

# CHAPTER 7

## Vitamins

- Introduction
- Definition and Classification of Vitamins
- Water Soluble Vitamins
- Fat Soluble Vitamins
- Summary
- Exercise

### INTRODUCTION

The name '*Vitamine*' was proposed in 1911 by Polish chemist Casimir Funk for the nutrient compound required to prevent the nutritional deficiency disease *beriberi*, because of its vital (vita = life) need and because chemically it was found to be an *amine*. Later, after a number of other essential organic nutrients were discovered, the "*e*" was dropped, when it was found that not all of them are amines. The term '*Vitamin*' has now been adopted universally and applied to a group of biologically essential compounds that include 14 compounds which cannot be synthesized by human beings. They must, therefore, be supplied through food.

Since their chemical nature was unknown letter designations were applied for their nomenclature, e.g. vitamins A, B and C. Later, vitamin B was shown to consist of several substances and subscripts were added, i.e. vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, etc. and collectively called *vitamin B complex*.

### DEFINITION AND CLASSIFICATION OF VITAMINS

**Vitamins** are organic nutrients that are required in small quantities (in micrograms to milligram quantities per day) for a variety of biochemical functions and which generally cannot be synthesized by the body and must, therefore, be supplied by the diet. Some can be synthesized by intestinal microorganisms, but in quantities that are not

sufficient to meet our needs. They may be water or fat soluble.

### Classification

The vitamins are grouped into two categories based on their solubility:

1. Water soluble vitamins
  2. Fat soluble vitamins
- **Water soluble vitamins which include**
    - i. Vitamin B complex, e.g.
      - Thiamine (vitamin B<sub>1</sub>)
      - Riboflavin (vitamin B<sub>2</sub>)
      - Niacin (vitamin B<sub>3</sub>)
      - Pantothenic acid (vitamin B<sub>5</sub>)
      - Pyridoxine (vitamin B<sub>6</sub>)
      - Biotin
      - Folic acid
      - Cobalamin (vitamin B<sub>12</sub>)
    - ii. Vitamin C or ascorbic acid.
  - **Fat soluble vitamins, which include**
    - Vitamin A or retinol
    - Vitamin D or cholecalciferol
    - Vitamin E or tocopherol
    - Vitamin K.

**Table 7.1** summarizes the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins.

Table 7.1: Summary of the best food sources, dietary allowances, the active coenzyme forms, the principal metabolic functions and the major clinical manifestations of deficiencies of the water soluble and fat soluble vitamins

Name	Active form	Sources	Daily requirements	Functions	Deficiency manifestations
<b>WATER SOLUBLE VITAMINS</b>					
Thiamine (Vitamin B <sub>1</sub> )	TPP	Cereals, meat, nuts green vegetables, eggs	1.0–1.5 mg	Coenzyme for oxidative decarboxylation and transketolase reactions	Beriberi, Wernicke Korsakoff syndrome
Riboflavin (Vitamin B <sub>2</sub> )	FMN, FAD	Yeast, germinating seeds, green leafy vegetables, milk, eggs, liver, meat	1.3–1.7 mg	Coenzyme for oxidation-reduction reactions	Cheilosis, glossitis, dermatitis, vascularization of cornea
Niacin (Vitamin B <sub>3</sub> )	NAD <sup>+</sup> and NADP <sup>+</sup>	Yeast, legumes, liver, meat	15–20 mg	Coenzyme for oxidation reduction reactions	Pellagra
Pantothenic acid (Vitamin B <sub>5</sub> )	Coenzyme A, (CoA-SH)	Wheat germs, cereals, yeast, liver, eggs	5–10 mg	Acyl carrier	Burning feet syndrome
Pyridoxin (Vitamin B <sub>6</sub> )	PLP	Yeast, unrefined cereals, pulses, vegetables, meat, fish, egg yolk	1.6–2 mg	Coenzyme for transamination, decarboxylation, non-oxidative deamination, trans-sulfuration reactions	Epileptic convulsions, dermatitis, hypochromic microcytic anemia
Biotin (Vitamin B <sub>7</sub> )	Biocytin (Enzyme bound biotin)	Liver, kidney, egg yolk, vegetables	150–300 µg	Coenzyme for carboxylation reactions	Rare dermatitis
Folic acid (Vitamin B <sub>9</sub> )	<b>THF</b> (Tetrahydrofolic acid)	Green leafy vegetables, liver, yeast	200 µg	Carrier of one carbon unit. Synthesis of methionine, purines and pyrimidines	Megaloblastic anemia, Neural tube defects
Cynocobalamin (Vitamin B <sub>12</sub> )	Methylcobalamin, Deoxyadenosylcobalamin	Only animal origin, meat, egg, liver, fish	3 µg	Coenzyme for reactions: Homocysteine to Methionine. Methylmalonyl-CoA to Succinyl-CoA	Pernicious anemia Megaloblastic anemia Neuropathy (dementia) Methylmalonic aciduria
Ascorbic acid (Vitamin C)	Ascorbic acid	Citrus fruits, Amla, leafy vegetables, tomatoes	60–70 mg	Antioxidant, involved in hydroxylation reactions in the synthesis collagen, steroid hormones, adrenaline, etc. facilitates absorption of iron from intestine	Scurvy

Contd...

Contd...

Table 7.1

Name	Active form	Sources	Daily requirements	Functions	Deficiency manifestations
<b>FAT SOLUBLE VITAMINS</b>					
Vitamin A (Retinol)	Retinol, Retinal, Retinoic acid	Fish liver oils, milk, milk products, Green leafy vegetables, carrots, yellow and red fruits	800–1000 retinol equivalents	Retinal and retinol are involved in vision. Retinoic acid regulates the expression of gene during growth and development. Antioxidant	Night blindness xerophthalmia formation of Bitot's spots, dry, rough and scaly skin. Retardation of growth in children
Vitamin D (Cholecalciferol)	1, 25-Dihydroxy-cholecalciferol	Cod liver oil, sunlight induced synthesis of vitamin D <sub>3</sub> in skin, egg yolk	200–400 IU	Regulation of the plasma level of calcium and phosphorus, calcification of bone	Rickets (in children) Osteomalacia (in adults)
Vitamin E ( $\alpha$ -Tocopherol)	$\alpha$ -tocopherol,	Soya and corn oils, germ oil, fish oil, eggs, alfalfa	8–10 mg	Natural antioxidant and acts as a scavenger of free radicals. Protects the RBCs from hemolysis Prevents peroxidation of PUFA in cell membrane	Hemolytic anemia. Retrolental fibroplasia (RLF) in premature infants
Vitamin K	Phylloquinone (Vitamin K <sub>1</sub> ) Menakinone (Vitamin K <sub>2</sub> )	Green leafy vegetables, tomatoes, cheese, meat, egg yolk	70–140 $\mu$ g	Required for activation of blood clotting factors. Required for $\gamma$ carboxylation of glutamic acid residue in clotting and oseo-calcin proteins	Hemorrhagic disorder, increased clotting time

### DIFFERENCE BETWEEN FAT SOLUBLE AND WATER SOLUBLE VITAMINS

- Water soluble vitamins function as precursor for coenzymes and antioxidants while fat soluble vitamins function as coenzymes, hormones and antioxidants.
- Water soluble vitamins are usually *non-toxic* since excess amounts of these vitamins are excreted in the urine, while fat soluble vitamins are *toxic* and even lethal when taken in excessive quantities.
- Water soluble vitamins are not stored extensively **except vitamin B<sub>12</sub>**, and so their intake has to be more frequent than that of other fat soluble vitamins which are stored.

### WATER SOLUBLE VITAMINS

#### Thiamine (Vitamin B<sub>1</sub>)

##### Structure

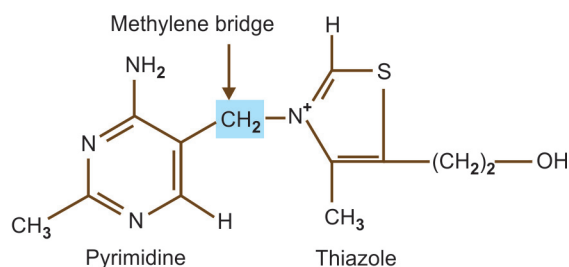
Thiamine consists of a pyrimidine ring attached to a thiazole ring (**Figure 7.1**) by methylene bridge.

##### Active Form of Thiamine

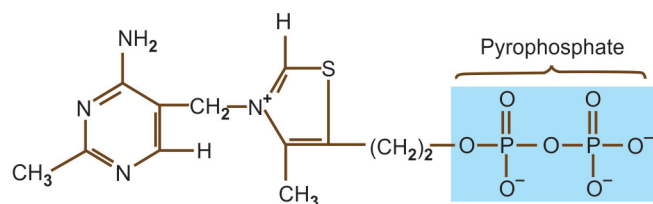
Thiamine pyrophosphate (TPP) is an active coenzyme form of vitamin thiamine (**Figure 7.2**).

##### Sources

- It is present in all natural foods but particularly good dietary sources are unrefined cereals, meat, nuts, green vegetables, eggs, etc.



**Figure 7.1:** Structure of thiamine



**Figure 7.2:** Thiamine pyrophosphate, active coenzyme form of thiamine (OH-group of thiazole is replaced by pyrophosphate)

- White bread and polished rice are very poor sources of the vitamin thiamine.

##### Functions

- Thiamine is required mainly for *carbohydrate metabolism*
- Thiamine pyrophosphate (TPP) is a coenzyme involved in several enzymatic reactions mainly for *oxidative decarboxylation* and *transketolase* reactions as follows:
  - 1 TPP is a coenzyme for *pyruvate dehydrogenase complex* which catalyzes the conversion of pyruvate into acetyl CoA by oxidative decarboxylation (**Figure 12.5**). Acetyl-CoA is a precursor for the synthesis of the **neurotransmitter acetylcholine** and also for the synthesis of myelin. *Thus, thiamine is required for the normal functioning of the nervous system.*
  - 2 TPP is a coenzyme for *α-ketoglutarate dehydrogenase* which catalyzes the conversion of α-ketoglutarate to succinyl-CoA in TCA cycle (*See Figure 12.6*).
  - 3 TPP is a coenzyme for the enzyme *transketolase*, in the pentose phosphate pathway of glucose oxidation (*See Figure 12.16*).

##### Nutritional Requirements

- Nutritional Research Council recommends daily intake of **1.0 to 1.5 mg** of thiamine for adults which is increased with increased muscular activity, dietary carbohydrates and in pregnancy and lactation.

##### Deficiency Manifestations

- The deficiency of vitamin B<sub>1</sub> results in a condition called **beriberi**. Deficiency of thiamine occurs in population who consume exclusively **polished rice** as staple food. Polishing of rice removes thiamine.
- The early symptoms of thiamine deficiency are anorexia, nausea, mental confusion, peripheral neuritis, muscle fatigue and irritability.
- Thiamine deficiency leads to three types of beriberi namely
  1. Dry beriberi
  2. Wet beriberi
  3. Infantile beriberi

##### Dry beriberi (neuritic beriberi)

- It develops when the diet chronically contains slightly less than the thiamine requirements.
- This form of beriberi is characterized primarily by **peripheral neuritis**, severe muscular weakness and fatigue. Other symptoms of dry beriberi include dry skin, mental confusion and poor appetite.

### Wet beriberi (cardiac beriberi)

- It develops when the deficiency is more severe in which cardiovascular system is affected in addition to neurological symptoms.
- Wet beriberi is characterized primarily by **edema** of extremities, heart enlargement and cardiac insufficiency. Other symptoms include tachycardia or bradycardia and palpitation.
- Both forms of beriberi may overlap to a varying degree and patients of beriberi may die due to heart failure, if not treated.

### Infantile beriberi

- Infantile beriberi is observed in breast fed infants born to mother suffering from thiamine deficiency. The breast milk of these mothers is deficient in thiamine.
- It is characterized by cardiac dilation (enlargement of heart), tachycardia, convulsions, edema and GI disturbances such as vomiting, abdominal colic, etc. In acute condition, the infant may die due to cardiac failure.

### Wernicke-Korsakoff Syndrome

- It is also known as **cerebral beriberi** and mostly seen in alcoholics.
- In chronic alcoholics, the nutritional deficiencies result from either poor intake of food or malabsorption of nutrients from intestine.
- Wernicke-Korsakoff syndrome is characterized by anorexia, nausea, vomiting, nystagmus, depression, ataxia, loss of memory, mental confusion, peripheral paralysis, muscular weakness, etc.

### Antimetabolites

Thiamine can be destroyed if the diet contains **thiaminase**. Thiaminase is present in raw fish and seafood.

### Thiamine Assay

Whole blood or Erythrocyte **transketolase** (requiring **TPP** as a coenzyme) activity is used as a measure of thiamine deficiency.

### Riboflavin (Vitamin B<sub>2</sub>)

#### Structure

Riboflavin is a yellow compound (Flavus = **yellow** in Latin) consisting of a **isoalloxazine ring** with a **ribitol** (sugar alcohol) side chain (**Figure 7.3**). Riboflavin is relatively **heat stable** but decomposes in the presence of visible light (photosensitive).

#### Active Form of Riboflavin

The active or coenzyme forms of the riboflavin are:

- Flavin mononucleotide (FMN), and
- Flavin adenine dinucleotide (FAD).

#### Sources

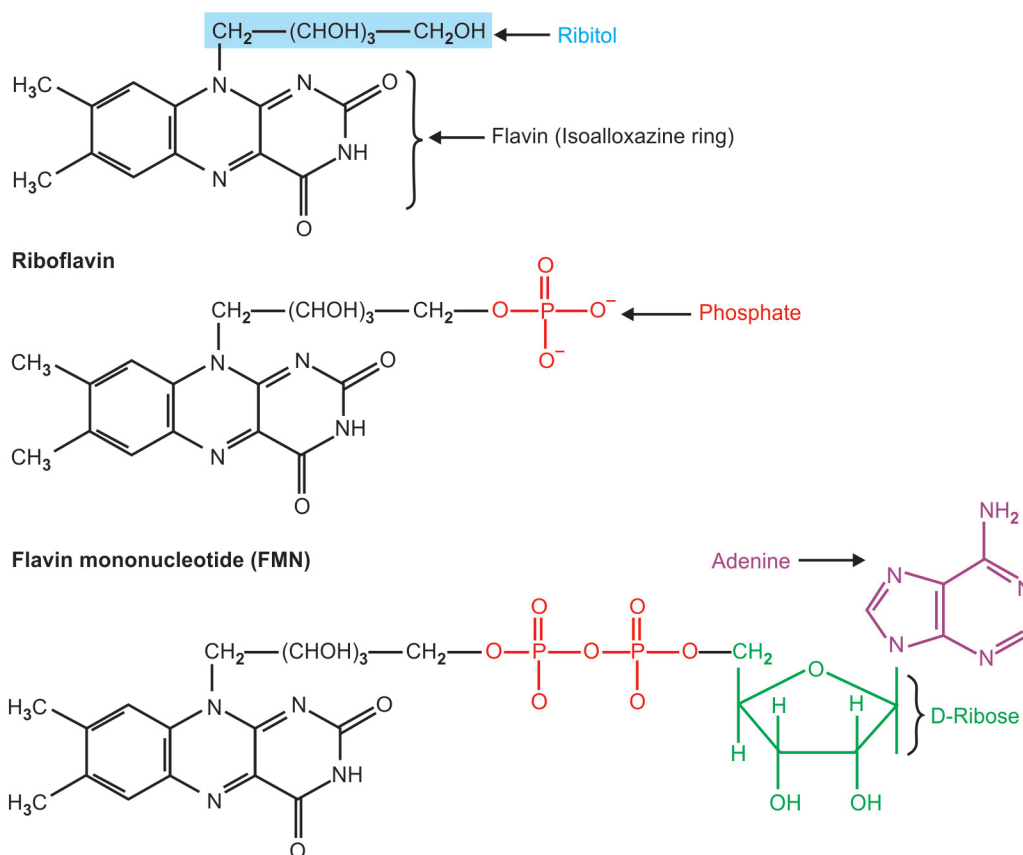
- The main dietary sources of riboflavin are yeast, germinating seeds, green leafy vegetables milk and milk products, eggs, liver and meat.
- Cereals are a poor source.

#### Functions

- Riboflavin is a precursor of coenzymes **FMN** and **FAD**, which are required by several **oxidation-reduction** reactions in metabolism. FMN and FAD serve as coenzymes for **oxidoreductase enzymes** involved in carbohydrate, protein, lipid, nucleic acid metabolism and electron transport chain. Some examples are given in **Table 7.2**.
- It is needed for maintenance of mucosal epithelial and the ocular tissues.
- They are also involved in protection against peroxidation in **metabolism of xenobiotics**.

**Table 7.2: Examples of enzymes requiring FMN or FAD as a coenzyme and reaction where they are involved**

Flavoprotein enzyme	Pathway/Reaction
Amino acid oxidase	Deamination of amino acids
Xanthine oxidase	Purine degradation
Succinate dehydrogenase	Citric acid cycle
Acyl-CoA dehydrogenase	Fatty acid oxidation
NADH dehydrogenase	Respiratory chain into mitochondria
Pyruvate dehydrogenase, and $\alpha$ -Ketoglutarate dehydrogenase	Oxidative decarboxylation of pyruvate and $\alpha$ -ketoglutarate



**Figure 7.3:** Structure of riboflavin and its active coenzyme forms FMN and FAD

### Nutritional Requirements

- The RDA for vitamin B<sub>2</sub> is **1.3 to 1.7 mg** for adults.
- It is related to protein use and increases during growth, pregnancy, lactation and wound healing.

### Deficiency Manifestations

- Riboflavin deficiency is quite rare as it has a wide distribution in food stuffs. It is usually seen along with deficiencies of other vitamins of B-complex group. It is most commonly seen in chronic alcoholics.
- The characteristic symptoms of riboflavin deficiency are:
  - Cheilosis: Fissures at the angles of the mouth,
  - Glossitis: Inflammation of the tongue that becomes swollen and magenta colored
  - Dermatitis: Rough and scaly skin
  - Vascularization (the development of blood vessels) of cornea, etc.

### Riboflavin Assay

A commonly used method for assessment of riboflavin

status uses the determination of FAD dependent **glutathione reductase** activity in freshly lysed erythrocytes.

### Niacin (Vitamin B<sub>3</sub>)

#### Structure

**Niacin** is a general name for the *nicotinic acid* and *nicotinamide*, either of which may act as a source of the vitamin in the diet. Niacin is a simple derivative of *pyridine*.

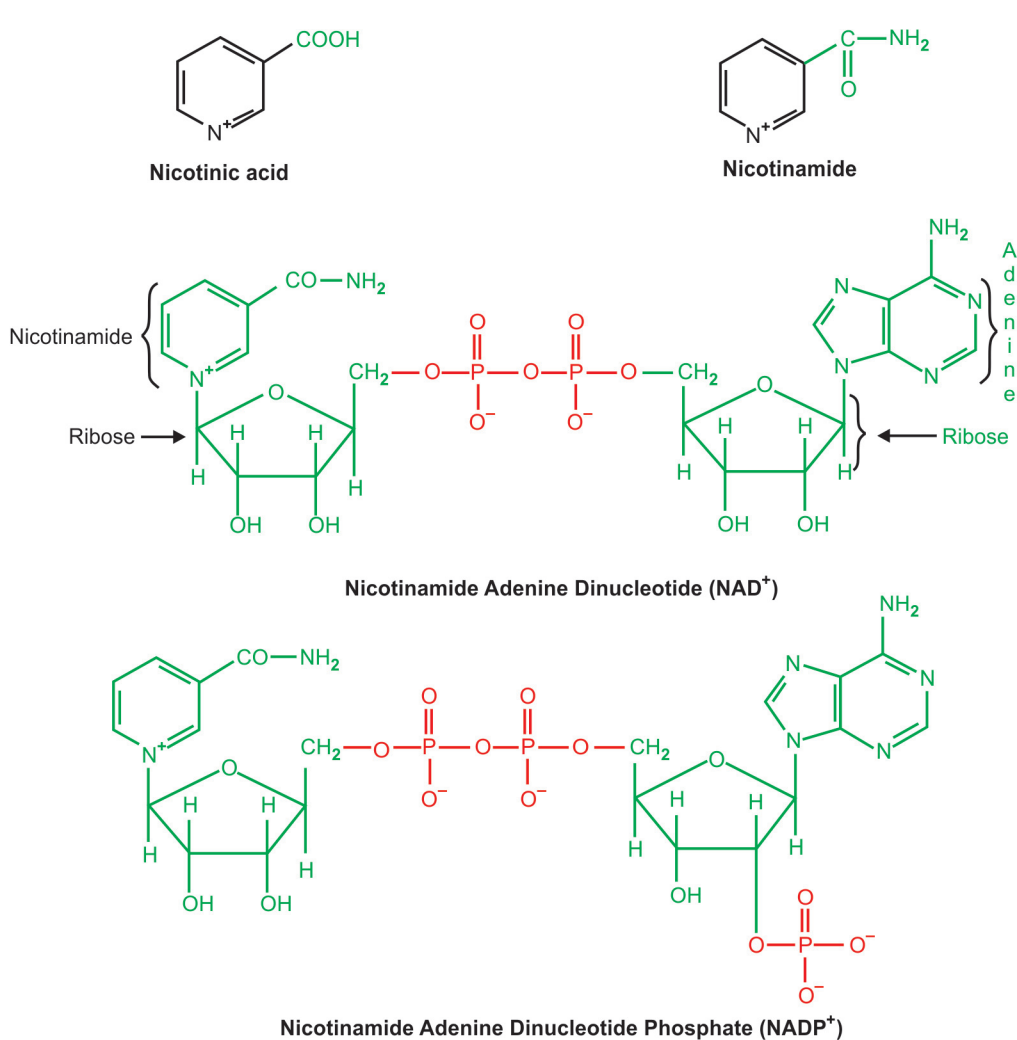
#### Active Form

Active forms of niacin are:

- Nicotinamide adenine dinucleotide (NAD<sup>+</sup>)
- Nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>) (**Figure 7.4**).

#### Sources

- Yeast, liver, legumes and meats are major sources of niacin.
- Limited quantities of niacin can also be obtained from the metabolism of tryptophan. *For every 60 mg of*



**Figure 7.4:** Structure and active coenzyme forms of niacin

*tryptophan, 1 mg equivalent of niacin can be generated.*

### Functions

- Niacin is a precursor of coenzymes, *nicotinamide adenine dinucleotide ( $\text{NAD}^+$ )* and *nicotinamide adenine dinucleotide phosphate ( $\text{NADP}^+$ )*.
- $\text{NAD}^+$  and  $\text{NADP}^+$  are involved in various **oxidation** and **reduction reactions** catalyzed by dehydrogenases in metabolism.
- They are, therefore involved in many metabolic pathways of carbohydrate, lipid and protein. Generally,  $\text{NAD}^+$  linked dehydrogenases catalyze oxidation-reduction reactions in **oxidative pathways**, e.g. citric acid cycle and glycolysis.
- Whereas  $\text{NADP}^+$  linked dehydrogenases or reductases are often found in pathways concerned with **reductive synthesis**, e.g. synthesis of cholesterol, fatty acid and pentose phosphate pathways.
- Selected examples of enzymes and the reactions they catalyze are given in **Table 7.3**.

### Nutritional Requirement

- The RDA for niacin is **15 to 20 mg**.
- Tryptophan** can only provide about 10% of the total *niacin* requirement.

### Deficiency Manifestation

#### Pellagra

- Deficiency of niacin in human causes pellagra, a disease involving the:



Table 7.3 : Examples of enzymes requiring NAD<sup>+</sup> or NADP<sup>+</sup> or NADPH and reaction where they are involved

Enzyme	Pathway/Reaction
<b>NAD dependent</b>	
Glyceraldehyde-3-phosphate dehydrogenase	Glycolysis: Glyceraldehyde-3 phosphate to 1,3-bisphosphoglycerate
Pyruvate dehydrogenase	Oxidative decarboxylation of pyruvate to acetyl-CoA
$\alpha$ -Ketoglutarate dehydrogenase	TCA cycle: $\alpha$ -ketoglutarate to succinyl-CoA
$\beta$ -Hydroxy acyl-CoA dehydrogenase	$\beta$ -Oxidation of fatty acid: $\beta$ - Hydroxy acyl-CoA to $\beta$ -Keto acyl-CoA
<b>NADP dependent</b>	
Glucose-6-phosphate dehydrogenase	Pentose phosphate pathway: Glucose 6-phosphate to 6-phosphogluconolactone
Malic enzyme	Transfer of acetyl-CoA from mitochondria to cytosol
<b>NADPH dependent</b>	
3-Ketoacyl reductase	Fatty acid synthesis: 3 Ketoacyl enzyme to 3-Hydroxyacyl enzyme
HMG CoA reductase	Cholesterol synthesis: HMG-CoA to Mevalonate

- Skin
- Gastrointestinal tract
- Central nervous system.
- The symptoms of pellagra are characterized by **three 'Ds'**:
  1. Dermatitis
  2. Diarrhea
  3. Dementia and if not treated death.

**Dermatitis:** Skin inflammation is seen in any area exposed to direct sunlight.

**Diarrhea:** Frequent diarrhea nausea, vomiting, anorexia are the disorders of GI tract.

**Dementia:** Dementia (loss of memory) is associated with degeneration of nervous tissues.

- To produce niacin deficiency, diet must be poor in both available niacin and tryptophan. Niacin deficiency occurs in:
  - Population dependent on maize (corn) or sorghum (*jowar*) as the staple food.
  - **Deficiency of vitamin B<sub>6</sub>** (pyridoxal phosphate) leads to **niacin deficiency** as it is involved as a coenzyme in the pathway of synthesis of niacin from tryptophan.
  - **Malignant carcinoid syndrome** in which tryptophan metabolism is diverted to formation of serotonin.
  - In **Hartnup disease**, a genetic disorder in which tryptophan absorption and transportation is impaired.

### Therapeutic Uses of Niacin

Nicotinic acid (not nicotinamide), used at high doses (1–2 gm/day), has been shown to lower total cholesterol, LDL cholesterol and VLDL triglyceride in patients with hyperlipoproteinemias.

### Toxicity

High intake of the vitamin has undesirable side effects, mainly vasodilation and flushing and liver damage.

### Pantothenic Acid (Vitamin B<sub>5</sub>)

The name pantothenic acid is derived from the Greek word '*pantothene*,' meaning from "*everywhere*" and gives an indication of the wide distribution of the vitamin in foods.

### Structure

Pantothenic acid is formed by a combination of *pantoic acid* and  $\beta$ -alanine (Figure 7.5).

### Active form

Active forms of pantothenic acid are:

- Coenzyme-A (CoA-SH)
- Acyl carrier protein (ACP).

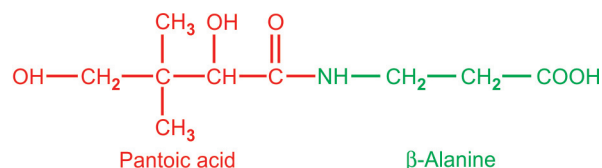


Figure 7.5: Structure of pantothenic acid



### Source

Eggs, liver, yeast, wheat germs, cereals, etc. are important sources of pantothenic acid, although the vitamin is widely distributed.

### Functions

- Pantothenic acid is a component of *coenzyme-A (CoA-SH)* and *acyl carrier protein (ACP)*. The thiol (-SH) group of CoA-SH and ACP acts as a carrier of acyl groups.
- Coenzyme-A participates in reactions concerned with:
  - Reactions of citric acid cycle
  - Fatty acid synthesis and oxidation
  - Synthesis of cholesterol
  - Utilization of ketone bodies.
- ACP participate in reactions concerned with fatty acid synthesis.

### Nutritional Requirement

The RDA of pantothenic acid is not well established. A daily intake of about 5–10 mg is advised for adults.

### Deficiency Manifestations

No clearcut case of pantothenic acid deficiency has been reported (because the substance is widely distributed in foods) except in malnourished prisoners of war in the far East in 1940s, where *neurological condition*, known as the *burning feet syndrome*, was reported and ascribed to pantothenic acid deficiency. As these people were severely malnourished and were deficient in other vitamins as well, it is not possible to attribute this specific effect to pantothenic acid deficiency. Clinical signs observed in experimentally induced deficiencies are:

- Paresthesia (abnormal tingling sensation)
- Headache
- Dizziness
- Gastrointestinal malfunction.

### Pyridoxine (Vitamin B<sub>6</sub>)

#### Structure

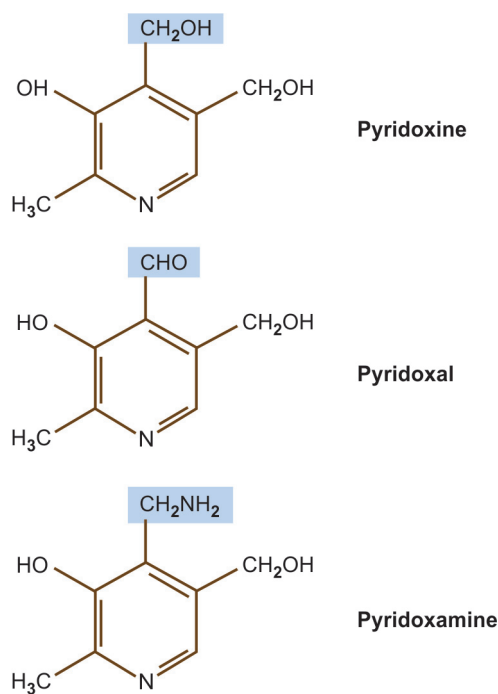
Vitamin B<sub>6</sub> consists of a mixture of three different closely related pyridine derivatives (**Figure 7.6**) namely:

1. Pyridoxine
2. Pyridoxal
3. Pyridoxamine.

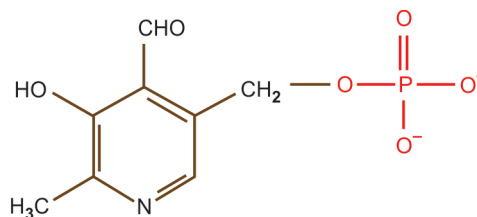
All the three have equal vitamin activity, as they can be interconverted in the body.

#### Active Form of Vitamin B<sub>6</sub>

**Pyridoxal phosphate (PLP)** is the active form of vitamin B<sub>6</sub> (**Figure 7.7**). PLP is formed from phosphorylation of all three forms of vitamin B<sub>6</sub>.



**Figure 7.6:** Structure of three different forms of vitamin B<sub>6</sub>



**Figure 7.7:** Structure of pyridoxal phosphate: an active form of vitamin B<sub>6</sub>

### Sources

- Pyridoxine occurs mainly in plants, whereas pyridoxal and pyridoxamine are present mainly in animal products.
- Major dietary sources of vitamin B<sub>6</sub> are yeast, unrefined cereals, pulses, meat, poultry fish, potatoes and vegetables.
- Dairy products and grains contribute lesser amounts.

### Functions

- Active form of vitamin B<sub>6</sub>, pyridoxal phosphate (PLP) acts as coenzyme in large number of reactions of amino acid metabolism. For example:
  - Transamination
  - Decarboxylation
  - Nonoxidative deamination
  - Trans-sulfuration
  - Condensation reactions of amino acids.

**Transamination reactions:** Transamination reactions are catalyzed by transaminases and PLP acts as coenzyme converting amino acid to keto acid, e.g. aspartate transaminase (AST) and alanine transaminase (ALT) (Figure 14.6).

**Decarboxylation reaction:** PLP acts as coenzyme in decarboxylation of some amino acids. The amino acids are decarboxylated to corresponding amines. The important biogenic amines synthesized by PLP decarboxylation are given below. (Figure 14.45).

- **$\gamma$ -Amino butyric acid (GABA):** It is an inhibitory neurotransmitter derived from glutamate on decarboxylation hence in vitamin B<sub>6</sub> deficiency underproduction of GABA leads to convulsions (epileptic seizures) in infants and children.
- **Serotonin and melatonin:** These are produced from tryptophan. Serotonin is a neurotransmitter and stimulates the cerebral activity. Melatonin is a sleep inducing substance and is involved in regulation of **circadian rhythm** of body.
- **Histamine:** Histamine produced by decarboxylation of histidine. It is a vasodilator and lowers blood pressure. It is involved in allergic reactions.
- **Catecholamines** (dopamine, norepinephrine and epinephrine) Synthesis of catecholamines from tyrosine requires PLP-dependent DOPA decarboxylase. Catecholamines are neurotransmitters and involved in metabolic and nervous regulation.

**Non-oxidative deamination:** Hydroxyl group containing amino acids (serine, threonine) are non-oxidatively deaminated to  $\alpha$ -keto acids and ammonia, which requires PLP. (Figure 14.9).

**Trans-sulfuration reaction:** PLP is a coenzyme for **cystathionine synthase** involved in synthesis of cysteine from methionine (Figure 14.25). In these reactions transfer of sulfur from methionine to serine occurs to produce cysteine.

**Condensation reactions:** Pyridoxal phosphate is required for the condensation reaction of L-glycine and succinyl CoA to form  $\delta$ -aminolevulinic acid, a precursor of heme (Figure 18.2).

**Other PLP-dependent reactions:**

- Pyridoxal phosphate is required for niacin coenzyme (NAD<sup>+</sup>/NADP<sup>+</sup>) synthesis from tryptophan (Figure 14.22).
- PLP is required for synthesis of serine from glycine (Figure 14.12).
- The enzyme glycogen phosphorylase contains covalently bound PLP.

- PLP is required for the synthesis of sphingosine, a component of sphingomyelin.

### Nutritional Requirement

The RDA for vitamin B<sub>6</sub> is **1.6 to 2.0 mg**. Requirements increase during pregnancy and lactation.

### Deficiency Manifestations

As pyridoxine occurs in most foods, the dietary deficiency of vitamin B<sub>6</sub> is rare. The main clinical symptoms of deficiency are given below:

- Vitamin B<sub>6</sub> deficiency causes neurological disorders such as **depression, nervousness and irritability**. These symptoms are due to decreased production of neurotransmitters, catecholamines, GABA and serotonin.
- Severe deficiency of pyridoxine causes epileptic seizures (convulsions) in infants due to reduced production of GABA.
- Demyelination of nerves causes peripheral neuropathy. Since vitamin B<sub>6</sub> is required for synthesis of sphingolipids needed for myelin formation.
- Vitamin B<sub>6</sub> deficiency causes **hypochromic microcytic anemia** due to decreased heme synthesis. Since PLP is required for the synthesis of heme.
- The commonest cause of pyridoxine deficiency is:
  - **Drug antagonism, e.g. isoniazide (INH)**, used in the treatment of tuberculosis and **penicillamide** used in the treatment of Wilson's disease and rheumatoid arthritis can combine with pyridoxal phosphate forming an inactive derivative with pyridoxal phosphate.
  - **Alcoholism:** Alcoholics may be deficient owing to metabolism of ethanol to acetaldehyde, which stimulates hydrolysis of the phosphate of the pyridoxal phosphate.

### Vitamin B<sub>6</sub> Assay

Activities of blood **transaminases** have been used frequently as indirect measurements of vitamin B<sub>6</sub> status. Erythrocyte levels of aspartate and alanine aminotransferase provide a better information of vitamin B<sub>6</sub> status.

### Therapeutic Uses

Pyridoxine is used for the treatment of:

- **Seizures.**
- **Down's syndrome**, a state of mental subnormality (incomplete development of mind) due to chromosomal defect.
- **Autism**, psychiatric disorder of childhood.
- **Premenstrual tension syndrome (PMS).**

### Toxicity

Pyridoxine seems to be safe at levels of 100–150 mg but taking 500–5000 mg per day, has shown peripheral neuropathy within 1–3 years.

### Biotin

Biotin was known formerly as vitamin H.

### Structure

Biotin is an imidazole derivative (**Figure 7.8**). It consists of a **tetrahydrothiophene** ring bound to an imidazole ring and a **valeric acid** side chain.

### Sources

- It is widely distributed in foods.
- Liver, kidneys, vegetables and egg yolk are the important sources of biotin.
- Biotin is also synthesized by intestinal bacteria.

### Active Form of Biotin

Enzyme-bound biotin, **biocytin** is an active form of biotin. Biotin is covalently bound to  $\epsilon$ -amino group of lysine of an enzyme to form **biocytin**.

### Functions

**Biotin is a coenzyme of carboxylase reactions**, where it is a carrier of  $\text{CO}_2$ . Some of the carboxylation reactions requiring biotin are given below.

- Conversion of acetyl-CoA into malonyl-CoA catalyzed by **acetyl-CoA carboxylase** in fatty acid synthesis (**Figure 13.17**).
- Conversion of pyruvate into oxaloacetate, catalyzed by **pyruvate carboxylase** in gluconeogenesis (**Figure 12.18**).
- Conversion of propionyl-CoA to D-methyl malonyl-CoA catalyzed by **propionyl-CoA carboxylase** in the pathway of conversion of propionate to succinate (**Figure 13.8**).
- It is also involved in the catabolism of branched chain amino acid catalyzed by  **$\beta$ -methyl-crotonyl-CoA carboxylase** (**Figure 14.32**).

### Biotin Independent Carboxylation Reaction

There are few carboxylation reactions which do not require biotin. For example:

- Formation of carbamoyl phosphate by **carbamoyl phosphate synthetase** in urea cycle.
- Addition of  $\text{CO}_2$  to form  $\text{C}_6$  in purine ring.
- Conversion of pyruvate to malate by malic enzyme.

### Nutritional Requirements

A daily intake of about 150–300  $\mu\text{g}$  is recommended for adults. Biotin is synthesized by intestinal micro-organisms in such a large quantities that a dietary source is probably not necessary.

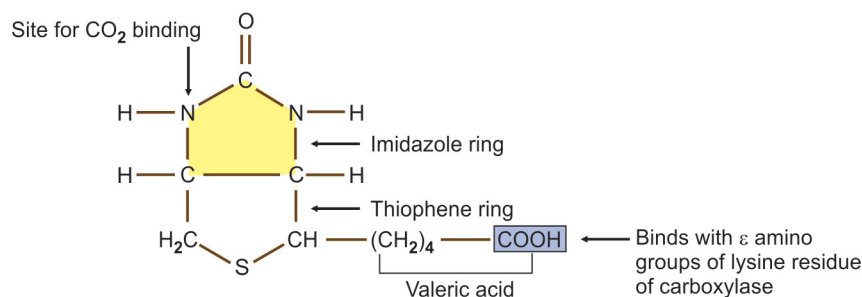
### Deficiency Manifestation

- Since biotin is widely distributed in plant and animal foods and intestinal bacterial flora supply adequate amounts of biotin, the natural deficiency of biotin is not well characterized in humans.
- The experimentally induced symptoms of biotin deficiency are nausea, anorexia, glossitis, dermatitis, alopecia (loss of hair), depression and muscular pain.
- Deficiency of biotin occurs in:
  - The people with the unusual dietary habit of consuming large amounts of uncooked eggs. Egg white contains the glycoprotein **avidin**, which binds the imidazole group of biotin and prevents biotin absorption.
  - Use of antibiotics, that inhibit the growth of intestinal bacteria, eliminates this source of biotin and leads to deficiency of biotin.

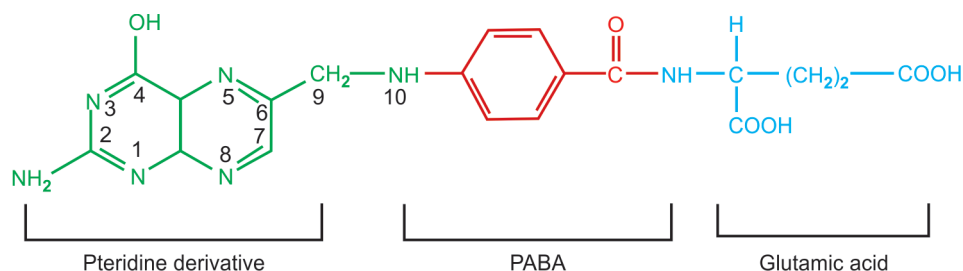
### Folic Acid

#### Structure

Folic acid consists of three components, **pteridine ring**, **p-amino benzoic acid (PABA)** and **L-glutamic acid** (**Figure 7.9**). In a folic acid molecule, the number of glutamic acid residues varies from one to seven. Folic acid usually has one glutamic acid residue.



**Figure 7.8:** Structure of biotin



**Figure 7.9:** The structure of folic acid

### Active Form of Folic Acid

**Tetrahydrofolate (THF)** is the active form of folic acid. Folate is enzymatically reduced in a two-stage process in tissues to yield the dihydro and then tetrahydrofolate, which requires vitamin C (**Figure 7.10**).

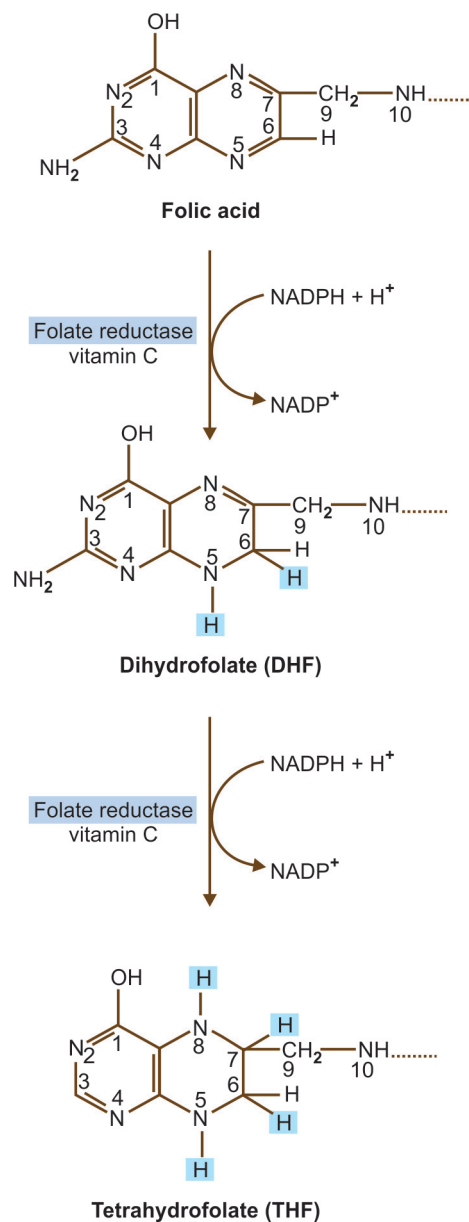
### Source

Folic acid is found in green leafy vegetables, liver, yeast. The word folate is related to folium which means *leaf* in Latin.

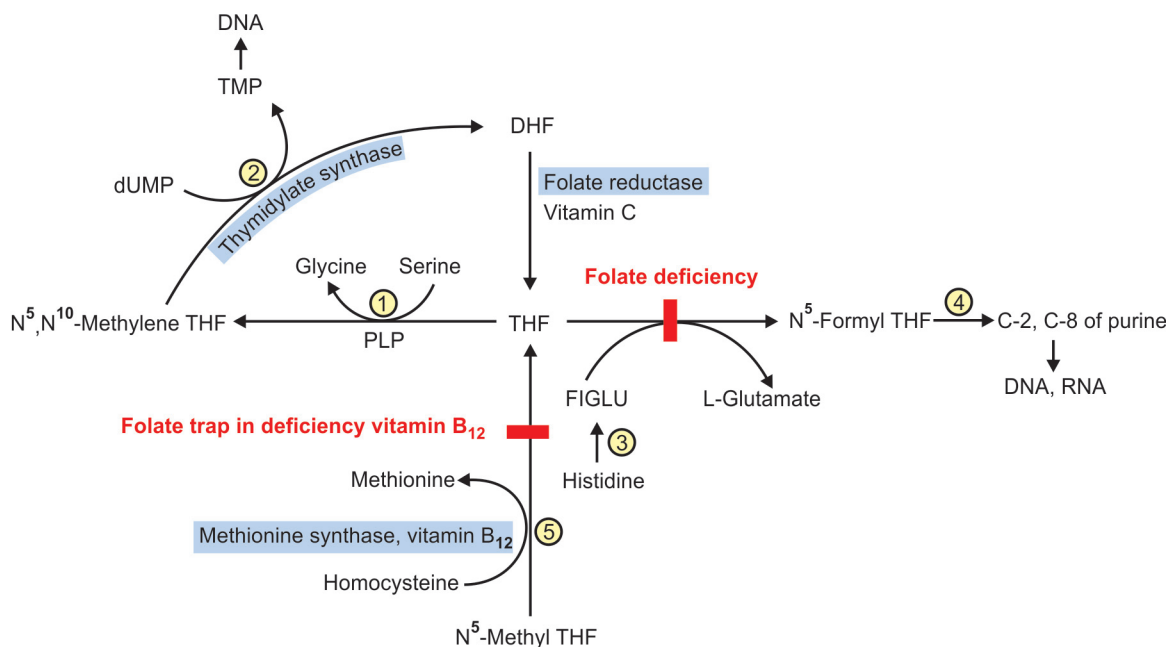
### Functions

- THF acts as a carrier of one carbon units. The one carbon units are:
 

Methyl	$\text{CH}_3$
Methylene	$\text{CH}_2$
Methenyl	$\text{CH}$
Formyl	$\text{CHO}$
Formimino	$\text{CH}=\text{NH}$
- One carbon unit binds to THF through  $\text{N}^5$  or  $\text{N}^{10}$  or both  $\text{N}^5$ ,  $\text{N}^{10}$  position. The THF coenzymes serve as acceptors or donors of one carbon units in a variety of reactions involved in amino acid and nucleic acid metabolism. Five of the major reactions in which THF is involved are given below (**Figure 7.11**).
  - Conversion of serine to glycine:** The conversion of serine to glycine is accompanied by the formation of  $\text{N}^5, \text{N}^{10}$ -methylene THF.
  - Synthesis of thymidylate (pyrimidine nucleotide):** The enzyme *thymidylate synthase* that converts deoxyuridylate (dUMP) into thymidylate (TMP) uses  $\text{N}^5, \text{N}^{10}$ -methylene THF as the methyl donor for this reaction. Thus, folate coenzyme plays a central role in the biosynthesis of **nucleic acids**.
  - Catabolism of histidine :** Histidine in the course of its catabolism is converted into *formimino-glutamate (FIGLU)*. This molecule can donate



**Figure 7.10:** Formation of tetrahydrofolate from folic acid



**Figure 7.11 :** Role of folic acid in one carbon metabolism  
 DHF: Dihydrofolate; FIGLU: Formiminoglutamate; THF: Tetrahydrofolate; dUMP: Deoxyuridine monophosphate; TMP: Thymidine monophosphate; PLP: Pyridoxal phosphate

the formimino group to THF to produce N<sup>5</sup> formimino THF. In case of folic acid deficiency, FIGLU accumulates and is excreted in urine.

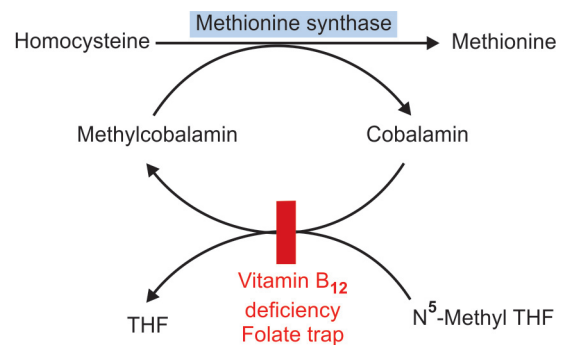
4. **Synthesis of purine:** N<sup>5</sup>-Formyl THF intermediate formed in histidine catabolism is used in the biosynthesis of purine and therefore in the formation of both DNA and RNA.
5. **Synthesis of methionine from homocysteine:** Homocysteine is converted to methionine in presence of N<sup>5</sup>-methyl THF, and vitamin B<sub>12</sub>.

In this reaction the methyl group bound to cobalamin (Vitamin B<sub>12</sub>) is transferred to homocysteine to form methionine and the cobalamin then removes the methyl group from N<sup>5</sup>-methyl THF to form THF (**Figure 7.12**).

This step is essential for the liberation of free THF and for its repeated use in one carbon metabolism. In B<sub>12</sub> deficiency, conversion of N<sup>5</sup>-methyl THF to free THF is blocked.

### Nutritional Requirements

- The RDA of folate is **200 µg**.
- Requirements increase during pregnancy and lactation.



**Figure 7.12:** The combined roles of vitamin B<sub>12</sub> and folate in the synthesis of methionine

### Deficiency Manifestations

Folate deficiency frequently occurs particularly in pregnant women and in alcoholics. Clinical symptoms of folic acid deficiency include:

- **Megaloblastic or macrocytic anemia:**  
 The deficiency of folic acid leads to impairment of synthesis of DNA. Impaired DNA synthesis, impairs the maturation of erythrocytes. Consequently,



megaloblasts are formed instead of normoblast. These megaloblasts are accumulated in the bone marrow and leads to megaloblastic anemia.

- **Accumulation and excretion of FIGLU in the urine:** Folate deficiency blocks the last step of histidine catabolism, due to lack of THF. This results in accumulation of FIGLU in body, which leads to increased excretion of FIGLU in urine (Figure 7.13).

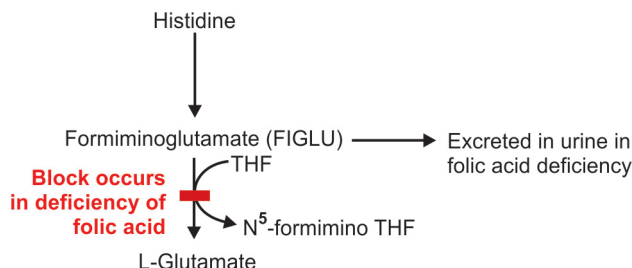


Figure 7.13: Excretion of FIGLU in folic acid deficiency

- **Hyperhomocysteinemia:** Due to folic acid deficiency the methylation of homocysteine to methionine is impaired which leads to hyperhomocysteinemia. Increased level of homocystein is a risk factor for cardiovascular disease.
- **Neural tube defect in fetus:** Since, folate is required for the formation of neural tube in early stage of gestation, the folate deficiency during early stage of pregnancy increases the risk of neural tube defect.

### Therapeutic Uses

N<sup>5</sup>-methyl THF called **folinic acid** or **citrovorum factor** is used as therapeutic drug to overcome the folate deficiency.

### Cobalamin (Vitamin B<sub>12</sub>)

#### Structure

Vitamin B<sub>12</sub> bears a complex *corrin ring* (containing pyrroles similar to porphyrin), linked to a *cobalt atom* held in the center of the corrin ring, by four coordination bonds with the nitrogen of the pyrrole groups. The remaining coordination bonds of the cobalt are linked with the nitrogen of *dimethylbenzimidazole nucleotide* and sixth bond is linked to either *methyl* or *5'-deoxyadenosyl* or *hydroxy* group to form *methylcobalamin*, *adenosylcobalamin* or *hydroxycobalamin* respectively (Figure 7.14).

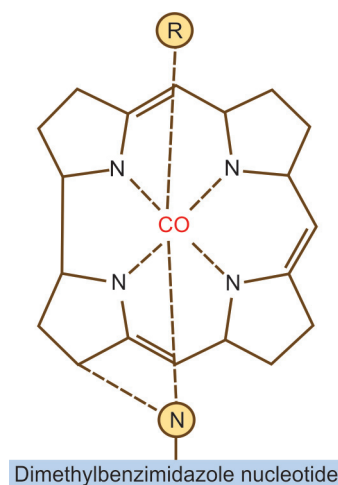


Figure 7.14: Structure of cobalamin (vitamin B<sub>12</sub>)  
R: Either methyl or deoxyadenosyl or hydroxy group

Thus, cobalamin exists in three forms that differ in the nature of the chemical group attached to cobalt. *Cynocobalamin* is the commercial available form of vitamin B<sub>12</sub>.

#### Active form of Vitamin B<sub>12</sub>

The active coenzyme forms of vitamin B<sub>12</sub> are:

- Methylcobalamin
- Deoxyadenosylcobalamin.

#### Sources

- *Dietary sources of vitamin B<sub>12</sub> are of animal origin* and include meat, eggs, milk, dairy products, fish, poultry, etc.
- *Vitamin B<sub>12</sub> is absent in plant foods.*
- Humans obtain small amounts of vitamin B<sub>12</sub> from their intestinal flora.

#### Absorption, Transport and Storage

- The intestinal absorption of vitamin B<sub>12</sub> requires an **intrinsic factor (IF)**, a glycoprotein secreted by parietal cells of the stomach.
- In stomach IF binds the dietary vitamin B<sub>12</sub> to form **vitamin B<sub>12</sub>-IF complex**. This complex binds to specific receptors on the surface of the mucosal cells of the ileum.
- After binding to the receptor, the bound vitamin B<sub>12</sub> is released from the complex and enters the ileal mucosal cells through a Ca<sup>2+</sup> dependent process.
- The vitamin in mucosal cell is converted into its main plasma transport form to **methylcobalamin**. It is then transported by a vitamin B<sub>12</sub> binding protein known as **transcobalamin (TC-I and TC-II)**.

- Methylcobalamin which is in excess is taken up by the liver, **stored in deoxyadenosyl B<sub>12</sub>** form.
- Liver can store about 4-5 mg of vitamin B<sub>12</sub> in adults, an amount sufficient to meet the body requirements of vitamin B<sub>12</sub> for 3–6 years.
- Vitamin B<sub>12</sub> is the only water soluble vitamin that is stored in significant amounts in the liver.

### Functions

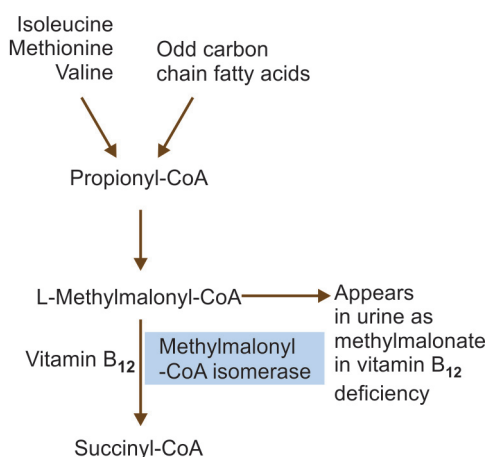
There are only two human enzyme systems that are known to require vitamin B<sub>12</sub> coenzyme.

#### 1. Isomerization of methylmalonyl-CoA to succinyl-CoA

- Propionyl-CoA is produced as catabolic end product of some aliphatic amino acids and in  $\beta$ -oxidation of odd chain fatty acids. The propionyl-CoA is then converted to succinyl-CoA.
- During conversion of propionyl-CoA to succinyl-CoA vitamin B<sub>12</sub> coenzyme, deoxyadenosyl cobalamine is required for the isomerization of L-methylmalonyl-CoA to succinyl-CoA.
- In vitamin B<sub>12</sub> deficiency methylmalonyl CoA accumulates and is excreted in urine as methylmalonic acid (**Figure 7.15**).

#### 2. Conversion of homocysteine to methionine

- Methylcobalamin is a coenzyme in the conversion of homocysteine to methionine, which joins the metabolic roles of vitamin B<sub>12</sub> and those of folic acid (for explanation see functions of folic acid and **Figure 7.12**). This is the only mammalian reaction known to require both vitamins.



**Figure 7.15:** Role of vitamin B<sub>12</sub> in the metabolism of propionyl-CoA

### Nutritional Requirements

RDA for adult is **3 µg** with higher allowances for pregnancy and lactating women.

### Deficiency Manifestations

Deficiency may arise due to decreased absorption or decreased dietary intake. Dietary deficiency is seen in strict vegetarians, since the vitamin found only in foods of animal origin or in microorganisms. Deficiency of vitamin B<sub>12</sub> leads to:

- Pernicious anemia
- Megaloblastic anemia
- Methylmalonic aciduria
- Neuropathy.

1. **Pernicious anemia:** It is caused by a deficiency of **intrinsic factor** in the stomach, which leads to impaired absorption of vitamin B<sub>12</sub>. It is characterized by megaloblastic anemia and **low hemoglobin** level with **neurological** disorders.

2. **Megaloblastic anemia:** It occurs due to **functional folate deficiency**. The functional folate deficiency is seen in vitamin B<sub>12</sub> deficiency due to folate trap (**Figure 7.12**).

3. **Methylmalonic aciduria:** Because vitamin B<sub>12</sub> is necessary for the conversion of methylmalonic acid to succinic acid, individuals deficient in vitamin B<sub>12</sub> excrete excess amounts of methylmalonic acid in the urine (**Figure 7.15**).

4. **Neuropathy:** In vitamin B<sub>12</sub> deficiency, many neurological symptoms appear due to progressive degeneration of myelinated nerves. Degeneration of myelinated nerves is due to accumulation of L-methylmalonyl-CoA, which impairs the myelin sheath formation. The neurological symptoms include numbness and tingling of fingers and toes, mental confusion, poor muscular coordination and dementia.

#### FOLATE TRAP

- Methylation of homocysteine to methionine depends on vitamin B<sub>12</sub> and N<sup>5</sup>-methyl THF. When vitamin B<sub>12</sub> is deficient N<sup>5</sup>-methyl THF cannot be converted to free THF. *Thus, most of folic acid of the body is irreversibly "trapped" as its methyl derivative (N<sup>5</sup>-methyl THF). This is called folate trap (Figure 7.12).*

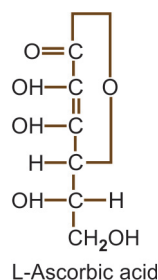
Folate trap creates folate deficiency and an adequate supply of free THF is not available for the synthesis of purine and pyrimidine bases. Thus, a B<sub>12</sub> deficiency can lead to a folate deficiency. Although the tissue folate levels are adequate or high, there is a functional folate deficiency due to the lack of THF.



## Vitamin C (Ascorbic Acid)

### Structure

Vitamin C is also known as *ascorbic acid*. It is a six-carbon sugar derivative (**Figure 7.16**). Most animals can synthesize ascorbic acid. But humans cannot synthesize ascorbic acid, due to lack of the enzyme *gluconolactone oxidase* which is required for the synthesis of ascorbic acid. Thus, humans have a dietary requirement of ascorbic acid.



**Figure 7.16:** Structure of ascorbic acid

### Active Form of Ascorbic Acid

Ascorbic acid itself is an active form.

### Sources

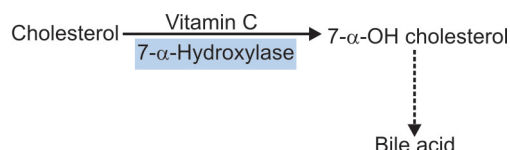
- The main dietary sources of vitamin C are leafy vegetables and fruits, especially citrus fruits, strawberries, tomatoes, spinach and potatoes.
- Cereals contain no vitamin C.
- Animal tissues and dairy products are very poor sources.

### Functions

Ascorbic acid functions as a reducing agent in many metabolic processes as follows:

- **Collagen biosynthesis:** Vitamin C is required for formation of collagen, where it is needed for the hydroxylation of *proline* and *lysine* residues, of procollagen. Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and collagen strength and stability. Since vitamin C is required for normal collagen formation, vitamin C is also involved in bone and dentin formation as well as wound healing process.
- **Steroid synthesis:** In adrenal cortex, vitamin C is involved in the hydroxylation reactions of steroids.

- **Adrenaline synthesis:** In adrenal medulla it serves as a reducing agent in hydroxylation reactions in the synthesis of adrenaline and noradrenaline from tyrosine.
- **Carnitine synthesis:** Vitamin C functions in the hydroxylation of  $\gamma$ -butyrobetaine to carnitine.
- **Bile acid formation:** Vitamin C is required for the hydroxylation of cholesterol in bile acid synthesis.



- **Degradation of tyrosine:** The oxidation of P-hydroxyphenylpyruvate to homogentisate requires vitamin C. The subsequent step is catalyzed by homogentisate oxidase, which is a ferrous ion containing enzyme that also requires vitamin C.
- **Folate metabolism:** Folic acid is converted to its active form tetrahydrofolate (THF) with the help of vitamin C (**See Figure 7.10**).
- **Absorption of iron:** Ascorbic acid facilitates the absorption of iron from intestine by reducing it to the  $\text{Fe}^{++}$  (ferrous) state.
- **Ascorbic acid is a water soluble antioxidant:** (Ascorbic acid is a strong reducing agent and acts as an antioxidant).
  - It reduces oxidized vitamin E (tocopherol) to regenerate functional vitamin E.
  - Vitamin C, thought to be involved in the prevention of atherosclerosis and coronary heart disease by preventing oxidation of LDL.
  - Antioxidant property of vitamin C is also associated with prevention of cancer by inhibiting nitrosamine formation from naturally occurring nitrates during digestion.

### Nutritional Requirements

The recommended daily allowance is about **60–70 mg**. Additional intakes are recommended for women during pregnancy and lactation.

### Deficiency Manifestation

Deficiency of ascorbic acid causes *scurvy*. Symptoms of scurvy are related to *deficient collagen formation* (Refer functions of vitamin C). These include:

- Spongy, swollen, bleeding gums, loosening of teeth
- Abnormal bone development and osteoporosis
- Poor wound healing
- Anemia due to impaired erythropoiesis
- Easy bruising and bleeding due to fragile capillaries.

### Therapeutic Uses

Use of vitamin C in preventing cold and cancers has not been scientifically supported. Although the incidence of common cold is not reduced by vitamin C, the duration of cold episodes and severity of symptoms can be decreased.

### Toxicity

Vitamin C can be taken in doses up to 2–3 g/day without undesirable effects. Above these levels, however, it cannot be absorbed from the intestine and can cause severe diarrhea and deposition of oxalate stones in kidneys.

## FAT SOLUBLE VITAMINS

### Vitamin A

#### Structure

Vitamin A contains a single 6-membered ring to which is attached an 11-carbon side chain (Figure 7.17). Vitamin A is an *alcohol (retinol)*, but can be converted into an *aldehyde (retinal)*, or an *acid (retinoic acid)*.

#### Active Form

Vitamin A consists of three biologically active molecules which are collectively known as **retinoids**.

1. Retinol: Primary alcohol ( $\text{CH}_2\text{OH}$ ) containing form
  2. Retinal: Aldehyde ( $\text{CHO}$ ) containing form
  3. Retinoic acid: Carboxyl ( $\text{COOH}$ ) containing form
- Each of these compounds are derived from the plant precursor molecule,  **$\beta$ -carotene** (a member of a family of molecules known as carotenoids).

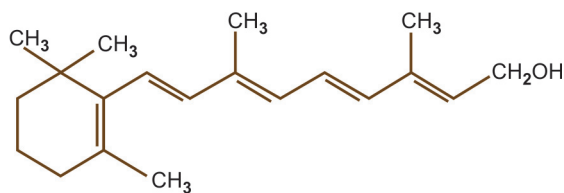


Figure 7.17: Structure of vitamin A, retinol

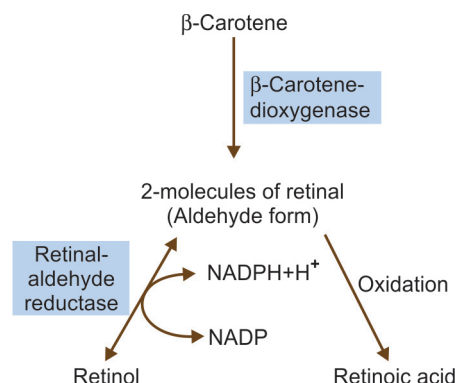


Figure 7.18: Conversion of  $\beta$ -carotene (provitamin) to biologically active forms of vitamin A

- $\beta$ -carotene which consists of two molecules of retinal linked at their aldehyde ends is also referred to as the *provitamin* form of vitamin A.
- The retinol and retinal are interconverted by enzyme **retinal aldehyde reductase**. The retinoic acid is formed by oxidation of retinal. The retinoic acid cannot be reduced to either retinol or retinal (Figure 7.18).

### Absorption, Transport and Storage

- Ingested  $\beta$ -carotene is cleaved in the intestine by  **$\beta$ -carotene dioxygenase** to yield retinal. Retinal is reduced to retinol by **retinaldehyde reductase**, an NADPH requiring enzyme within the intestine (Figure 7.18).
- Retinol is esterified with palmitic acid incorporated into chylomicrons together with dietary lipid and delivered to the liver for storage.
- Transport of retinol from the liver to extrahepatic tissues, occurs by binding of retinol to **retinol binding protein (RBP)**.
- Transport of retinoic acid is accomplished by binding to albumin.

### Sources

- The richest dietary sources of vitamin A are fish liver oils (cod liver oil). Animal livers are also rich sources but meat is rather low in vitamin A.
- Other good sources are milk and dairy products, dark-green leaves, such as spinach and yellow and red fruits and vegetables, such as carrots, tomatoes, and peaches.

### Functions of Vitamin A

- Vitamin A is required for a variety of functions such as vision, cell differentiation and growth, mucus secretion, maintenance of epithelial cells, etc.
- The role of vitamin A in vision has been known through the studies of **G Wald**, who received the Nobel prize in 1943 for this work.
- Different forms of the vitamin have different functions.
  - Retinal and retinol are involved in vision.
  - Retinoic acid is involved in cellular differentiation and metabolic processes.
  - $\beta$ -carotene is involved in antioxidant function.

### Role of Vitamin A in Vision

The cyclic events occur in the process of vision, known as *rhodopsin cycle* or *Wald's visual cycle* (Figure 7.19). Both rod and cone cells of retina contain a photoreceptor pigment in their membrane and vitamin A is a component of these pigments. *Rhodopsin* or *visual purple*, the visual pigment of rod cells in the retina consists of **11-cis-retinal** bound to protein *opsin*.

- When rhodopsin absorbs light, the 11-cis-retinal is converted to all-trans retinal.
- The isomerization is associated with a conformational change in the protein opsin.
- Conformational changes in opsin generates a nerve impulse that is transmitted by the optic nerve to the brain.
- This is followed by dissociation of the all-trans retinal from opsin.
- The all-trans retinal is immediately isomerized by retinal isomerase to 11-cis-retinal.
- This combines with opsin to regenerate rhodopsin and complete the visual cycle.

The conversion of all-trans retinal to 11-cis-retinal is incomplete and therefore remaining all-trans retinal which is not converted to 11-cis-retinal is converted to all-trans retinol by *alcohol dehydrogenase* and is stored in the liver. When needed, retinol re-enters the circulation and is taken up by the retina, where it is converted back to 11-cis-retinal which combines with opsin again to form rhodopsin (Figure 7.19).

**Dark adaptation time:** When a person enters from bright light to dark there is difficulty in seeing due to depletion of rhodopsin, but after few minutes the vision improves. During these few minutes, rhodopsin is resynthesized and vision is improved. The time taken for regeneration of rhodopsin is known as **dark adaptation time**. Dark adaptation time is increased in vitamin A deficient individuals.

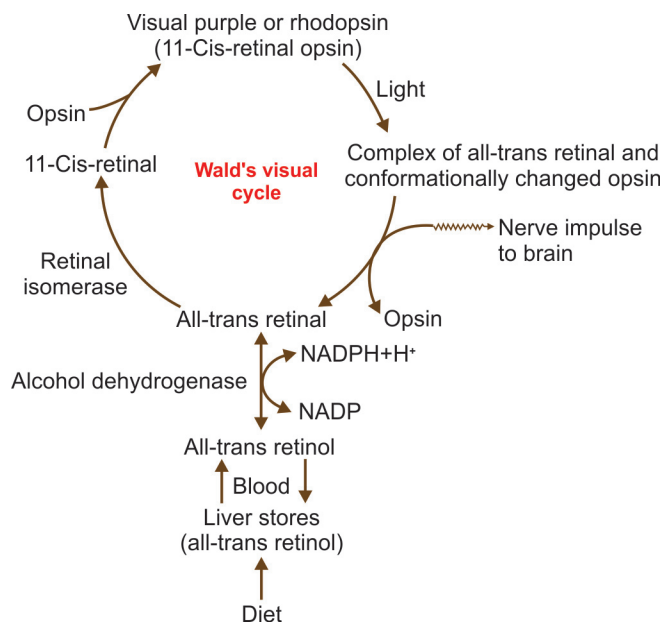


Figure 7.19: Wald's visual cycle

### Color Vision

- While vision in dim light is mediated by rhodopsin of the rod cells, color vision is mediated by three different retinal containing pigments in the cone cells, the three pigments are called *porphyropsin*, *iodopsin* and *cyanopsin* and are sensitive to the three essential colors: **red**, **green** and **blue** respectively. All these pigments consist of **11-cis-retinal** bound to protein *opsin*.
- Thus, when light strikes the retina, it bleaches one or more of these pigments, depending on the color quality of the light. The pigments are converted to all-trans retinal, and the protein moiety opsin is released as in the case of rhodopsin. This reaction gives rise to the nerve impulse that is read out in the brain as color:
  - Red if porphyropsin is split
  - Green if iodopsin is split
  - Blue if cyanopsin is split.
- If mixtures of the three are converted, the color read out in the brain depends on the proportions of the three split.

### Cellular Differentiation and Metabolic Effect

- **Retinoic acid** is an important regulator of **gene expression** especially during growth and development. Retinoic acid is essential for normal gene expression during embryonic development such as

cell differentiation in spermatogenesis and in the differentiation of epithelial cells.

- Retinoic acids exert a number of metabolic effects on tissues. These include:
  - Control of biosynthesis of membrane *glycoproteins* and *glycosaminoglycans* (mucopolysaccharide) necessary for mucus secretion. The normal mucus secretion maintains the epithelial surface moist and prevents keratinization of epithelial cell.
  - Control of biosynthesis of *cholesterol*.

#### Antioxidant Function

- $\beta$ -carotene is an antioxidant and may play a role in trapping peroxy free radicals in tissues.
- The antioxidant property of lipid soluble vitamin A may account for its possible anticancer activity.
- High levels of dietary carotenoids have been associated with a decreased risk of cardiovascular disease.

#### Nutritional Requirements

The RDA of vitamin A for adults is **800–1000 retinol equivalents**. (1 retinol equivalent = 1  $\mu\text{g}$  retinol = 6  $\mu\text{g}$   $\beta$ -carotene).

#### Deficiency Manifestation

Clinically, degenerative changes in eyes and skin are observed with vitamin A deficiency.

#### Effect on Vision

##### Night blindness (nyctalopia)

- Night blindness is one of the earliest symptoms of vitamin A deficiency. This is characterized by loss of vision in night (in dim or poor light) since **dark adaptation time** is increased. Prolonged deficiency of vitamin A leads to an irreversible loss of visual cells.
- Severe vitamin A deficiency causes dryness of cornea and conjunctiva, a clinical condition termed as **xerophthalmia** (dry eyes).
- If this situation prolongs, **keratinization** and **ulceration** of cornea takes place. This results in destruction of cornea. The cornea becomes totally opaque resulting in permanent loss of vision (blindness), a clinical condition termed as **keratomalacia**. Xerophthalmia and keratomalacia are commonly observed in children.
- White opaque spots develop on either side of cornea in vitamin A deficiency are known as **Bitot's spot**.

#### Effect on Skin and Epithelial Cells

- Vitamin A deficiency causes keratinization of epithelial cells of skin which leads to keratosis of hair follicles, and dry, rough and scaly skin.
- Keratinization of epithelial cells of respiratory, urinary tract makes them susceptible to infections.

#### Other Symptoms of Vitamin A Deficiency

- Failure of growth in children.
- Faulty bone modelling producing thick cancellous (spongy) bones instead of thinner and more compact ones.
- Abnormalities of reproduction, including degeneration of the testes, abortion or the production of malformed offspring.

#### Therapeutic Use of Vitamin A

- The use of retinoic acid preparations, in the treatment of psoriasis, acne and several other skin diseases, is related to its involvement with epithelial cell differentiation and integrity
- Some precancerous lesions seem to respond to treatment with carotenoids.

#### Hypervitaminosis A

- The symptoms of hypervitaminosis A include nausea, vomiting, diarrhea, loss of hair (alopecia), scaly and rough skin, bone and joint pain, enlargement of liver, loss of weight, etc.
- In pregnant women, the hypervitaminosis A may cause congenital malformation in growing fetus (teratogenic effect).
- The excess intake of carotenoids is not toxic like vitamin A.

#### Why Vitamin A is Considered as a Hormone?

- Within cells both retinol and retinoic acid function by binding to specific receptor proteins present in the nucleus of target tissues.
- Following binding, the receptor-vitamin complex interacts with several genes involved in growth and differentiation and affects expression of these genes. In this capacity, retinol and retinoic acid are considered as hormones.

#### Vitamin D (Cholecalciferol)

Vitamin D is also known as **calciferol** because of its role in calcium metabolism and **antirachitic factor** because it prevents rickets.

*Vitamin D could be thought of as a hormone rather than a vitamin because:*

- As it can be synthesized in the body
- It is released in the circulation
- Has distinct target organs
- Action of vitamin D is similar to steroid hormones. It binds to a receptor in the cytosol. Following binding, the receptor vitamin complex interacts with DNA to stimulate the synthesis of calcium binding protein.

### Structure

Vitamin D is a steroid compound. There are two forms of vitamin D.

1. The naturally produced  $D_3$  or **cholecalciferol** (Figure 7.20), is the form obtained from animal sources in the diet, or made in the skin by the action of ultraviolet light from sunlight on **7-dehydrocholesterol** (Figure 7.21).
2. Artificially produced form  $D_2$  or **ergocalciferol**, is the form made in the laboratory by irradiating the plant sterol, **ergosterol**.

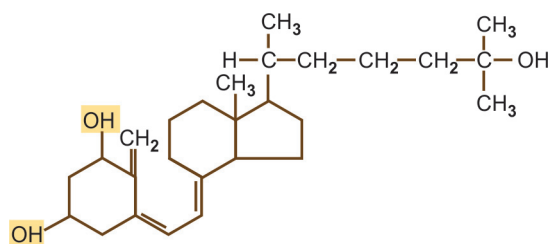


Figure 7.20: Structure of 1,25-dihydroxycholecalciferol: an active form of vitamin  $D_3$

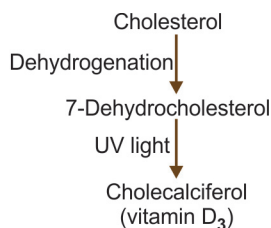


Figure 7.21: The formation of vitamin  $D_3$  in the body

### Absorption, Transport and Activation of Vitamin D

Exogenous or dietary vitamin D is absorbed in the duodenum along with lipids. It is transported to the liver through chylomicron.

#### Active Form of Vitamin

Cholecalciferol is an inactive form of vitamin D. It needs further metabolism to produce the active form of the vitamin. **1,25 dihydroxycholecalciferol** also known as **calcitriol** is the active form of vitamin D. The steps involved in activation are:

1. The first step is the conversion of cholecalciferol to 25-hydroxycholecalciferol. The **25-hydroxylation occurs in liver** and is catalyzed by **hydroxylase**.

2. The 25-hydroxycholecalciferol formed transported to the kidney, where it is further hydroxylated by, **1,α-hydroxylase enzyme** in the 1-position to 1,25 dihydroxycholecalciferol. (Figure 7.22).

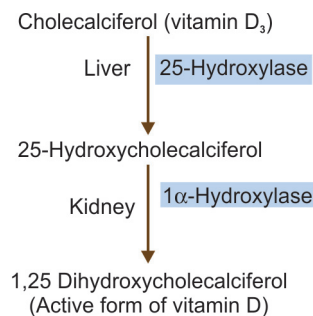


Figure 7.22: Activation of vitamin D

### Sources

- Best sources are cod liver oil and often fish oils and sunlight induced synthesis of vitamin  $D_3$  in skin.
- Egg yolk and liver are good sources.

### Functions

Vitamin D (Calcitriol) plays an essential role as a hormone in the regulation of **calcium** and **phosphorus** metabolism.

It maintains the normal plasma level of calcium and phosphorus by acting on **intestine**, **kidneys** and **bones** (Figure 7.23).

#### Action of calcitriol on intestine

It increases the plasma calcium and phosphorus concentration by stimulating the absorption of calcium and phosphorus from the intestine by enhancing the synthesis of calcium binding proteins **calbindins**. This protein increases the calcium uptake by the intestine.

#### Action of calcitriol on kidney

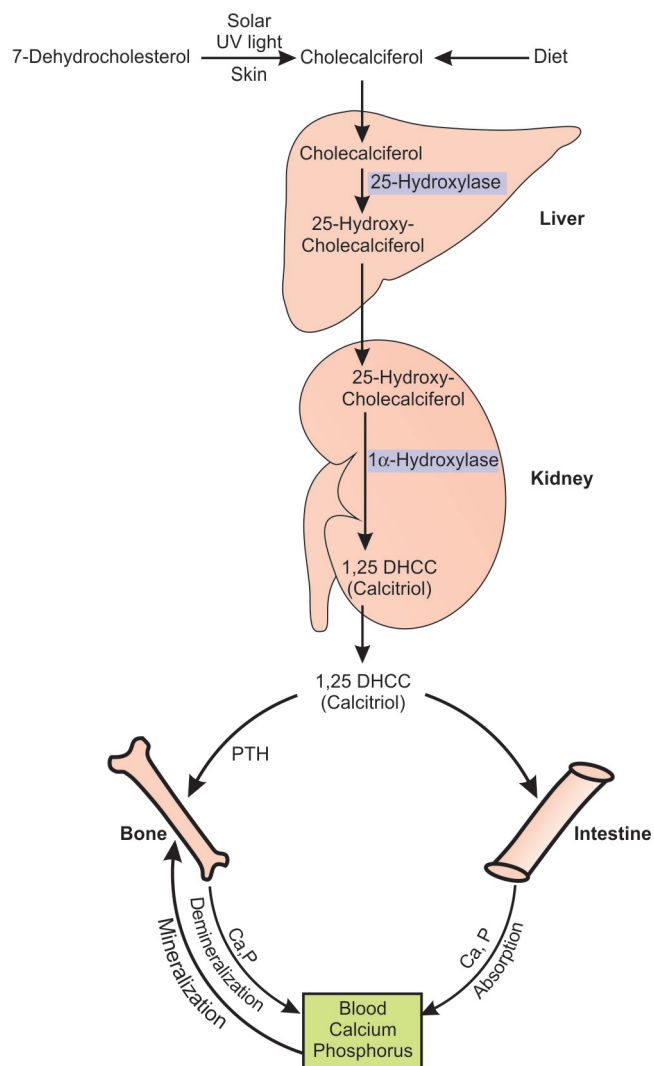
It stimulates the reabsorption of calcium and phosphorus from the kidney and decreases their excretion.

#### Action of calcitriol on bone

It is believed that calcitriol has both **anabolic** and **catabolic** role on bone.

- Calcitriol promotes the mineralization of bones by deposition of calcium and phosphorus.
- Calcitriol along with PTH stimulates the mobilization of calcium and phosphorus from bone by stimulating the synthesis of **osteocalcin** (a calcium binding protein in bone). This causes elevation of plasma calcium and phosphorus levels.





**Figure 7.23:** Metabolism and functions of Vitamin D where 1,25 DHCC: 1,25 dihydroxycholecalciferol; PTH: Parathyroid hormone

### Nutritional Requirement

The daily requirements of vitamin D is **200–400 IU**.

### Deficiency Manifestation

Deficiency of vitamin D causes **rickets (rachitis)** in growing children and **osteomalacia** in adults.

### Rickets

- Rickets is characterized by formation of soft and pliable bones due to poor mineralization and calcium deficiency. Due to softness, the weight bearing bones are bent and deformed.



**Figure 7.24:** Bowing of legs in rickets

- The main features of the rickets are, a large head with protruding forehead, pigeon chest, bow legs, (curved legs), knock knees (**Figure 7.24**) and abnormal curvature of the spine (kyphosis).
- Rachitic children are usually anemic or prone to infections. Rickets can be fatal when severe.
- Rickets is characterized by low plasma levels of calcium and phosphorus and high alkaline phosphatase activity.

### Osteomalacias (Adult Rickets)

- The deficiency of vitamin D in adults causes **osteomalacia**. This is a condition similar to that of rickets.
- Osteomalacia characterized by demineralization of previously formed bones, Demineralization of bones makes them soft and susceptible to fractures.

### Renal Rickets (Renal Osteodystrophy)

In chronic renal failure synthesis of calcitriol in kidney is impaired. As a result, the deficiency of calcitriol occurs which leads to hypocalcemia and hyperphosphatemia. It can be treated by oral or intravenous administration of calcitriol (active form of vitamin D).

### Vitamin D Resistant Rickets

As the name implies, this is a disease which does not respond to treatment with vitamin D. There are various possible causes of this condition and all involve a defect in the metabolism or mechanism of action of 1,25 dihydroxycholecalciferol as follows:

- Due to **defective vitamin D receptor**

- Due to a *defective 1,  $\alpha$ -hydroxylase activity in kidney*
- Due to liver disease and kidney failure as the production of 25-hydroxycholecalciferol and 1,25 dihydroxycholecalciferol respectively will be inefficient in the damaged tissue.

### Hypervitaminosis D

High doses of vitamin D over a long period are toxic.

- The early symptoms of hypervitaminosis D include nausea, vomiting, anorexia, increased thirst, loss of weight, etc.
- Hypercalcemia is seen due to increased bone resorption and intestinal absorption of calcium.
- The prolonged hypercalcemia causes calcification of soft tissues and organs such as kidney and may lead to formation of stones in the kidneys.

### Therapeutic Use

Vitamin D analogues have been used in the treatment of psoriasis.

## Vitamin E (Tocopherol)

### Structure

Vitamin E consists of eight naturally occurring tocopherols, of which  $\alpha$ -tocopherol is the most active form (Figure 7.25).

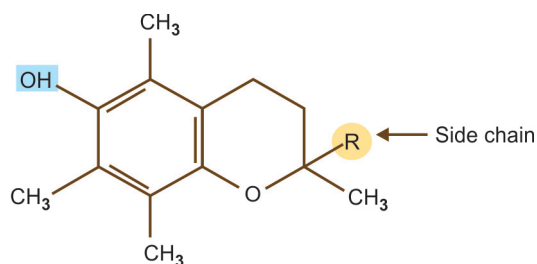


Figure 7.25: Structure of  $\alpha$ -tocopherol

### Sources

The major dietary sources of vitamin E are fats and oils. The richest sources are germ oil, corn oil, fish oil, eggs, lettuce and alfalfa.

### Absorption, Transport and Storage

Vitamin E is absorbed from intestine together with dietary lipid. It is incorporated in chylomicrons. It is delivered to the liver via chylomicron. The liver can export vitamin E into very low density lipoprotein (VLDL) to target cells. In cells, tocopherols are

distributed where antioxidant activity is required. *The major site of vitamin E storage is in the adipose tissue.*

### Functions

- Vitamin E acts as a natural antioxidant by scavenging free radicals and molecular oxygen.
- Vitamin E is important for preventing peroxidation of polyunsaturated fatty acids in cell membranes.
- Protection of erythrocyte membrane from oxidant is the major role of vitamin E in humans. It protects the RBCs from hemolysis.
- Vitamin E also helps to prevent oxidation of LDL. Oxidized LDL may be more atherogenic than native LDL and thus vitamin E may protect against atherosclerotic coronary heart disease.
- Whether vitamin E affects human fertility is unknown.
- In animals, vitamin E is required for normal reproduction and prevents sterility.

### Nutritional Requirements

A daily consumption of about:

- 10 mg (15 IU) of  $\alpha$ -tocopherol for a man
- 8 mg (12 IU) for a woman is recommended. One mg of  $\alpha$ -tocopherol is equal to 1.5 IU.

### Deficiency Manifestation

Vitamin E deficiency in humans is rare.

- The major symptom of vitamin E deficiency in human is *hemolytic anemia* due to an increased red blood cell fragility.
- Another symptom of vitamin E deficiency is *retrolental fibroplasia (RLF)* observed in some premature infants of low birth weight. Children with this defect show *neuropathy*.

### Hypervitaminosis E

Unlike other fat soluble vitamins such as A and D, vitamin E does not seem to have toxic effects.

## Vitamin K

This vitamin is called an *anti-hemorrhagic factor* as its deficiency produced uncontrolled hemorrhages due to defect in blood coagulation.

In 1929, H Dam gave the name coagulation vitamin from the Danish word *koagulation*. It is now called vitamin K.

### Structure (Figure 7.26)

- There are two naturally occurring forms of vitamin K:



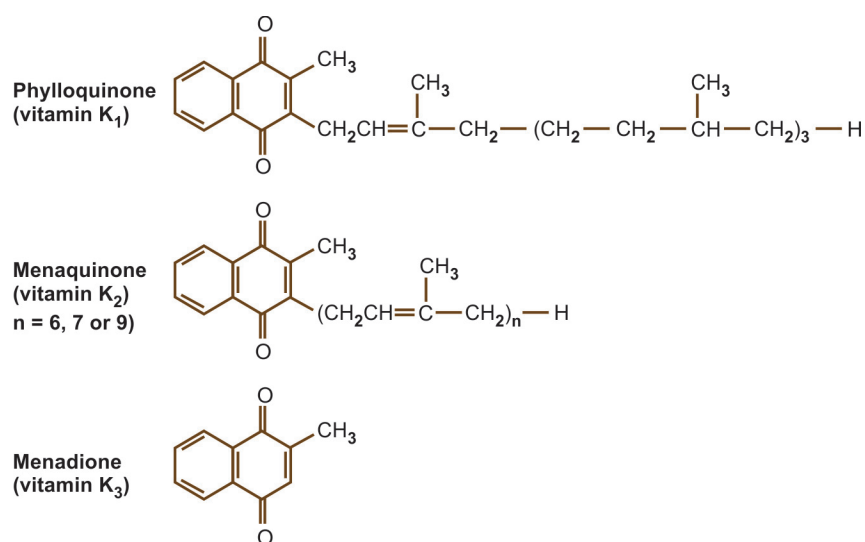


Figure 7.26: Structure of vitamin K

- Vitamin K<sub>1</sub>** or **phylloquinone** derived from plant.
- Vitamin K<sub>2</sub>** or **menaquinones**, produced by microorganisms.

Both these natural types have the same general activity.

- Vitamin K<sub>3</sub>** or **menadione** is a synthetic product, which is an alkylated form of vitamin K<sub>2</sub>.

#### Sources

- Excellent sources are cabbage, cauliflower, spinach and other green vegetables.
- Good sources include tomatoes, cheese, dairy products, meat, egg yolk, etc.
- The vitamin is also synthesized by microorganisms in the intestinal tract.

#### Absorption, Transport and Storage

The naturally occurring vitamin K derivatives are absorbed only in the **presence of bile salts**, like other lipids. It is transported to the liver in the form of chylomicrons, where it is stored.

Menadione (synthetic vitamin K), being water soluble, is absorbed even in the absence of bile salt, passing directly into the hepatic portal vein.

#### Functions of Vitamin K

- Vitamin K plays an important role in **blood coagulation**. Vitamin K is required for the activation of blood clotting factors, prothrombin (II), factor VII, IX and X. These blood clotting proteins are synthesized in liver in inactive form, and are

converted to active form by **vitamin K dependent carboxylation** reaction. In this, vitamin K dependent carboxylase enzyme adds the extra carboxy group at  $\chi$ -carbon of glutamic acid residues of inactive blood clotting factors.

- Vitamin K is also required for the carboxylation of glutamic acid residues of **osteocalcin**, a  $\text{Ca}^{2+}$  binding protein present in bone.

Anticoagulants, **dicumarol** and **warfarin** are structurally similar to vitamin K and inhibit the action of vitamin K.

#### Nutritional Requirements

The suggested intake for adults is **70–140  $\mu\text{g/day}$** .

#### Deficiency Manifestation

- Vitamin K deficiency is associated with **hemorrhagic disease**. In vitamin K deficiency, clotting time of blood is increased. Uncontrolled hemorrhages occur on minor injuries as a result of reduction in prothrombin and other clotting factors.
- Vitamin K is widely distributed in nature and its production by the intestinal microflora ensures that dietary deficiency does not occur. Vitamin K deficiency, however, is found in:
  - Patients with liver disease** and **biliary obstruction**. Biliary obstruction inhibits the entry of bile salts to the intestine.
  - In newborn infants**, because the placenta does not pass the vitamin to the fetus efficiently, and the gut is sterile immediately after birth.

- **Following antibiotic therapy** that sterilizes the gut.
- **In fat malabsorption**, that impairs absorption of vitamin K.

### Hypervitaminosis K

Excessive doses of vitamin K produce a **hemolytic anemia** (due to increased breakdown of RBCs) and **jaundice** (in infants).

### Therapeutic Use

An important therapeutic use of vitamin K is an antidote (drug that counteracts the effects of a poison) to poisoning by dicumarol type drugs.

### SUMMARY

- Vitamins are organic nutrients, essential for growth and development. They must be taken in the diet, because the body either cannot synthesize them at all or not in sufficient amounts for its needs.
- Most of the water soluble vitamins function as coenzymes.
- Thiamine (vitamin B<sub>1</sub>) is a component of thiamine pyrophosphate, a coenzyme in oxidative decarboxylation of keto acid and of an enzyme of the pentose phosphate pathway, transketolase.
- Riboflavin and niacin are each important coenzymes in oxidation reduction reactions. Riboflavin is a component of FMN and FAD, whereas niacin is present in NAD and NADP.
- Pantothenic acid is present in coenzyme-A and acyl carrier protein which acts as a carrier for acyl groups in many reactions.
- Pyridoxine is an essential precursor of pyridoxal phosphate and is the coenzyme for several enzymes of amino acid metabolism including the trans aminases.
- Biotin is the coenzyme for several carboxylase enzymes.
- Folic acid in the form of tetrahydrofolate and vitamin B<sub>12</sub>, takes part in providing one carbon residues for nucleic acid synthesis.
- Ascorbic acid is a water soluble antioxidant.
- In vegetables, vitamin A exists as provitamin,  $\beta$ -carotene, vitamin A is required in vision, cell differentiation, growth, mucus secretion, etc.
- Vitamin D is steroid prohormone, whose activity is carried out by its active form 1,25-dihydroxycholecalciferol (calcitriol). It is involved in the regulation of calcium and phosphate metabolism,

- Vitamin E (tocopherol) is the most important antioxidant in the body. It protects against the effect of toxic free radicals.
- Vitamin K is needed for the synthesis of several blood clotting factors, (e.g. II, VIII, IX and X). It functions as a cofactor to a carboxylase enzyme.
- In contrast to water soluble vitamins only one of the fat soluble vitamins (vitamin K) has a coenzyme function.

### EXERCISE

#### Solve

#### Case History 1

A 15-year-old male has polished rice as a major component of his diet. He is hospitalized with symptoms of poor appetite, peripheral neuropathy and muscular weakness.

#### Questions

- Name the probable disorder and its different types.
- Which factor is deficient in the diet?
- Give the active form of the deficient factor.
- Give any reaction where this factor is required.

#### Case History 2

A male infant, 6 months of age, was admitted to the hospital in a coma. Blood investigation indicated that the child was anemic and that his vitamin B<sub>12</sub> level was very low. A urine sample contained increased amount of methylmalonate and homocysteine.

#### Questions

- Why was the infant anemic?
- What are the sources of vitamin B<sub>12</sub> in the diet?
- Explain the high level of methylmalonate and homocysteine in the infant's urine.
- Give active form of vitamin B<sub>12</sub>.

#### Case History 3

A 42-year-old woman with a chronic inflammatory bowel disease was on intravenous feeding containing fat free and carbohydrate rich diet. After 3 months she began to complain of being unable to see appropriately in dim light.

#### Questions

- Name the probable disorder.
- Which factor is deficient in the diet?
- What is the daily requirement of this factor?
- Name the rich sources of this factor.

**Case History 4**

A 6-year-old girl is hospitalized with symptoms of digestive disorders, dermatitis, depression and dementia.

**Questions**

- Name the disorder.
- Disorder is due to deficiency of which biomolecule?
- Give active form of this biomolecule.
- Give any reaction requiring the active form of this biomolecule.

**Case History 5**

A ten-year-old boy presented with spongy bleeding gums with loose teeth.

**Questions**

- What is the disease he is suffering from?
- What is the cause?
- What is the biochemical basis of the disease?
- Give RDA for the concerned biomolecule.

**Case History 6**

A small 3-year-old child was brought with bow legs, protruding forehead, pigeon chest, depressed ribs and kyphosis.

**Questions**

- Name the disease.
- Which biomolecule is deficient?
- What are the functions of the concerned biomolecule?
- RDA of the concerned biomolecule.

**Multiple Choice Questions (MCQs)**

1. Which of the following coenzymes is not derived from vitamins?

- CoASH
- TPP
- Pyridoxal phosphate (PLP)
- Coenzyme Q

2. A deficiency of vitamin B<sub>12</sub> causes:

- Scurvy
- Rickets
- Pernicious anemia
- Beriberi

3. Rickets is due to deficiency of:

- Vitamin D
- Vitamin A
- Vitamin C
- Vitamin B<sub>1</sub>

4. Which of the following vitamins would most likely become deficient in a person who developed a completely vegetarian lifestyle?

- Vitamin C
- Niacin
- Cobalamin
- Vitamin E

5. Pyridoxal phosphate is a coenzyme for the reactions, *except*:

- Transamination
- Deamination
- Decarboxylation
- Oxidation-reduction

6. Both folic acid and methylcobalamin are required in:

- Phosphorylation
- Deamination
- Methylation of homocysteine to methionine
- Conversion of pyruvate to acetyl-CoA

7. Beriberi is caused by a deficiency of:

- Thiamine
- Thymine
- Threonine
- Tyrosine

8. Precursor of CoA is:

- Folic acid
- Thiamine
- Riboflavin
- Pantothenic acid

9. Biotin is involved in:

- Oxidation-reduction
- Carboxylation
- Decarboxylation
- Dehydration

10. Antihemorrhagic vitamin is:

- Vitamin A
- Vitamin E
- Vitamin K
- Vitamin D

11. Both Wernicke's disease and beriberi can be treated by administering vitamin:

- Thiamine
- Niacin
- Riboflavin
- Ascorbic acid

12. Pellagra occurs due to deficiency of:

- Biotin
- Niacin
- Pantothenic acid
- Folic acid

13. All of the following vitamins have antioxidant property, *except*:

- $\beta$ -carotene
- Ascorbic acid
- Tocopherol
- Cholecalciferol

14. Increased prothrombin time is observed in the deficiency of:

- Vitamin K
- Vitamin B
- Vitamin A
- Vitamin B<sub>12</sub>

15. Thiamine pyrophosphate is required for the following enzymatic activity:

- Hexokinase
- Transketolase