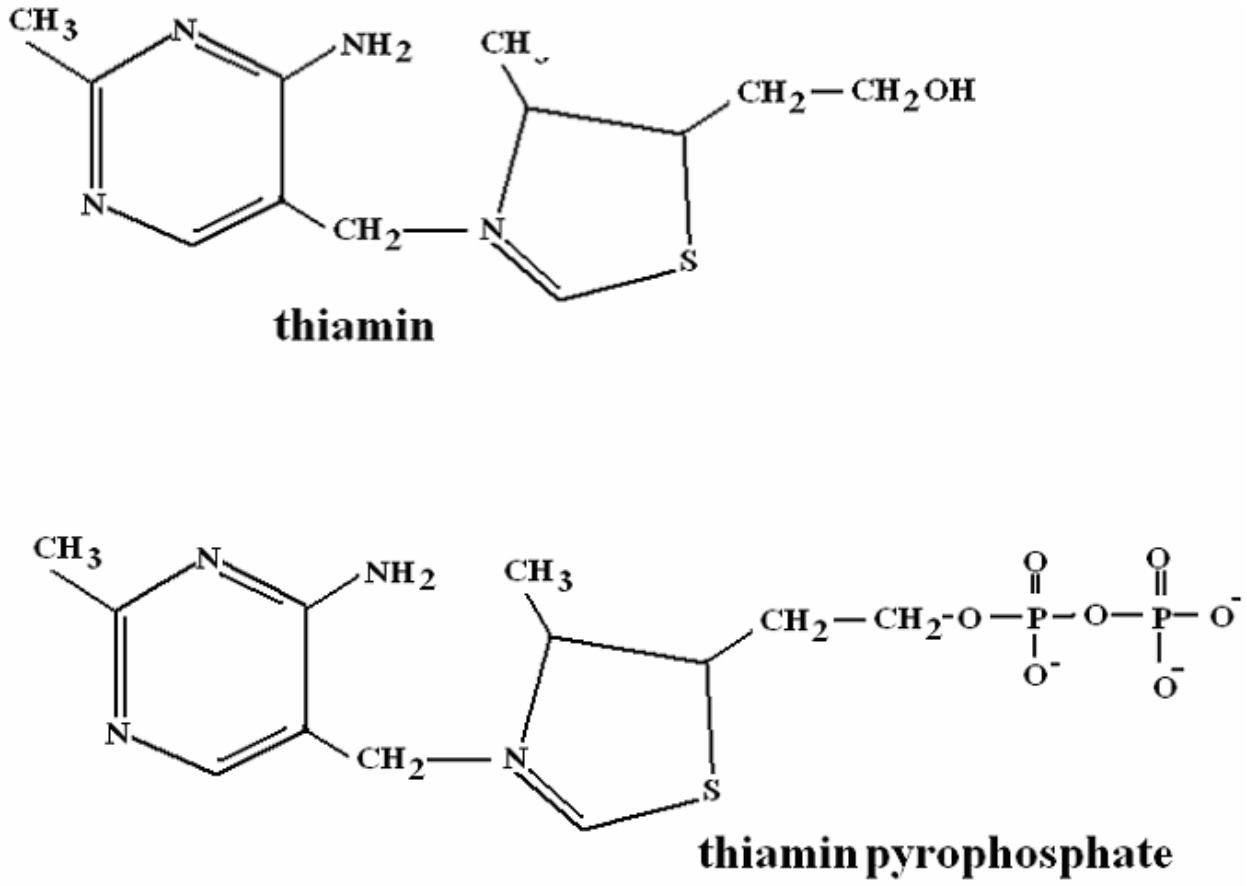


Figure 16: Structure of thiamin and its active form thiamin pyrophosphate

Sources

Both vegetarian and animal foods contain thiamin. Yeast (6-24mg/100gm), lean pork (0.72-1.04 mg/100gm) and legumes (0.53-1 mg/100gm) are rich source of thiamin. In outer part of rice seed, thiamin is present. Hence, polished milled rice (0.02-0.04mg/100gm) contains low amount of thiamin whereas rice-bran (2-4mg/100gm) is rich in it. Parboiling can introduce thiamin into the core of rice seed. Hence, chance of development of thiamin deficiency is much less among the people who eat parboiled rice in comparison to those who eat polished milled rice. Loss of thiamin is less in home-pounded rice ((0.08-0.14mg/100gm).

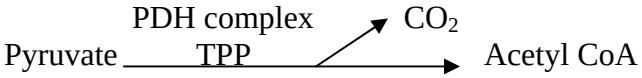
Metabolism

Thiamin is absorbed from gut predominantly by passive diffusion. But part of the absorption is by Na-dependant secondary active process. In blood, it is mostly transported in free form. However, 20-30% remains bound to protein. It enters the tissues by active transport. But in RBC, it enters by facilitated diffusion. Total body pool is approximately 30mg (80% is in thiamin pyrophosphate form). Muscles, heart, liver, kidneys and brain have high concentration of thiamin. Its half-life is 9-18days in our body. It is excreted mostly through urine and a less amount through milk and bile. The half-life being so low, it needs a continuous supply. Otherwise deficiency state sets in.

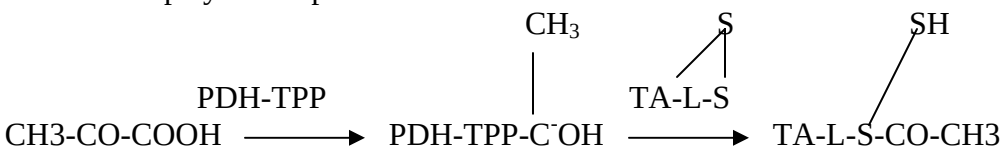
Functions

Thiamin pyrophosphate performs the following important functions:

1. Oxidative decarboxylation of ketoacids:
    a. Decarboxylation of pyruvate: Pyruvate is decarboxylated to acetyl CoA by pyruvate dehydrogenase (PDH) complex which contains thiamin pyrophosphate as one of its coenzyme.

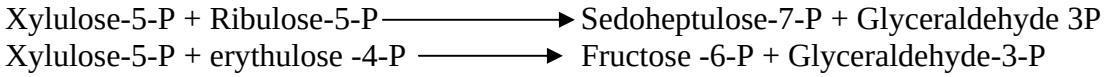


TPP plays an important role in this reaction



    b. Decdecarboxylation of α-ketoglutarate to succinyl CoA by α-ketoglutarate dehydrogenase complex also occur by a similar reaction.
    c. Decarboxylation of branched chain aminoacids: Leucine, valine and isoleucine are first transaminated to their corresponding ketoacids (α-ketocaproate, α-ketoisovalerate and α-keto-β-methylvalerate respectively) and then undergo oxidative decarboxylation to isoveleryl CoA, isobutyryl CoA and α-methyl butyryl CoA. This also requires TPP as coenzyme.

2. Transketolation reactions also need TPP as coenzyme:



3. Neurological functions: In nervous system, thiamin is found to decrease actyl CoA turnover, acetyl CoA utilization, synthesis of catecholamines, uptake of 5-HT and levels of glutamate, aspartate, glutamine and γ-aminobutyrate. It is found to influence Na+ conductance and thereby nerve conductance.

Requirement

For adults, the daily requirement is 0.5mg/1000kcal. For infants, it is 0.4mg/100kcal. During pregnancy, the extra requirement (over usual requirement of 0.5mg/100kcal) is 0.4mg/day.

Deficiency disorders

Thiamin deficiency leads to beriberi and in alcoholics, the deficiency causes Wernicke-Korsakoff syndrome. The important causes of deficiency are decreased dietary intake, loss due to food processing and cooking, alcoholism and intake of thiamin antagonists (e.g., oxythiamin, pyrithiamin, amprolium, thiaminase-I, thiaminase-II etc). Some food contains these anti-thiamin factors. Deficiency predominantly affects nervous system, heart and muscles. The beriberi has three types: infantile, dry and wet beriberi. Infants of 2-3months of age in thiamin deficiency develop infantile beriberi. Infantile beriberi has three different type of presentation: cardiac, pseudomeningitic and aphonic form. The features of different form of thiamin deficiency are shown in the table:

Form of thiamin deficiency	Features
Dry beriberi	Calf-muscle tenderness, peripheral neuropathy- loss of motor reflexes and sensory loss.
Wet beriberi	Peripheral neuropathy, edema, tachycardia, wide pulse pressure, cardiomegaly and high out-put cardiac failure.
Infantile beriberi	Cardiac form: Cardiomegaly, tachycardia, cyanosis, dyspnoea, vomiting and loud piercing cry. If it is not treated urgently, death occurs. Pseudomeningitic form: Vomiting, nystagmus, convulsion and purposeless movement of limbs. Aphonic form: Decreased tone of cry (aphonic).
Wernicke-Korsakoff syndrome	Korsakoff dementia and psychosis. Wernicke's component: Ataxia, mental dysfunction and ocular motor sign.

Few alcoholics are very prone to develop Wernicke-Korsakoffe syndrome. It is seen that they have a form of transketolase enzyme that have ten times less avidity to thiamin pyrophosphate. However, it is known how lower avidity of transketolase leads to Wernicke-korsakoff syndrome. Cardiac and muscle effects are due to defect in oxidative decarboxylation and thereby ATP (energy) metabolism. Deficiency of ATP contributes to other features also. But the exact pathogenesis of clinical features is not known.

Status assessment

Erythrocyte transketolase activity is commonly assayed to evaluate thiamin deficiency state. The activity is decreased in thiamin deficiency. On addition of thiamin pyrophosphate in the sample if the activity of transketolase is increased by 16% or more, it confirms thiamin deficiency state. This effect is called TPP effect.

Assay of thiamin in blood and CSF also indicates thiamin status in body. Blood pyruvate, lactate and α-ketoglutarate are increased in thiamin deficiency and can be used as index of thiamin deficiency.

Toxicity

Headache, irritability, tachycardia and dermatitis with pruritus are some important features of toxicity of the vitamin.

Riboflavin

Chemistry

Riboflavin contains isoalloxazine ring with D-ribityl group attached at 10^{th} position and substituent methyl groups at 7 and 8^{th} position (Figure 17). It is also called vitamin B_2. Active or coenzyme forms are flavinmononucleotide (FMN) and flavin adeninedinucleotide (FAD). This vitamin is water-soluble, heat-stable but light sensitive.

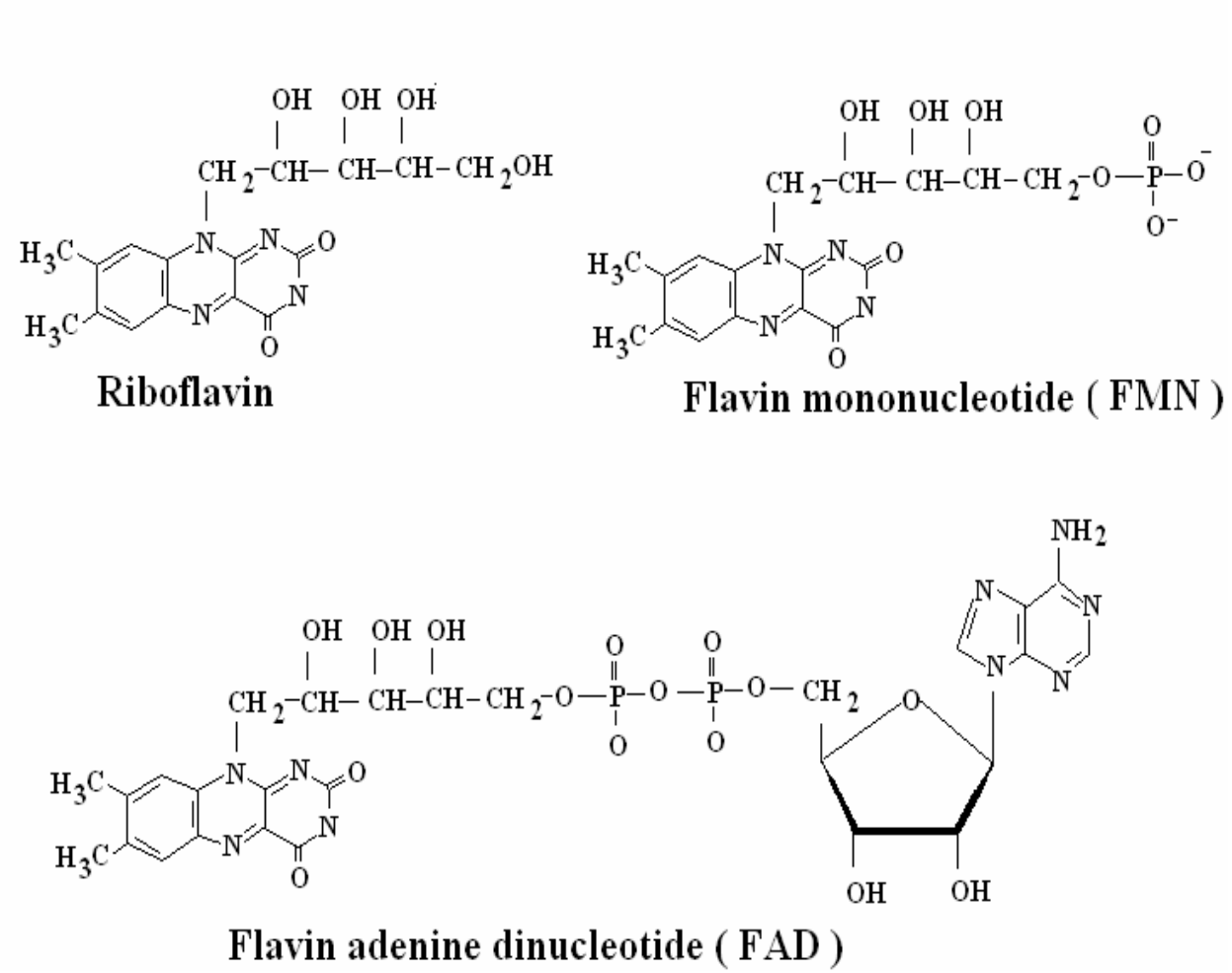


Figure 17: Structure of riboflavin, FMN and FAD

Sources

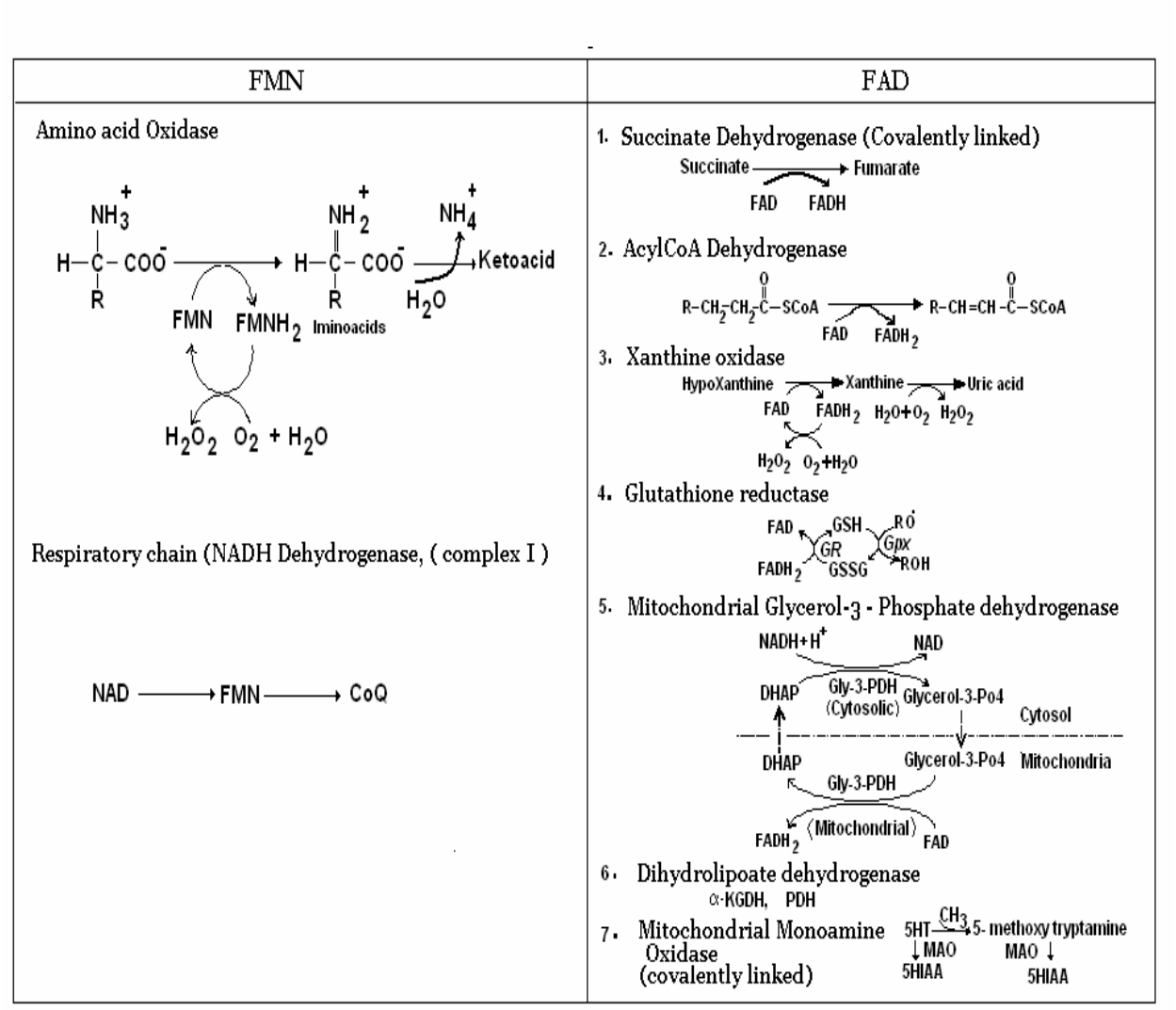
Liver, dried yeast, egg, milk etc are rich sources of riboflavin. Fish, whole cereals, legumes and greens are good sources.

Metabolism

The dietary sources mostly contain protein-bound FAD or FMN. Gastric acid make FAD and FMN free from proteins. Pyrophosphatase and phosphatase convert FAD and FMN into riboflavin which is absorbed probably by a sodium dependent active transport. Bile salt plays a role in the absorption of riboflavin. In blood, it remains mostly in free form and a part remains attached to albumin or immunoglobulin. The entry into tissues is by facilitated diffusion where it is converted to FMN by flavokinase, an ATP-dependant enzyme. A phosphatase can convert FMN into riboflavin. FAD synthetase converts FMN into FAD that requires ATP. Thyroid hormone plays a role in conversion of riboflavin to FMN and FAD. In hypothyroidism, this conversion is impaired. FAD and FMN form complex with protein to perform its function. Some FAD is covalently linked to proteins. Part of tissue riboflavin is acted upon by hydrolase and oxidase and produces oxidized product which is excreted through urine and stool.

Functions

Riboflavin mostly remains linked to protein as flavoprotein or metalloflavoprotein with Mb or Fe. The examples of FMN-dependant enzymes are amino acid oxidase and NADH dehydrogenase complex. A few FAD-dependant enzymes are succinate dehydrogenase, acyl CoA dehydrogenase, xanthine oxidase, glutathione reductase, mitochondrial glycerol 3-phosphate dehydrogenase, dihydrolipoate dehydrogenase and mitochondrial monoamine oxidase. The reactions are shown below in Box 2.



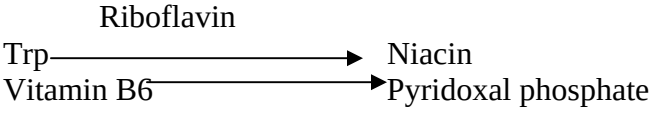
Box 2: FMN and FAD dependant reactions

Requirement

RDA of riboflavin is 0.6 mg/100kcal. In pregnancy and during lactation period, additional requirement is 0.3 and 0.26mg/day respectively. RDA is little less for infants and children.

Deficiency disorder

The important causes of riboflavin deficiency are inadequate dietary intake, poor storage and processing of food, lactose intolerance, malabsorption, hypothyroidism, oral contraceptives, phototherapy, phenobarbitone treatment and genetic defect of mitochondrial FAD dehydrogenase. Riboflavin deficiency is associated with other water-soluble vitamin deficiency because causes of deficiency are often same. Another reason is biosynthesis of some vitamin or active vitamin (e.g., niacin, pyridoxal phosphate) requires riboflavin.



The clinical features of riboflavin deficiency are listed below:

Cheilosis
Angular stomatitis
Glossitis (magenta tongue)
Seborrheic dermatitis
Anemia
Sore throat
Oedema and hyperemia of pharyngeal and oral mucosa

Status assay

RBC riboflavin concentration and RBC glutathione reductase activity are considered indices of the riboflavin status. GSH reductase activity coefficient i.e., ratio of activity with and without addition of FAD is also an index. Assay of urinary excretion of riboflavin in fasting, random and 24 hr specimen or a load return test may also be performed. The assay may be performed by fluorometric, microbiological or HPLC method.

Niacin

Chemistry

Niacin is pyridine-3-carboxylic acid. It is also known as nicotinic acid. It is present in active form as nicotinamide, also known as niacinamide. The active forms are nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) (Figure 18). It is water-soluble, heat-stable and stable in air. So there is hardly any loss of niacin during cooking.

Sources

Meat, liver and fishes are rich non-vegetarian food sources of niacin. Dried yeast is one of the richest source. Peanut, cereals, legumes and unpolished rice are rich vegetarian food source. Tryptophan can be converted to niacin by a biochemical pathway present in human. It is seen that approximately 60mg of tryptophan is converted to 1mg of niacin. This pathway needs thiamin, riboflavin, FAD, NADPH, vitamin B_6 and Fe. Limiting amino acid in corn is tryptophan. Hence, persons taking corn as staple food may develop niacin deficiency. Even other vitamin deficiency also may be associated with niacin deficiency because they have common source and conversion of tryptophan to niacin is dependant on these vitamins.

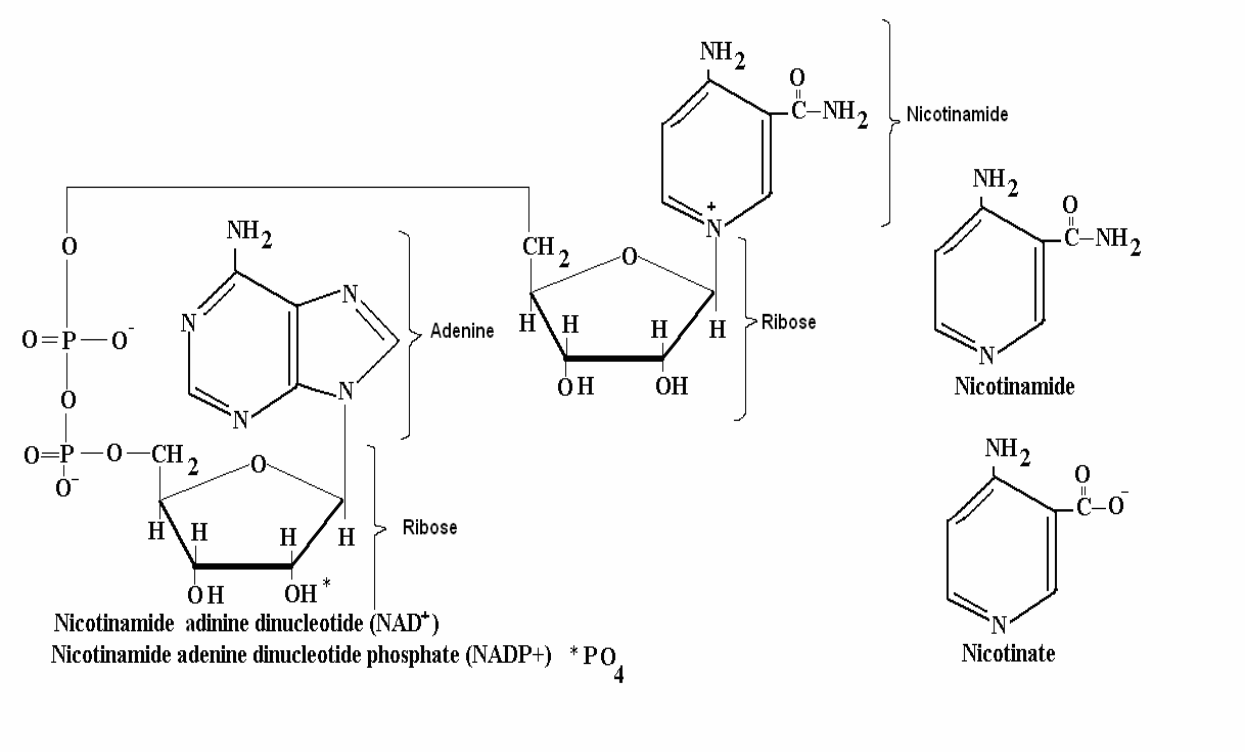


Figure 18: Niacin, Niacinamide, NAD and NADP

Metabolism

Niacin and nicotinamide are absorbed in gut by passive diffusion. In blood, 15-30% remains in protein bound form. Conversion of nicotinamide and niacin to NAD requires ATP. NAD can be converted to niacin by various reactions and thus, niacin is recycled. A part of it is catabolized in liver to its oxidation products, pyridones which are excreted in urine.

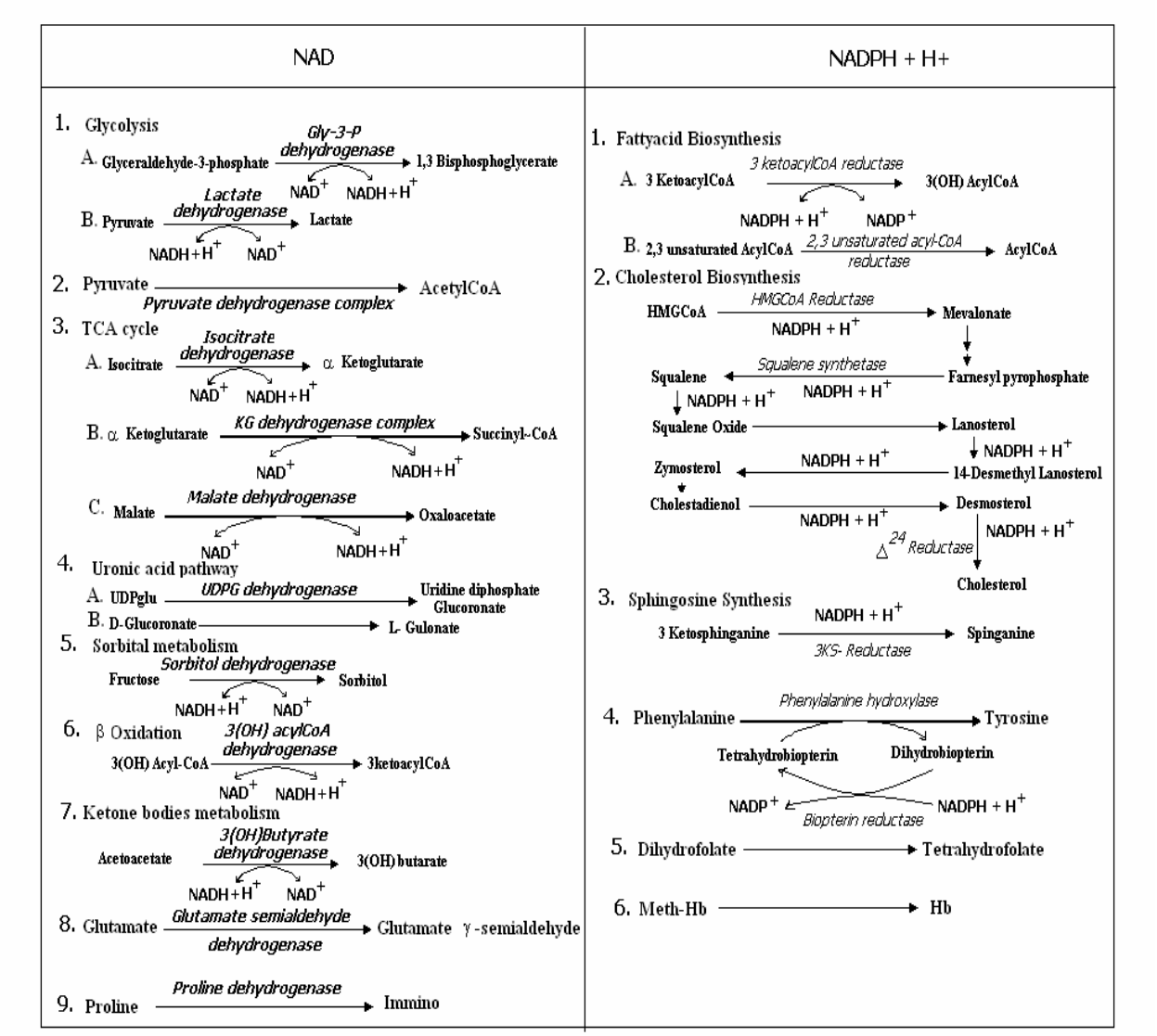
Functions

A few NAD dependant enzyme and their reactions are shown in the Box 3. The reactions that produce NADPH and a few those utilize NADPH are also shown in the Box 3. NADH produces ATP in respiratory chain and NADPH is used for siosynthesis reactions e.g., cholesterol, fatty acids etc. Niacin in therapeutic dose (1-3gm/day) acts as hypolipidemic agent. It reduces serum TAG level and increases HDL-cholesterol. It probably has effect on serum LDL-cholesterol also. Niacin therapy is found to prevent or delay type1 diabetes. It prevents cancer, probably by enhancing the DNA repair process.

Requirement

Niacin equivalent (NE) is the unit of niacin. One NE is equal to 1mg of niacin or equivalent to 60mg of tryptophan.

For adults, RDA is 11.3-13.3 NE/day. In infants and children, it is 5-9NE/day. For adolescent, the requirement is 18NE/1000Kcal. During pregnancy and lactation, the extra requirement is 2 and 5 NE/day respectively.



Box 3: NAD and NADP dependant reactions

Deficiency disorder

The malnutrition, decreased dietary intake of niacin, malabsorption, high corn diet, Hartnup's disease and drug therapy with INH are a few important causes of niacin deficiency. In Hartnup's disease, the deficiency is due to defect in absorption of tryptophan along with other neutral amino acids from gut. INH therapy leads to deficiency of vitamin B_6 that is required for niacin synthesis from tryptophan. Hence, it may precipitate niacin deficiency along with features of vitamin B_6 deficiency. Deficiency of niacin leads to *pellagra* which is *characterized by dermatitis, diarrhea and dementia. "Neckless lesion" is characteristic form of dermatitis* in pellagra.

Status assessment

For evaluation of deficiency status, the following parameters may be used: (a) Assay of pyridine nucleotide level in blood and tissues, (b) Assay of urinary catabolites e.g., 2-pyridone and N'-

methylnicotinamide, (c) Ratio of NAD: NADP less than one is also indicative of a deficiency state and (d) Level of tryptophan in RBC.

Pantothenic Acid

Chemistry

Pantoic acid and β-alanine together forms pantothenic acid. Active forms are coenzyme A (CoA) and acyl carrier protein (Figure 19). It is stable at neutral pH but cooking destroys 15-50% of the vitamin. RJ Williams and RT Major synthesized pantothenic acid in 1940. F. Lipman explored first time that CoA is the functional form and is cofactor for acetylation reaction. He got Noble Prize for this discovery in 1953.

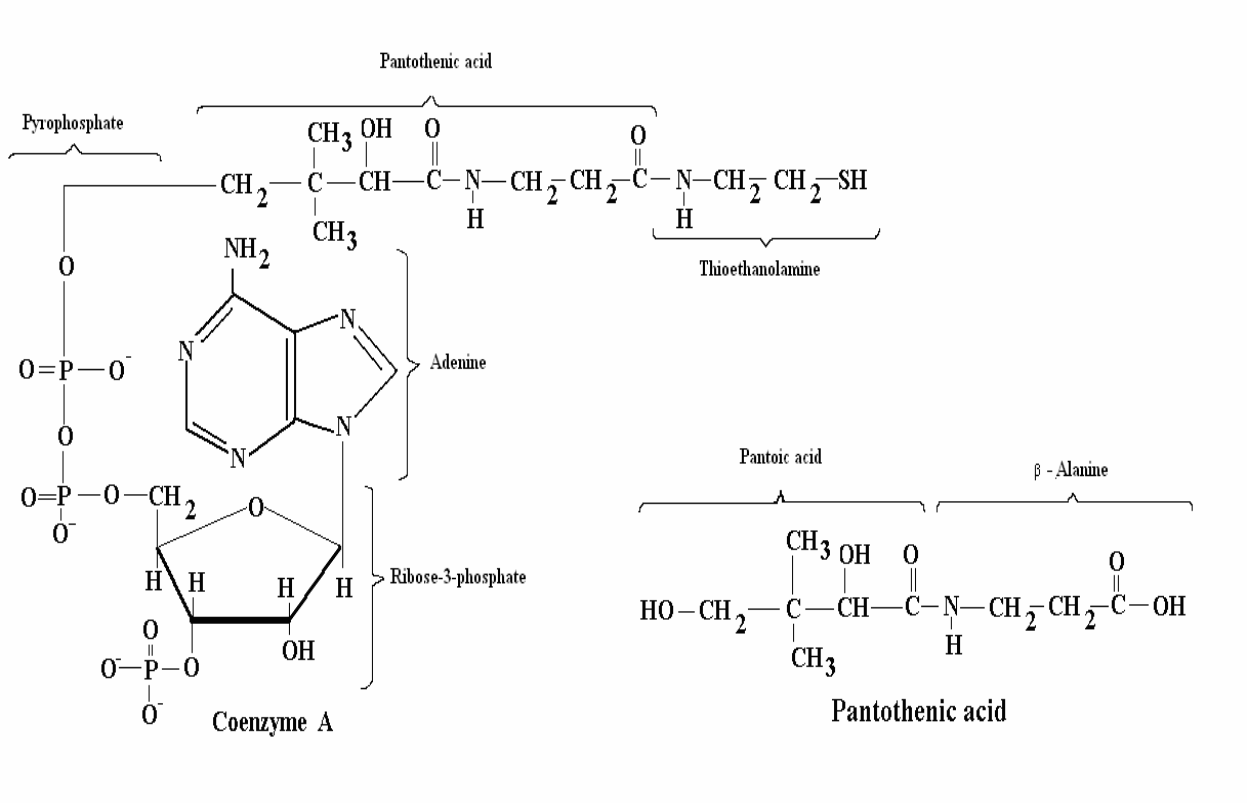


Figure 19: Structure of pantothenic acid and coenzyme A

Sources

This vitamin is widely distributed in food. Few rich sources of pantothenate are liver, kidney, tuna fish, egg yolk, milk etc. Yeast fermented food, brocoli and some other vegetables are also rich in this vitamin.

Metabolism

CoA is digested to pantothenic acid in gut. Then it is absorbed by enterocytes with the help of a sodium symport. From blood, pantothenic acid enters the cells by Na^+ symport. In cells, pantothenate is converted to ACP or CoA. It is excreted in urine in the form of cysteamine pantothenate.

Functions

(a) CoA plays very important role in energy metabolism. Acetyl CoA, succinyl CoA and fatty acyl CoA are very important molecules which produce ATP by being metabolized.
(b) CoA plays important role in biosynthesis of various biomolecules like fatty acid, phospholipids including sphingolipids, cholesterol, isoprenoids, haem, vitamin B_{12}, cytochromes, acetyl choline, melatonin, glycosaminoglycans etc.
(c) CoA is essential for acetylation, acylation (myristoylation) and isoprenylation of proteins. These reactions are crucial for some functions of proteins like membrane anchoring, signaling etc.

Requirement

There is no RDA for panthothenate. However, the amount that is suggested to be adequate for maintaining good health is 4-7mg (18-32μmol) per day for adults, 2mg/day for infants, 4-5mg/day for 7-10 yrs old children, 6mg/day during pregnancy and 7mg/day for lactating mother.

Deficiency disorder

The deficiency of pantothenate is rare because of its wide distribution in food. It is mostly found to be associated with severe form of malnutrition among the prisoners. The clinical features are listed below in the Box 4:

Burning foot syndrome
Fatigue
Headache
Insomnia
Intestinal disturbances
Paresthesia of hand and foot

Box 4: Clinical features of pantothenic acid deficiency

Status assessment

The status can be evaluated from serum or other biological fluids by microbial assay or by HPLC method.

Vitamin B_6

Chemistry

Vitamin B_6 is derivative of 3-hydroxy-5- hydroxymethy-2-methyl pyridine. Three forms of B_6 are pyridoxine, pyridoxal (PL) and pyridoxamine. The active forms are pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP) (Figure 20). PLP is the primary form. It is attached to lysine by a covalent link to ε-amino group.

Sources

It is available from both animal and plant food sources. In plants, it remains in 5'-O-β-D-glycopyranosyl pyridoxine (PGN) form and in animal foods, it remains either in pyridoxal or in pyridoxamine phosphate. The vegetables those are rich in B_6 are potato, spinach, carrot, cabbage, broccoli, beans, legumes, nuts, seeds, fruits like banana and raisins, cereals and grains. Chicken,

tuna, beef, etc are a few rich animal sources of the vitamins. Bioavailability of vitamin B_6 varies widely among foods. The bioavailability is very high from tuna and banana.

Metabolism

Dietary PLP is acted upon by alkaline phosphatase to produce pyridoxal which is absorbed from gut by passive diffusion and comes in circulation. A part of PL is converted to PLP in enterocytes by which a part of absorbed vitamin B_6 is metabolically trapped. In circulation, PL remains in free or bound with albumin. Three forms for vitamin B_6 can form their corresponding phosphates by kinases and can be converted back by phosphatases. PLP may be obtained from PMN and pyridoxine phosphate by oxidases. Pyridoxal can be oxidized to pyridoxic acid which is excreted in urine.

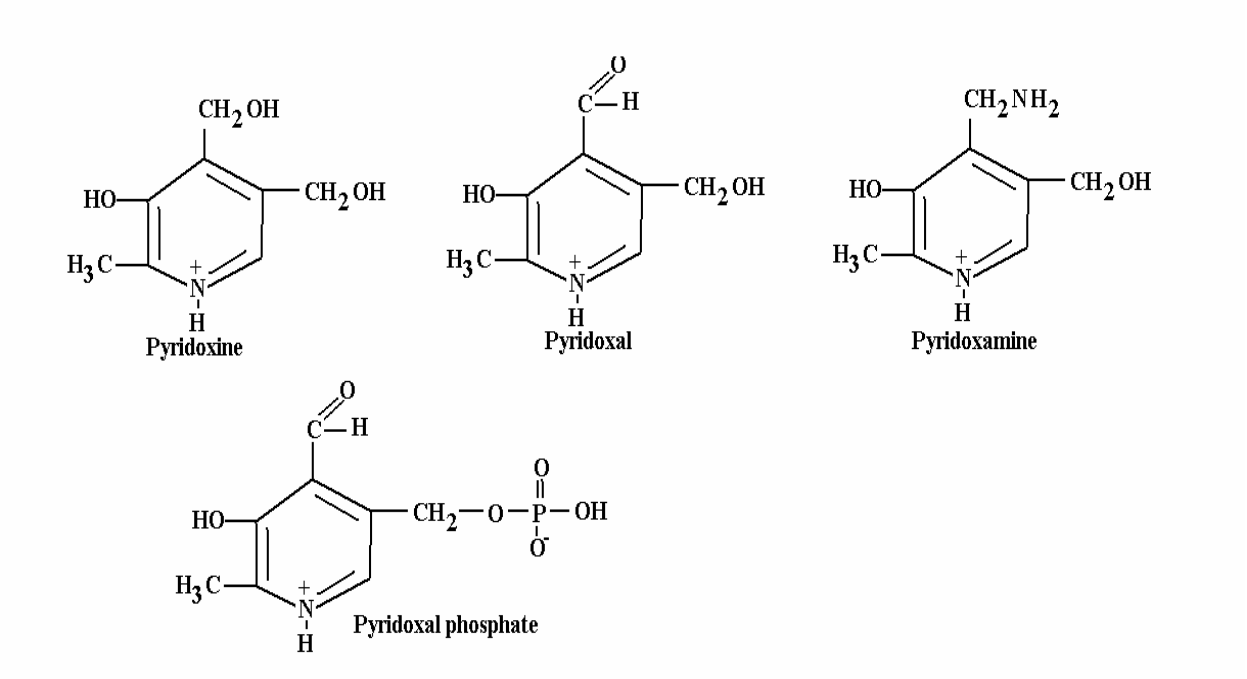


Figure 20: Structure of vitamin B_6

Functions

More than 100 reactions in our body need this vitamin. Transamination reaction requires PLP and PLP shuttle twice between aldimine and ketimine form to transfer ammonia group of one amino acid to a ketoacid (Figure 21). The box below mentions few important reactions requiring PLP as coenzyme (Box 5). Vitamin B_6 plays important role in immune functions also. It is required for IL-2 production and lymphocyte proliferation. Vitamin B_6 enhances steroid hormone sensitivity.

Therapeutically vitamin B_6 has been tried with variable effect in many clinical conditions like post-natal depression, pre-menstrual syndrome, hyperemesis gravideram, tenosinuvitis causing carpal-tunnel syndrome etc.

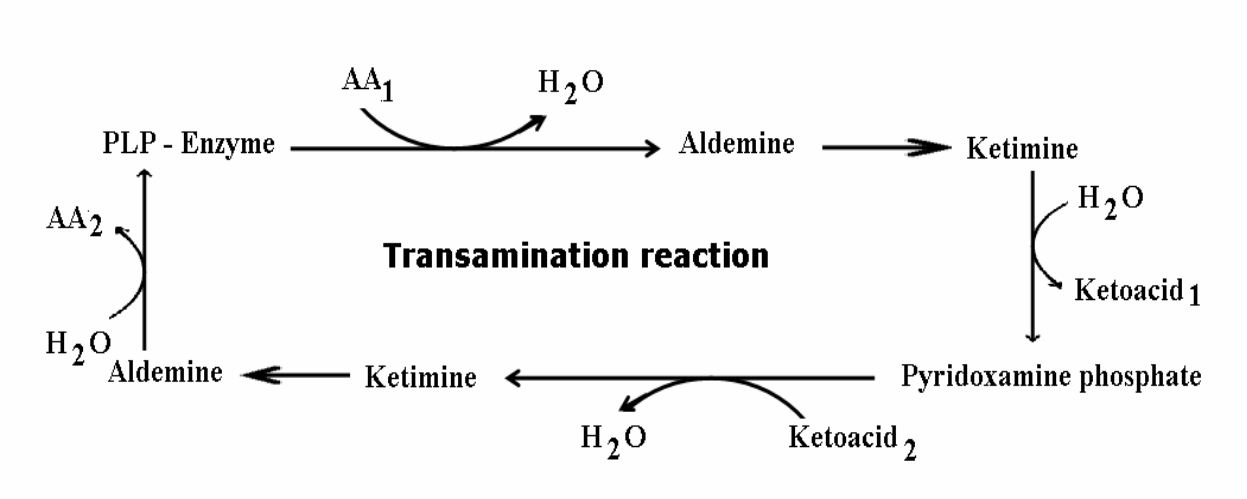


Figure 21: Transamination reaction

1. **Transamination Reaction**
   AST, ALT
2. **Glycogen** —Phosphorylase→ **Glucose-6-Phosphate**
3. **Decarboxylation Reaction**
   - Glutamate → GABA
   - 5(OH)Tryptophan → 5HT
   - Histidine → Histamine
   - Cysteine → Taurine
   - Serine → Ethanolamine
4. **Tryptophan** —PLP→ **Niacin**
5. **Glycine + SuccinylCoA** —PLP→ δ-ALA
6. **Serine** —PLP, THF→ **Glycine**
7. Cystathionine —PLP→ **Homoserine + Cysteine**
8. **PalmitoylCoA + Serine** —PLP→ **3-Ketosphinganine** → **Sphingosine**

Box 5: Reactions requiring pyridoxal phosphate.

Requirement

The RDA for the adult males is 1.7-2mg/day and 1.4-1.6mg/day for adult female. The requirement for infants is 0.3-0.6mg/day and for children, it is 1-1.4mg/day. In pregnancy and lactation, the requirement is 2.2 and 2.1mg/day respectively.

Deficiency

Alcoholics, babies of mother taking OCP, persons taking drugs like INH, cyclosporine, penecillamine, theophyline and OCP are prone to develop vitamin B_6 deficiency. The clinical features are listed in the Box 6 given below:

Stomatitis
Cheilosis
Glossitis
Irritability
Depression
EEG abnormality and convulsion in infants

Box 6: The clinical features of pyridoxine

Status assessment

There are some direct and indirect ways of assessing vitamin B_6. Measurement of plasma PLP, RBC PLP, plasma total vitamin B_6 and pyridoxal and urinary 4-pyridoxic directly reflect the vitamin B_6 status. GOT and GPT activity in RBC, tryptophan load test and methionine load tests are indirect way of assessing the status. In tryptophan load test, after giving a load of 2gm tryptophan urinary xanthuric and kynurenic acids are measured. In B_6 deficiecy, tryptophan can not be converted to niacin but arrested at xanthuric and kynuric acid stage of biosynthesis. Hence, these two acids are excreted in excess in B_6 deficiency state during the load test.

Toxicity

At high dose, it is found to have neurotoxicity and photosensitivity.

Folic acid

Chemistry

Folic acid is made up of a pteridine, a paraaminobenzoic acid (PABA) and a glutamic acid. The pteridine and PABA together is known as pteroyl moiety or pteroic acid (Figure 22). The active form is tetrahydrofolate polyglutamate. Polyglutamate conjugate is χ-linked polypeptide of seven glutamate residues.

Sources

Folic acid is ubiquitous in food stuff. The very rich sources are yeast, liver, fruits, leafy vegetables, etc. Cooking process mediated loss is 50-95% due to oxidation.

Metabolism

Food folate is present in polyglutamate form which is digested to folate monoglutamate by conjujugase enzyme. This enzyme is not abundant but just adequate in intestine. This enzyme is inhibited by alcohol, acid pH, some factors present in beans and some drugs like diphenylhydantoin and salicylazosulfapyridine. Folate monoglutamate thus produced enters into the intestinal cells by active transport which is converted to tetrahydrofolate (THF) and then to formyl- or methyl THF. In these forms, they appear in circulation. In circulation, it remains in free form or in bound form to proteins. Free form is taken up by liver and other tissues. In liver,

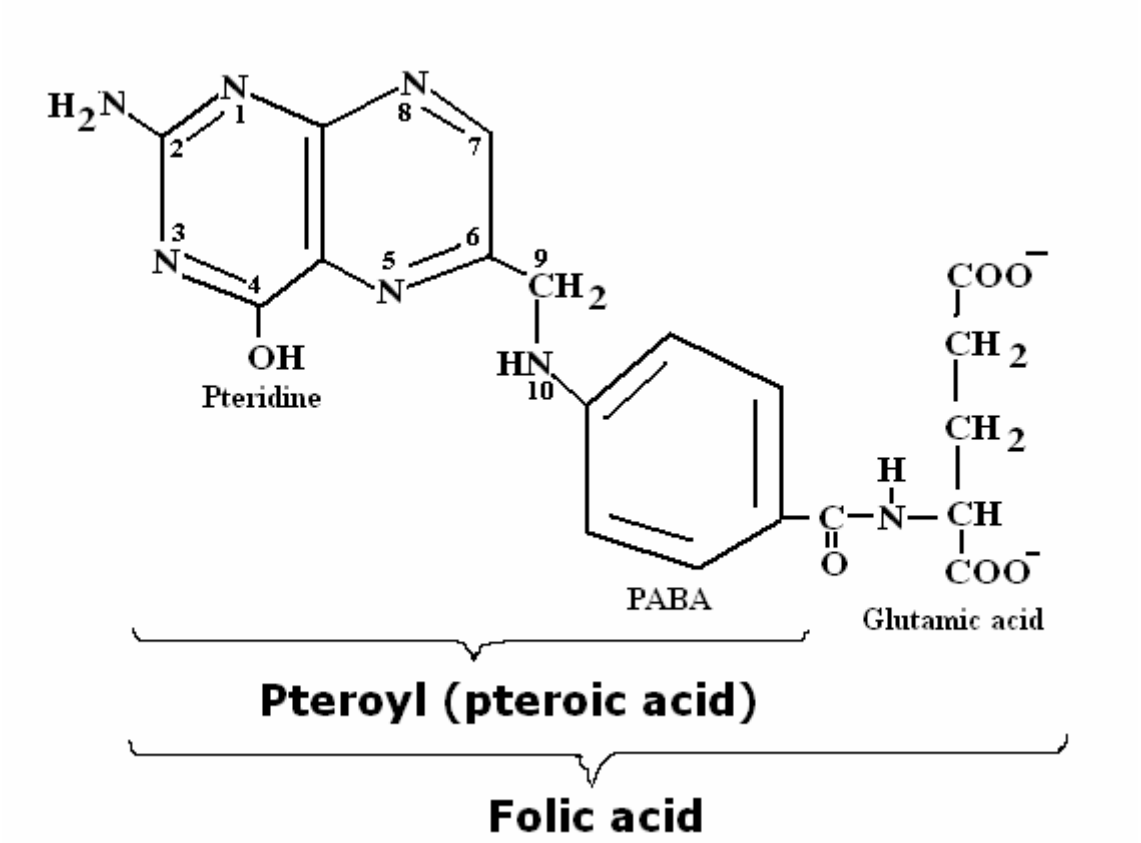


Figure 22: Structure of folic acid.

nearly 50% of total body pool (i.e., 2-5mg) of folate is stored in polyglutamate form. In tissues, THF acquires one carbon containing group and form various one carbon group containing THF e.g., formyl-(-CHO), formimino-(-CH=NH), methynyl- (-CH=), methylene- ($-CH_2-$), hydroxymethyl- ($-CH_2OH$), and methyl-($-CH_3$) THF. After donating one carbon group, THF is regenerated to perform the one carbon donation function repeatedly. The various one carbon containing form of THF are mostly interconvertable (Figure 23).

Functions

The important function of folic acid is in *de-nova* synthesis of purine and pyrimidine, metabolism of aminoacids and methylation of t-RNA.

(1) *De-novo* synthesis of purine and pyrimidine: Thymidylate synthetase that synthesizes TMP from dUMP needs N^5, N^{10}-methylene THF. Two carbons in purine nucleus are also donated by N^{10}-formyl THF and N^5, N^{10}-methylene THF (Figure 24). These are necessary for nucleotide and nucleic acid biosynthesis.

(2) Amino acid metabolism: The reactions of amino acid metabolism that need folic acid is listed in Box 7.

Folate supplementation is found to prevent neural tube defect. Hence, oral folic acid is recommended 6 months before the pregnancy is planned.

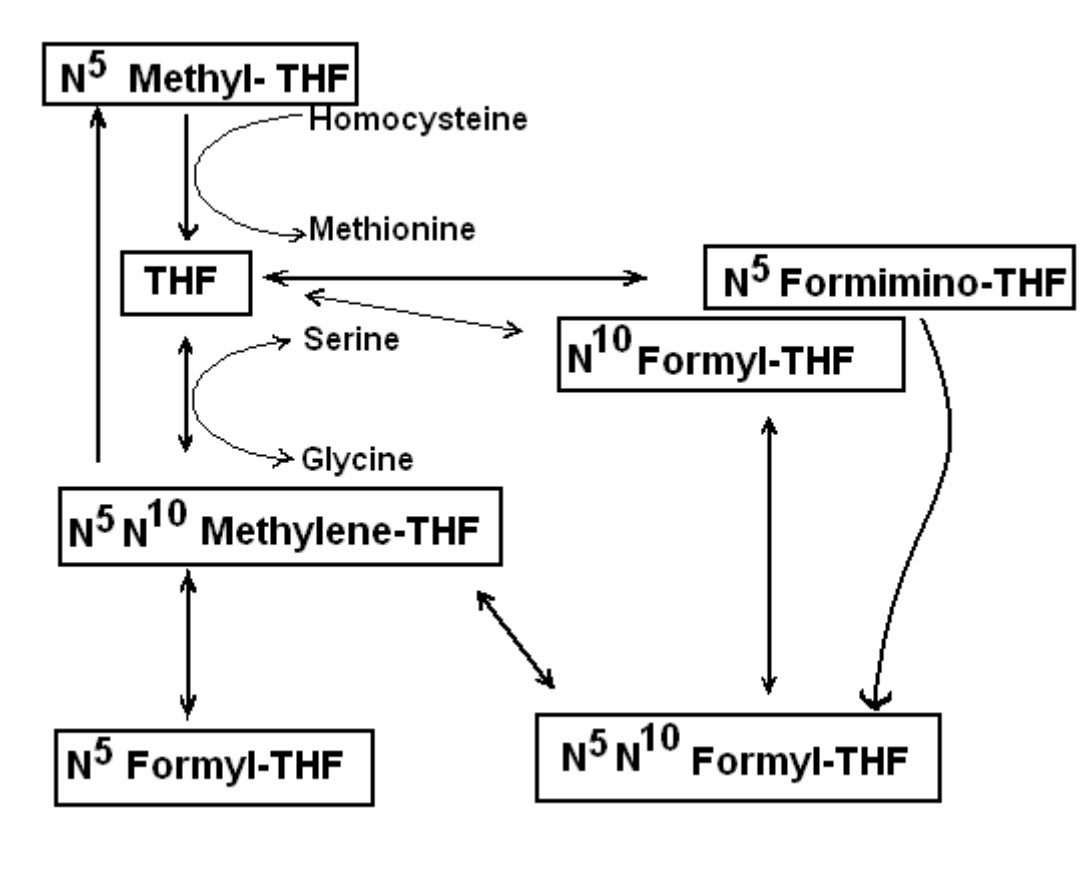


Figure 23: Metabolism of folate.

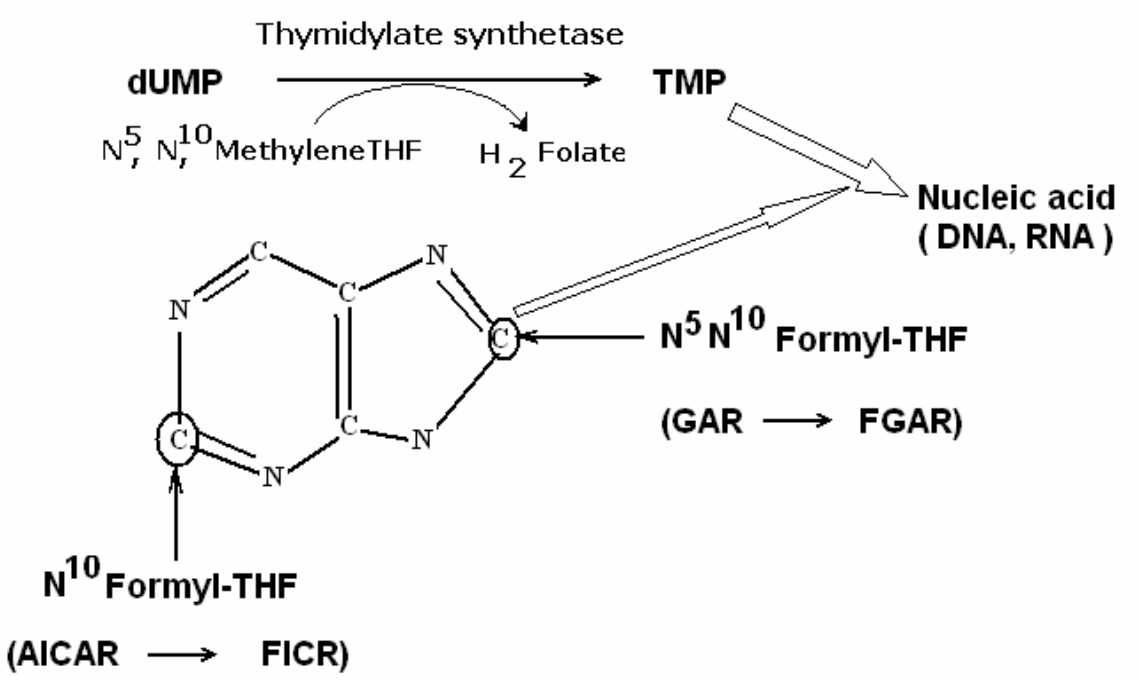
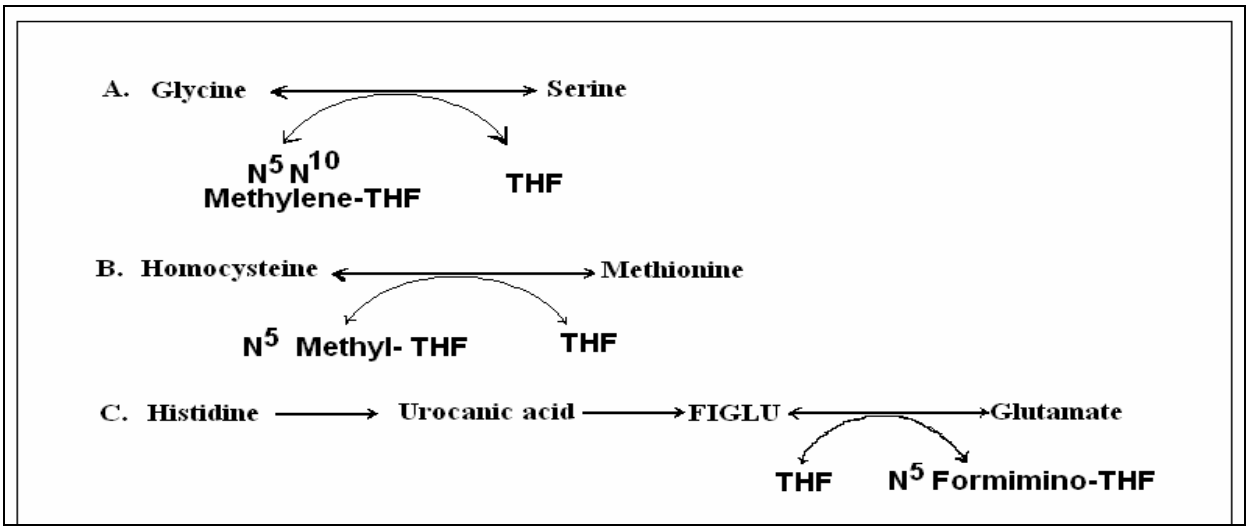
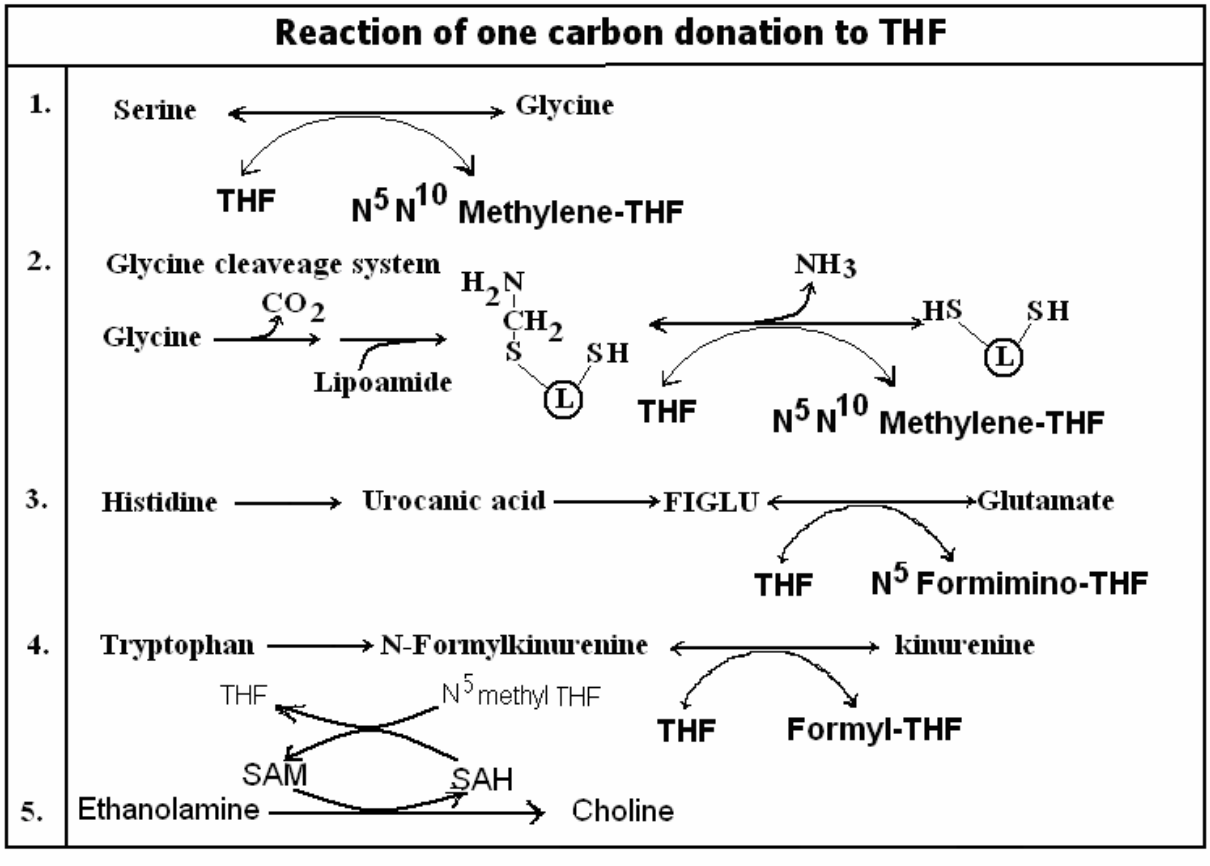


Figure 24: Contributions of THF in de-novo synthesis of purine and pyrimidine



Box 7: Reactions of amino acids requiring folic acid

Folate is necessary for transfer of one carbon from one molecule to other molecule. Major donor of methyline group to THF is serine. Glycine, histidine, tryptophan, choline and betaine are other doners of one carbon. The reactions leading to one carbon donation are shown in the Box 8.



Box 8: Reactions of one carbon donation to THF

Folate deficiency is found to be beneficial for treating malaria. There are reports to show that phenytoin that reduces blood folate level can cure malaria.

THF biosynthesis is necessary for bacterial growth. Trimethoprim that inhibit folate reductase is used as anti-bacterial agent. Methotrexate that inhibits folate reductase in human is used as anti-cancer agent. Thus, folate metabolism has become the therapeutic target for some diseases.

Relation between vitamin B_{12} and folate

As shown in the Figure 23, various forms of THF are interconvertable. But the reaction converting N^5, N^{10} methyline THF to N^5-methyl THF is unidirectional. Hence, unless N^5 methyl THF releases its methyl group, THF can not be formed or recycled. N^5-Methyl THF can donate methyl group to homocysteine to form metheonine. This reaction needs vitamin B_{12}. Hence, in B_{12} deficiency folate that forms N^5-methyl THF can not come back to THF form. This is called folate trap (Figure 25).

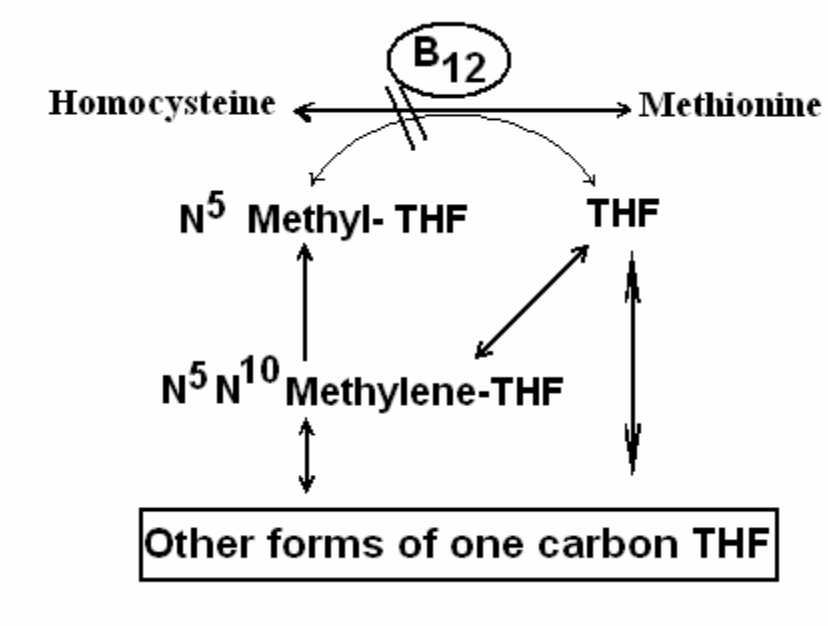


Figure 25: Folate trap

In combined deficiency of folate and vitamin B_{12}, if the patient is treated only with folic acid, it aggravates the sub-acute combined degeneration of spinal cord because folic acid stimulates RBC formation that utilize whatever minimum B_{12} is available in the system. This leads to decreased availability of B_{12} for CNS, precipitating sub-acute combined degeneration of spinal cord. However, this hypothesis is challenged by many.

Requirement

The RDA of folic acid for infants is 25-35μg, children 50-100μg and adolescent 150μg. For adult male and female, RDA is 200 and 180μg respectively. In pregnancy, RDA is 400μg. Hence folic acid along with iron is supplemented during pregnancy to prevent anemia. The requirement during lactation is 260-280μg/day.

Deficiency disorders

There are four stages of folate deficiency. Salient features of each stage are given below:

Stage 1: Folate store is not affected in this stage. But serum folate level goes below 3ng/ml, but RBC folate remains at level more than 200ng/ml.

Stage 2: In this stage, both serum and RBC folate levels decrease and RBC folate level goes below 160ng/ml.

Stage 3: DNA synthesis is defective and the result of dehydrouridine suppression test is abnormal. Nuclear hypersegmentation is seen in granulocytes.

Stage 4: Megaloblastic anemia is characteristic feature of this stage where mean corpuscular volume increases and macroovalocytes are seen.

Status assessment

Folate assay in serum and RBC along with peripheral blood picture gives an idea about folate status. Figlu excretion on histidine load test is increased. Because histidine catabolism is arrested at formiminoglutamate (FIGLU) and conversion of FIGLU to glutamate needs folate. 5-amino-4-imidazole carboxamide ribonuclotide (AICAR), an intermediate of *de novo* synthesis of purine can not be formylated and is excreted in excess in folate deficiency. Excretion AICAR can also be used as marker of folate deficiency. Deoxyuridylic acid (dU) suppression test is also positive in folate deficiency. The biochemical basis of the test is that conversion of deoxyuridylic acid (dU) to thymidylic acid requires folate. Hence, when lymphocytes not having folate deficiency are cultured in presence of dU, its ^{3}H-thymidine uptake is less because cells can synthesize thymidine from dU. But in folate deficient lymphocyte culture, even the presence of dU can not suppress ^{3}H-thymidine uptake because cells can not produce thymidine from dU in folate deficient state. Hence they take it up from the media. Hence, dU mediated suppression of ^{3}H-thymidine uptake has been used as test for folate deficiency.

Toxicity

Even at high dose, folate is non-toxic. Epileptic patients on phenobarbitone or phenytoin therapy can develop seizures at very high dose of folate. In rats, at very high dose, folate is precipitated to its crystalline form which is nephrotoxic.

Vitamin B_{12}

Chemistry

Vitamin B_{12} has cobalt whose four co-ordinates are linked to a tetrapyrole. The tetrapyrole with cobalt at centre is called corrin. Another co-ordinate of cobalt links corrin to a benzimidazole. The other co-ordinate may be linked to cyanide or hydroxyl or deoxyadenosyl or methyl group (Figure 26). The active forms of cobalamin are methyl and deoxyadenosyl cobalamin. Dorothy Hodgkin got noble prize in 1964 by doing X-ray diffraction of cobalamin and determining its structure. Robert Woodward got noble prize in 1965 after being able to synthesize it and confirming its structure.

Sources

Vegetarian items lack vitamin B_{12} unless it is contaminated by bacteria. Liver, kidney, heart, egg yolk, fish etc are rich source of vitamin B_{12}. Gut flora can synthesize it but it can not fulfill the whole requirement.

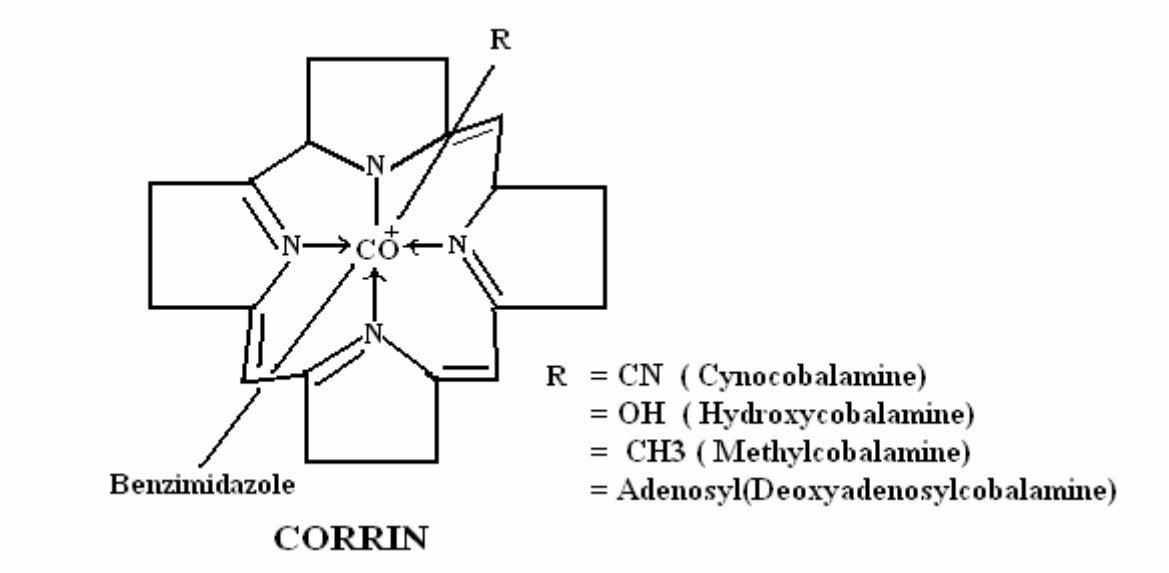


Figure 26: Cobalamin structure

Metabolism

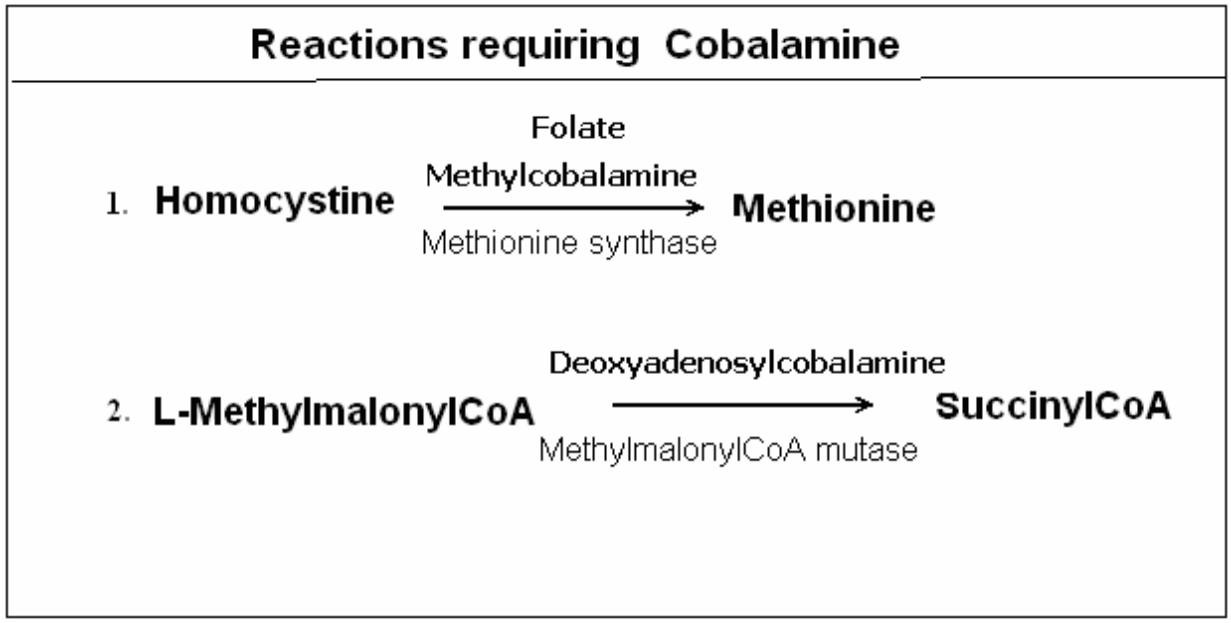
There is a protein present in saliva which binds to vitamin B_{12}. In stomach, vitamin B_{12} becomes free from that binder by the action of hydrochloric acid. Vitamin B_{12} then binds to another protein produced by stomach and known as intrinsic factor (IF). Vitamin B_{12} is taken from outside. Hence it is the extrinsic factor. Some amount of salivary binder attached to vitamin B_{12} that can not be separated by stomach HCl is digested in proximal part of duodenum and there also B_{12} can bind to IF. Intrinsic factor bound B_{12} is taken up through a receptor present in ileum. In the enterocytes, B_{12} gets free from IF and transported to circulation by a transport protein called transcobalamin II. B_{12} binds to transcobalamin I also in the circulation. But its role in circulation is not clear. B_{12} is stored in liver being bound to transcobalamin I. Vitamin B_{12} bound to transcobalamin II is delivered to the tissues to perform its functions. In cells, it performs its function in cytoplasm by being converted to methylcobalamin. The function of B_{12} in mitochondria is performed by deoxyadenosylcobalamin. Total body pool of B_{12} is 3-5mg and 50% of it remains in liver as a storage form. As the requirement is less, this stored amount is enough to prevent its deficiency disorder for few years. Excretion of B_{12} is very slow through apoptosis of liver and kidney cells and enterocytes as well.

Functions

The cytosolic enzyme methionine synthase use methylcobalamin as co-enzyme and the mitochondrial enzyme methyl malonyl-CoA mutase needs deoxyadenosylcobalamin as coenzyme for its function. The reactions catalyzed by these enzymes are shown in the Box 9.

Requirement

The RDA for vitamin B_{12} varies with age. For adults, it is 2.4μg/day and for infants 0.4-0.5 μg/day. In childhood, the requirement is 0.9-1.8 μg/day and in pregnancy and lactation 2.6 and 2.8 μg/day respectively.



Box 9: Reactions requiring cobalamin*e*

Deficiency disorders

The causes of vitamin B_{12} deficiency are:

(a) Dietary deficiency which is most common among vegetarians and elderly persons.
(b) Autoimmunity that produces anti-parital cell antibodies or anti-intrinsic factor antibody or both. This leads to pernicious anemia. Hypochlorhydria is a feature of this disorder. Low acid production also contributes to pathogenesis. B_{12} does not become free from salivary binder at low pH.
(c) Pancreatic insufficiency and malabsorption
(d) Infestation with fish-tape worm also leads to deficiency.
(e) Deficiency of transporter, transcobalamin II is a rare cause deficiency state.
(f) Disorders that affect conversion of B_{12} to its coenzymes and the mutation of methylmalonyl CoA produce the features of deficiency state.

The disorders which are known to be associated with vitamin B_{12} deficiency are: (a) megaloblastic anemia and (b) neuropathy (sub-acute combined degeneration of cord). Other possible associations are found with (c) atheroma, (d) neural tube defect and (e) hepatic steatosis. The possible biochemical pathogenesis of these disorders is depicted in Figure 27.

Status assessment

Anemia with macrocytosis and megaloblastic anemia is the hematologic feature of B_{12} deficiency. Serum methymalonic acid and homocysteine levels are increased but are not pathognomic of B_{12} deficiency. Anti-parital antibody and anti-intrinsic factor antibody may be high in pernicious anemia.

Schilling test is another test to check the deficiency of intrinsic factor. In this test, patient is given a dose of intramuscular injection of vitamin B_{12}. This B_{12} as it comes in the circulation

binds with the free transcobalamin. Then an oral dose of ^{60}Co-labelled B_{12} is administered and excretion of ^{60}Co-labelled B_{12} in urine is assessed. In intrinsic factor deficiency, absorption of B_{12} is less and hence excretion is also very less. Less excretion of B_{12} is interpreted as IF deficiency.

However, the most definitive and standard method of diagnosis of B_{12} deficiency is serum cobalamin assay.

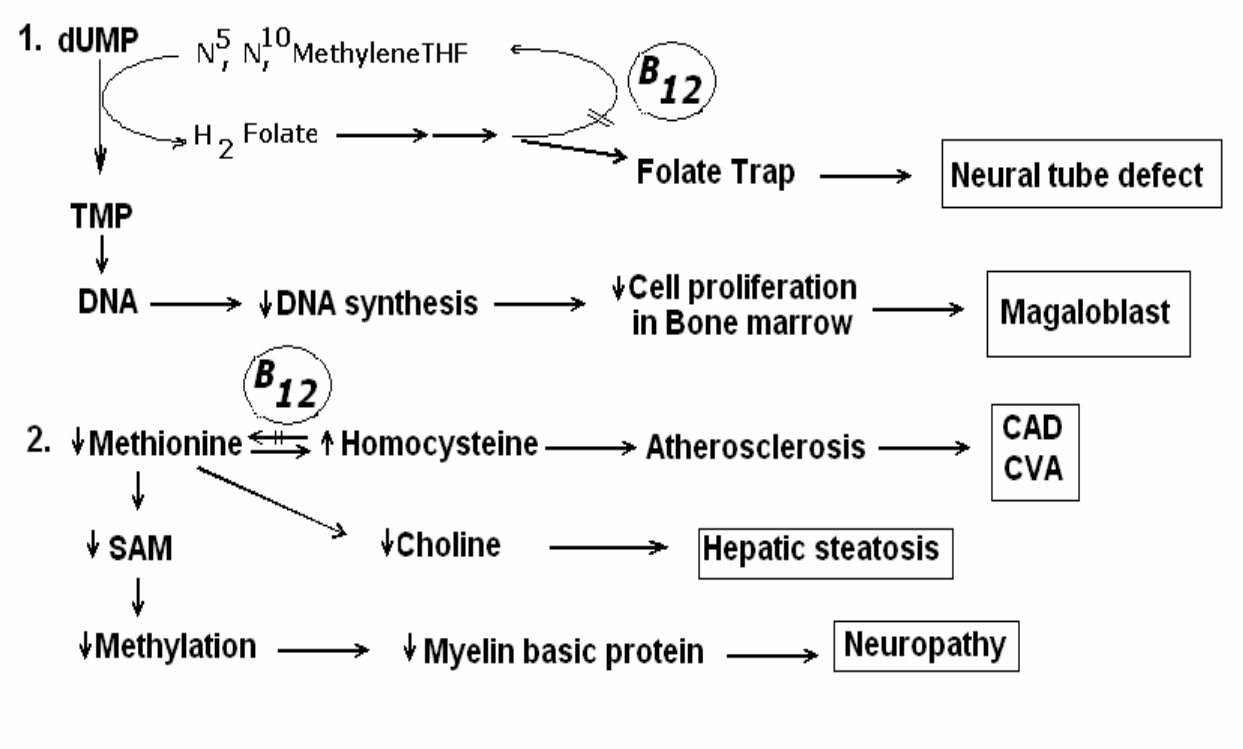


Figure 27: Biochemical pathogenesis of cobalamin deficiency disorders.

Biotin

Chemistry

The structure of biotin is shown in Figure 28. It has one ureido (-N-CO-N-) group. It remains attached to ε-amino group of lysine moiety of the enzyme. Biotin attached to lysine is known as biocytin.

Sources

Gut flora provides adequate amount of biotin. Other significant sources are liver, milk and egg yolk. Vegetarians can get biotin from yeast (fermented food), soyabean and peanuts.

Metabolism

Ingested holocarboxylases that contain biotin release it by the action of protein digesting enzyme. Biotin then is absorbed in gut by facilitated diffusion. Nearly 80% of biotin in blood remains in free form and the rests are attached covalently or reversibly to proteins. Free form

enters the target tissues by simple or facilitated diffusion. Biotin is incorporated to enzymes by being activated to biotenyl-5'-adenylate by holocarboxy synthetase enzyme. After performing its function, proteolytic degradation of holocarboxylase produce biocytin and then biotin is recycled (Figure 29).

A part of biotenyl-5'-adenylate is catabolized to biotinyl CoA and then to either binorbiotin or to biotin sulfons which are excreted in urine.

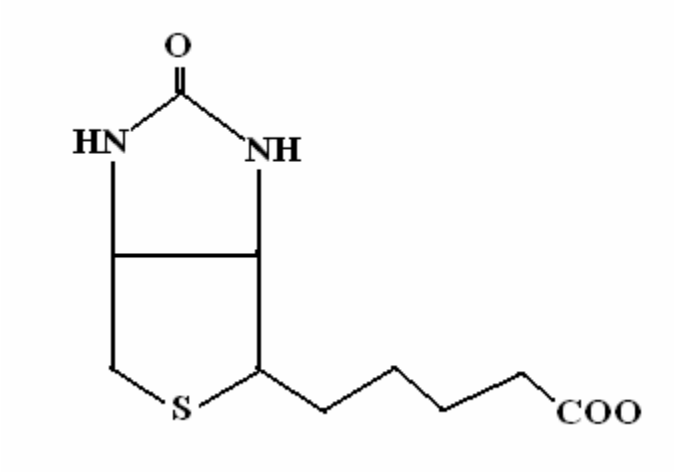


Figure 28: Structure of biotin

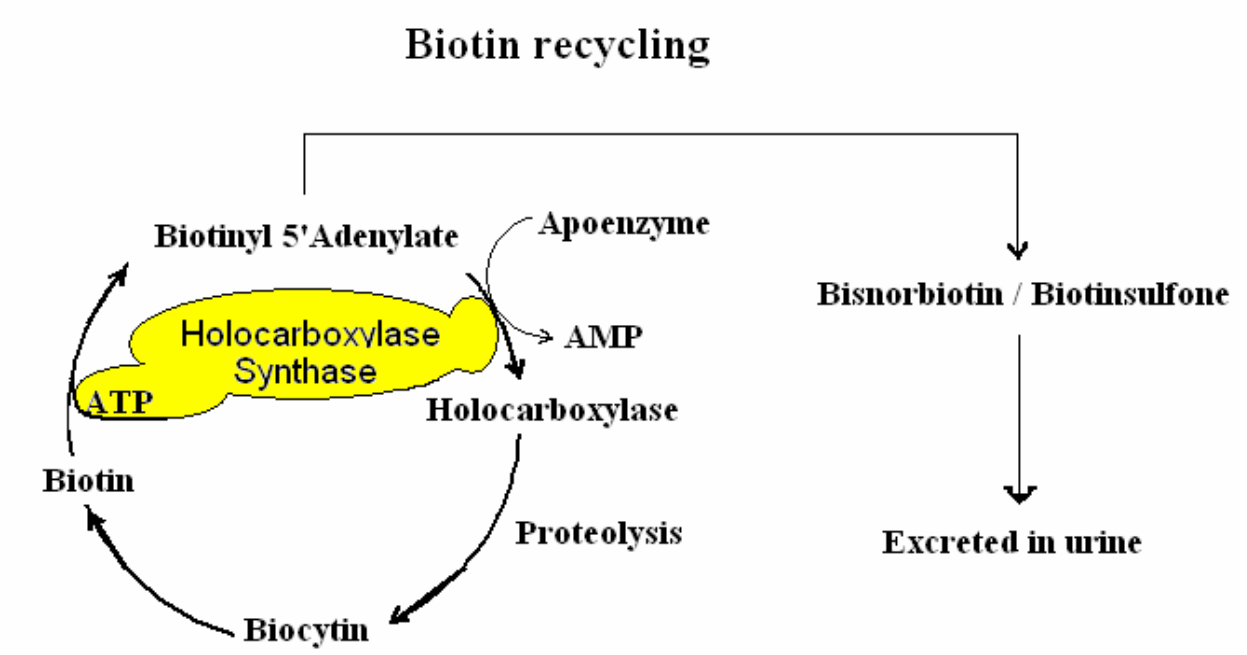


Figure 29: Biotin recycling

Functions

Biotin acts as coenzyme for four carboxylase enzymes. Besides vitamin K which helps in gamma carboxylation of glutamate residues of some clotting factor and other gla proteins, biotin is the most important vitamin for carboxylation of metabolic intermediates. The reactions are:

(a) Acetyl CoA $\xrightarrow[\text{Biotin}]{\text{Acetyl CoA carboxylase}}$ Malonyl CoA.

This is the key enzyme for fatty acid biosynthesis. Hence, in biotin deficiency fatty acid biosynthesis is impaired. Due to lack of fat biosynthesis, facial features change in these patients.

(b) Pyruvate $\xrightarrow[\text{Biotin}]{\text{Pyruvate carboxylase}}$ Oxaloacetate.

This is one of the regulatory enzymes for gluconeogenesis pathway.

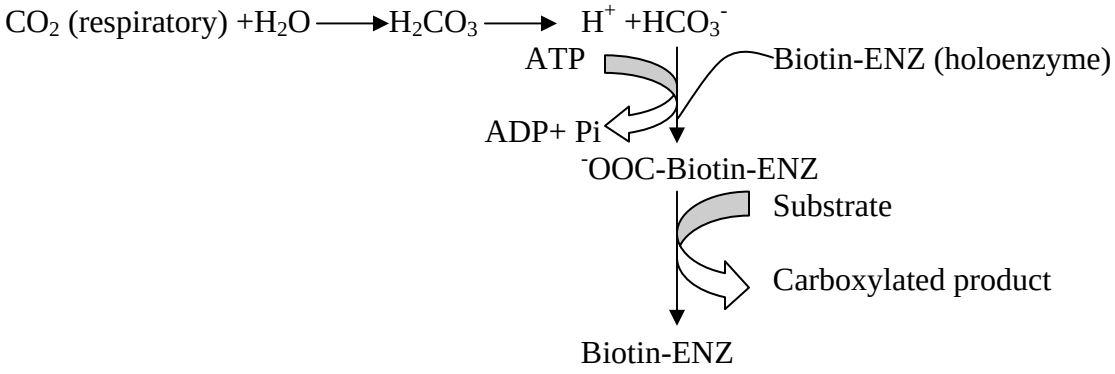
(c) Propionyl CoA $\xrightarrow[\text{Biotin}]{\text{Propionyl CoA carboxylase}}$ d-methylmalonyl CoA

The sources of propionate in our body are isoleucine, methionine, threonine, valine and odd-chain fatty acid oxidation. Hence, this reaction plays a role in metabolism of some amino acid, odd-chain fatty acid and gluconeogenic pathway.

(d) 3-Methylcrotonyl-CoA $\xrightarrow[\text{Biotin}]{\text{Methycrotonyl CoA carboxylase}}$ 3-Methyglutaconyl CoA.

This is an intermediary step in leucine metabolism.

All these enzymes prevent accumulation of organic acids which is raised in plasma and urine in biotin deficiency state. Biotin in holoenzyme plays an important role in transfer of carboxyl group to the substrate by forming a carboxybiotin complex as shown below:



Requirement

The assessment of requirement is yet little arbitrary. The amount that is recommended for adults is 30-100μg/day. For children, it is 20-30 μg/day and for infants, it is 10-15μg/day.

Deficiency disorders

The raw egg-white contains avidin which is an anti-vitamin to biotin. Rats fed with egg-white develop a biotin deficiency state known as 'egg-white injury'. The features of biotin deficiency in human are listed in the table:

Face	Unusual distribution of fats in face gives a 'biotin deficiency facies'
Skin	Thinning of hairs, scaly red skin rash, perioral rash, candidiasis
Neurological	Depression, lethargy, halocination, parasthesia, hypotonia
Development	Developmental delay

Status assessment

An improvement after supplementation gives a retrospective diagnosis of deficiency. Serum, plasma or urinary measurement of biotin also gives an idea of deficiency. In deficiency state, urinary organic acid e.g., 3-hydroxyisovaleric acid is increased and may be used as an indicator of deficiency state.

Toxicity

There is no known toxicity of biotin.

Final messages (what to tell to patients about vitamins?)

The deficiency of vitamins has diverse etiology and leads to ill-health. Vitamin supplementations can prevent the vitamin deficiency states and usual dose of supplementation is not known to cause any harm. Many are in favour of regular vitamin supplementation since high level of stress prevails in modern life style. If a healthy person should take regular vitamin supplementation is still a controversial issue. Human body is found to set the requirement of vitamin C at higher level on its chronic supplementation. Allowing the body to get adjusted at higher dose of vitamins may cause problems on sudden withdrawal of supplementation. Human race has survived thousands of years without extra vitamin supplementation. So prevention of vitamin deficiency states and leading a healthy life is possible without their supplementation, if a balanced diet made from naturally available foodstuffs is taken.

1. The food which is rich in one vitamin may be a poor source of other vitamins. Hence, adding varieties to diet is a natural way of fulfilling the requirement of vitamins. Use of proper method of cooking, food preservation and processing can reduce vitamin loss associated with these processes.
2. Requirement of vitamins increases in disease states. But it is yet to be proven how rational (or irrational) is it to prescribe multivitamin in each and every acute illnesses, which is found widely in medical practice. However, many chronic diseases need chronic vitamin supplementation. For example, folic acid should be given regularly to cases with chronic hemolytic anemia.
3. Consequences of vitamin A deficiency may be grave. It is a common but preventable disorder. Hence, vitamin A supplementation for children is practiced as a part of blindness control program in many countries.

4. Vitamin requirement is increased in pregnancy. To prevent neural tube defect, folic acid supplementation should ideally be started six months before conception and should be continued throughout the pregnancy. Intake of nutritious food is enough to meet the extra requirement of other vitamins during pregnancy.
5. Protein energy malnutrition is often associated with multiple vitamin deficiency. Presence of a single vitamin deficiency in a person should be investigated to find out the cause. Although dietary deficiency is the commonest cause of vitamin deficiency, a deficiency state may also occur in improper food habits (food faddism) and food processing, alcoholism, malabsorption, infestation (e.g., fish tape worm causes folic acid deficiency), in pathological and pathophysiological states, chronic administration of some medicines and some rare genetic disorders of vitamin metabolism (e.g., cobalamin transporter deficiency).
6. Vegetarians are susceptible to develop deficiency of some vitamins which are mostly present in non-vegetarian foods (e.g., vitamin B_{12}). Fermentation can enrich foods with folic acid and vitamin B_{12}. Hence, intake of fermented food can prevent it.
7. The knowledge is the key to health. Patients should be explained about the cause of deficiency, its effect and the remedial measures of vitamin deficiencies. Once the causes are removed or treated, vitamin therapy for a short duration is enough to treat the deficiency states. Even after the deficiency state is corrected, residual damage due to some vitamin deficiency (e.g., vitamin A) may persist throughout life. These cases need tertiary preventions.
8. There is no substantial scientific evidence to justify the belief that vitamins increase strength or libido in otherwise healthy subjects. Rather unjustified self-medication and overenthusiastic hypersupplementation, particularly of fat soluble vitamins, may cause problems (e.g., hypervitaminosis).

Suggested readings

1. Modern Nutrition in Health and disease 10th edition by ME Shils et al. (2004).
2. Handbook of Vitamins 2nd edition by LJ Machlin (1996)

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