
UNIT 8 WATER-SOLUBLE VITAMINS: B COMPLEX VITAMINS & VITAMIN C

Structure

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8.1 INTRODUCTION

Vitamins are essential nutrients found in foods. The requirements are small but they perform specific and vital functions essential for maintaining health. In the previous unit, we learnt about fat-soluble vitamins. Here in this unit, we shall focus our understanding on the another class of vitamins water-soluble vitamins i.e. vitamin B-complex and vitamin C.

You would recall that the major group of water soluble vitamins is the B-complex group of vitamins. Examples include thiamin, riboflavin, niacin, pyridoxin, folic acid, cyanocobalamin etc. Vitamin C or ascorbic acid though categorized under water soluble vitamins, is different from the B-complex group due to its different nature and mode of action in the body.

So let us begin our discussion on the similar lines as we had done for fat-soluble vitamins.

Objectives

After studying this unit, you will be able to:

- describe the structure and functions of water soluble vitamins,
- identify the food sources, bioavailability, consequences of deficiency and toxicity,
- recognize the recommended amount needed during various physiological stages, and
- appreciate their importance in relation to other nutrients.

8.2 WATER-SOLUBLE VITAMINS: AN OVERVIEW

Vitamins, we already know, are classified by the materials in which they will dissolve. Fat-soluble vitamins – vitamin A, D, E and K – about which we learnt in the last Unit, dissolve in fat before they are absorbed in the blood stream to carry out their functions. Excesses of these vitamins are stored in the liver. Because they are stored, they are not needed every day in the diet. By contrast, water-soluble vitamins dissolve in water and are not stored. They are eliminated in urine. We need a continuous supply of them in our diets. The water-soluble vitamins are the B-complex group and vitamin C.

Vitamin B-complex, as the name suggests, comprises of a group of vitamins which essentially are same in many respects. However, in this unit, we shall focus on six of these – thiamin, riboflavin, niacin, pyridoxin, folic acid and cyanocobalamin – which

along with other B-complex vitamins are considered to be essential from nutrition point of view. You would recall studying the structures of these vitamins in the Nutritional Biochemistry Course in Unit 3. All water-soluble vitamins have cyclic ring structures (refer to Figure 8.1) with side chains and are alcohols, amines or acids. All of them are enzymes, coenzymes or apoenzymes and have key roles in several metabolic reactions. Vitamin C or ascorbic acid, on the other hand, is a compound that is structurally similar to carbohydrate (refer to Figure 8.1) and plays important physiological roles.

Being water-soluble, the B-complex and C vitamins are readily absorbed from the different regions of the small intestine. Water-soluble vitamins are easily destroyed or washed out during food storage or preparation. Proper storage and preparation of food can minimize vitamin loss.

Clinical manifestations of deficiency of some B vitamins—such as beriberi (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) —were once major public health problems in some parts of the world. These manifestations have now declined, the decline being brought about through changes in the patterns of food availability and consequent changes in dietary practices. Although many clinical manifestations of B-vitamin deficiencies have decreased, there is evidence of widespread subclinical deficiency of these vitamins (especially of riboflavin and pyridoxin). These subclinical deficiencies, although less dramatic in their manifestations, exert deleterious metabolic effects.

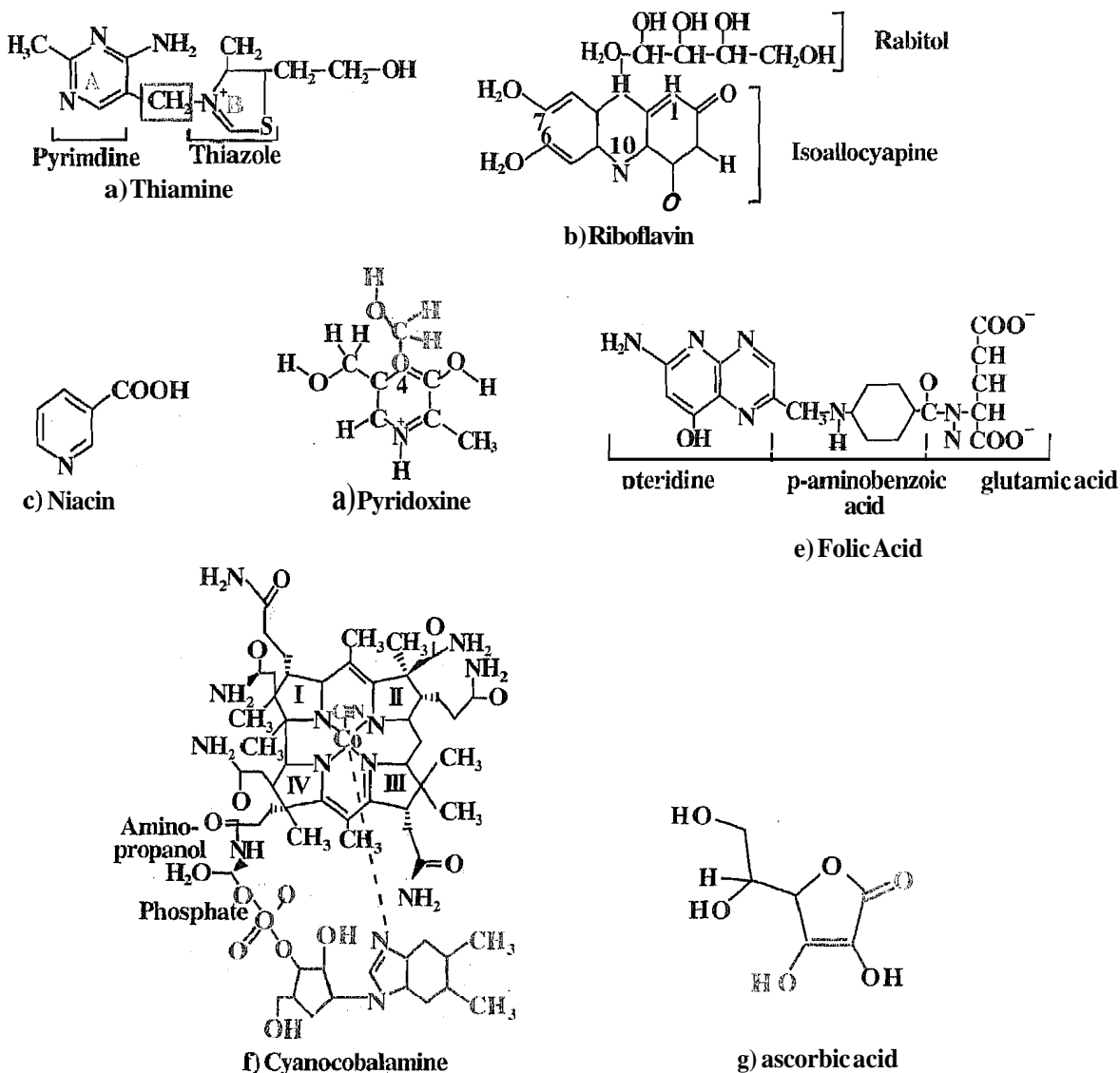


Figure 8.1: Structure of water soluble vitamins

In our discussion in this unit, we shall focus on the food sources, the mechanism of absorption, storage and elimination, important physiological roles, deficiency diseases and the concept of bioavailability for each of these vitamins.

Let's begin with thiamin, one of the essential B-complex vitamins.

8.3 THIAMIN (VITAMIN B₁ OR ANEURIN)

Thiamin is one of the earliest recognized vitamins. The chemical structure of thiamin (Figure 8.1) was established by *Williams* in 1936. The thiamin molecule consists of two linked organic ring structures: a pyrimidine ring bearing an amino group and a sulphur – containing thiazole ring linked to the pyrimidine by a methylene bridge (as shown in red in Figure 8.1(a) under the thiamin structure). The thiazole ring bears a primary alcohol side chain that becomes phosphorylated to give the thiamin phosphate esters that have cofactor activity. Phosphorylated forms of thiamin, you may recall studying in the Nutritional Biochemistry Course, Unit 3, include thiamin monophosphate (TMP), thiamin pyrophosphate (TPP) and thiamin triphosphate (TTP). TTP is the most abundant form and constitutes almost 80% of total thiamin.

Let us next study about the food sources of thiamin.

Food Sources

Thiamin is present in many food products and depending on the amount of vitamin present, we have categorized the foods as rich, good or fair sources as enumerated herewith:

- *Rich sources:* Rice polishings, wheat germ, dried yeast, yeast extract.
- *Good sources:* Whole cereals, whole wheat, millets, raw and hand-pounded or parboiled rice, pulses, soyabean, dried beans, oilseeds and nuts.
- *Fair sources:* Meat, fish, eggs, milk, vegetables and fruits.

Figure 8.2 highlights some of these sources of thiamin. Look up Table 8.2 as well given at the end of the unit which summarizes the rich sources of all water-soluble vitamins.

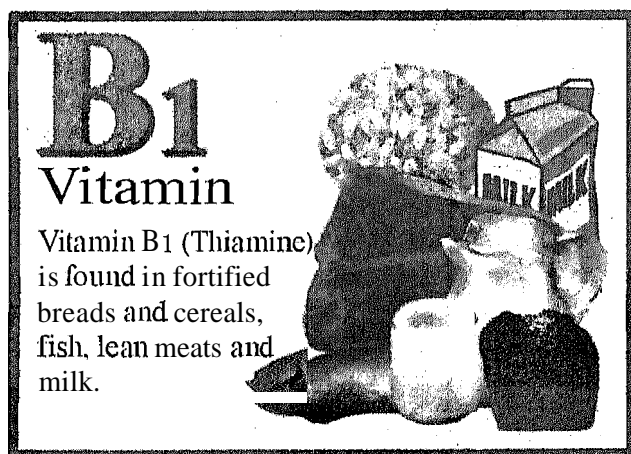


Figure 8.2: Food sources of thiamin

Next, let us learn about the metabolic fate of thiamin.

Absorption, Storage and Elimination

After a meal, thiamin is found in the intestine in the free form. Its absorption involves two mechanisms—both active and passive. At lower intraluminal concentrations (of <math><1-2 \mu\text{mol/L}</math>), thiamin is absorbed by an active sodium-dependent carrier-mediated system. This mechanism involves phosphorylation. At a higher concentration, passive diffusion occurs. Thiamin is absorbed primarily from the upper jejunum by diffusion and by an active transport mechanism but can also occur in the duodenum and ileum.

After absorption, only a small part passes into circulation as free thiamin while a greater part is converted into thiamin pyrophosphate (TBP) in the liver and intestinal mucosa with the help of the enzyme *thiamin kinase* and ATP. A small quantity of thiamin is also converted into thiamin triphosphate (TTP). Thiamin is transported in blood by facilitated diffusion – in erythrocytes in both free and phosphorylated forms and in plasma as free thiamin and TMP. Thiamin or its phosphorylated derivatives are present in negligible amounts in various tissues. Thiamin is excreted in urine.

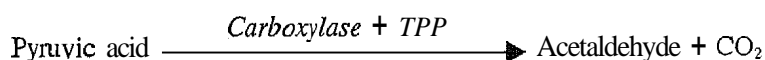
What is the role of thiamin in our body! Surely, you must be aware of the significant role of thiamin! Read the next sub-section and refresh your knowledge.

Functions of Thiamin

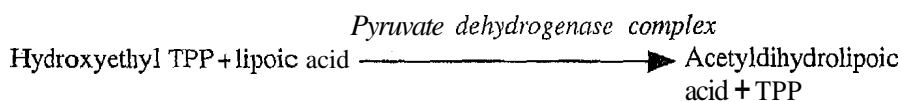
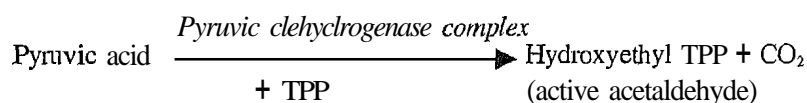
Thiamin has a key metabolic role in the cellular production of energy, mainly in glucose metabolism i.e. it helps the body cells convert carbohydrates into energy. Thiamin is also essential for the functioning of the heart, muscles, and nervous system. All these functions of thiamin are elaborated in this section. Before you read this section we suggest you look up section 4.9 in Unit 4, in the Nutritional Biochemistry Course. This will help you understand the functions of thiamin as a coenzyme better. So get started. We begin with the regulatory function of thiamin.

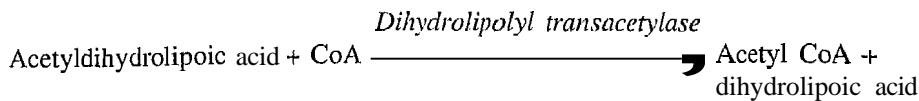
- 1) *Regulator of enzyme activity*: Thiamin regulates the enzymes involved in carbohydrate metabolism. These are:
 - a) *Pyruvate dehydrogenase*, which provides a key link between glycolytic pathway and citric acid cycle.
 - b) *α-ketoglutarate dehydrogenase* in citric acid cycle and transketolase involved in pentose phosphate pathway.
 - c) Each enzyme contains a decarboxylase moiety that binds TPP at the active site, a lipoic acid binding moiety, a flavoprotein and one or more regulatory components that toggle the enzyme complex between the active form and the inactive form.
 - d) A fourth thiamin requiring enzyme is a branched-chain *ketoacid dehydrogenase*, which plays a role in the metabolism of branched-chain amino acids.
- 2) *Coenzyme in enzyme catalyzed reactions*: Thiamin functions as the coenzyme thiamin pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. Specifically the Mg²⁺-coordinated TPP participates in the formation of α-ketols (e.g. among hexose and pentose phosphates as described in the reaction given at point iv below) as catalyzed, by *transketolase* and in the oxidation of α-keto acids (e.g. pyruvate, α-ketoglutarate, and branched chain α-keto acids) by *dehydrogenase complexes* (refer to reaction ii and iii). Hence, when there is insufficient thiamin, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism (via α-keto acids) has severe consequences, such as a decrease in the formation of acetylcholine for neural function.

i) *Decarboxylation of pyruvic acid*:

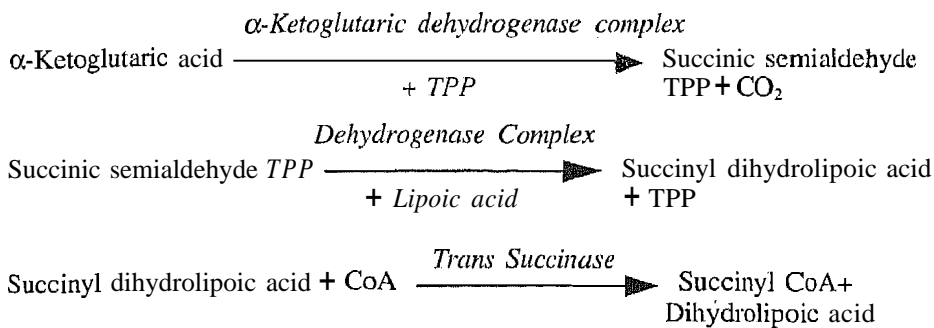


ii) *Oxidative Decarboxylation of Pyruvic acid*:

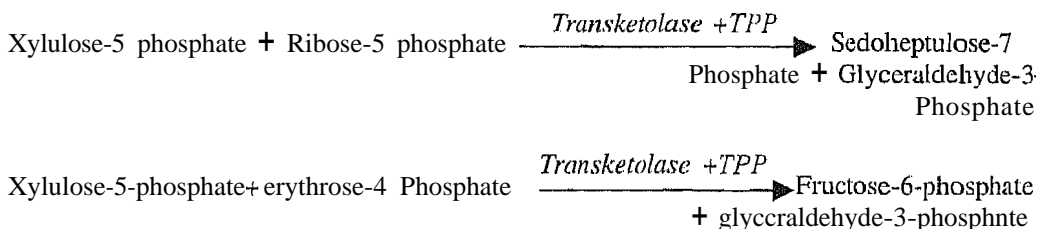




iii) *Oxidative Decarboxylation of α -Ketoglutaric acid:*



iv) *α -Keto₂ Formation in HMP shunt pathway:*



- 3) *TPP and TTP are vital for the nerves and cardiac tissues:* TPP and TTP are interconvertible and are involved in carbohydrate metabolism. As discussed above, when there is insufficient thiamin, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism (via α -keto acids) has severe consequences, such as a decrease in the formation of acetylcholine for neural function. Thus, deficiency in the tissues affects energy metabolism in nervous tissue and cardiac muscle.
- 4) *Role in the conversion of carbohydrate to fats:* Thiamin helps in the initial steps of fatty acid and sterol production. In this way, thiamin also helps convert carbohydrate to fat for storage of potential energy.

Having looked at the functions, next let us consider the bioavailability aspect for thiamin.

Bioavailability of Thiamin

Thiamin is readily available from the gut from food sources (as thiamin phosphate esters). Drugs and alcohol abuse may interfere with thiamin absorption and impair thiamin availability. Compared with most other vitamins, thiamin deficiency is seen more rapidly when low intake is encountered. Since thiamin is lost in cooking and is depleted by use of coffee, tannin from black teas, nicotine and alcohol, it is necessary to insure that intake of thiamin is optimal.

What would be the consequences of decreased intake of thiamin? The next subsection focuses on this aspect,

Deficiency of Thiamin

Thiamin deficiency causes the disease beriberi in human beings, which has been classically considered to exist in *dry* (paralytic) and *wet* (oedematous) forms. The early clinical features are anorexia and dyspepsia, associated with heaviness and weakness of the legs. There is tenderness of the calf muscles on pressure with complaints of 'pins and needles' pain and numbness in the legs. The knee jerks are usually sluggish but occasionally slightly exaggerated. The subjects feel **weak** and get easily exhausted while working. If not treated, the subjects may develop *polyneuritic beriberi*, that is, inflammation of many or all of the peripheral nerves, A detail discussion on the different forms of beriberi follows:

- 1) *Wet beriberi*: Oedema is the important feature of wet beriberi. It may develop rapidly and involve not only the legs but also the face, trunk and serous cavities. Palpitation and breathlessness are present. The calf muscles are frequently tense, slightly swollen and tender on pressure. The veins of the neck are distended and show visible pulsations. The diastolic blood pressure is low and systolic is high. The pulse is fast and bounding. The heart becomes weak and death occurs due to heart failure.
- 2) *Dry beriberi*: Early symptoms are similar to those found in wet beriberi. The muscles become progressively wasted and weak and walking becomes difficult. The emaciated subject needs the help of sticks to stand and walk and finally becomes bed-ridden. If not treated, the patients will die.

Beriberi occurs in human-milk-fed infants whose nursing mothers are deficient. Let us get to know about the infantile beriberi.

- 3) *Infantile beriberi*: Infantile beriberi is commonly seen in many South-East Asian countries where the diets consist mostly of "polished rice" and are deficient in thiamin. The occurrence of beriberi is due to:
 - a) inadequate thiamin intake, related mainly to poor thiamin content of breast milk, and
 - b) consumption of over-milled rice, deficient in thiamin by the mother.

The disease in infants is generally acute in onset while the chronic forms of the disease are seen in late infancy and childhood. Two types of infantile beriberi are known. These are: (i) cardiovascular type, and (ii) neuritic type.

- i) *The cardiovascular type (wet)*: It manifests itself in babies between the ages of 2 and 4 months. The onset is acute with classical signs and symptoms of congestive cardiac failure, tachycardia, dyspnoea, enlargement of the heart, elevated venous pressure, enlarged tender liver, oedema and oliguria. In some infants, cyanosis and pulmonary oedema may develop rapidly and death may ensue in a matter of few hours.
- ii) *The neuritic type (dry)*: It is also referred to as *Wernicke-Korsakoff syndrome* or *cerebral beriberi*. It shows typical manifestations of peripheral neuropathy, tenderness of calf muscles, diminished tendon jerks. The accent is predominantly on the central nervous system (CNS) with sensorial alteration (irritability, apathy, drowsiness and coma) signs of raised intracranial tension, staring expression and varying degrees of neurologic deficit.

Besides occurring in infants, beriberi also occurs in adults with high carbohydrate intakes (mainly from milled rice) and with intakes of anti-thiamin factors, such as the bacterial thiaminases that are in certain ingested raw fish. Beriberi is still endemic in Asia. In relatively industrialized nations, the neurologic manifestations of *Wernicke-Korsakoff syndrome* are frequently associated with chronic alcoholism in conjunction with limited food consumption. Some cases of thiamin deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy. Thiamin deficiency has also been observed in people with chronic alcoholism.

We have studied the deficiency symptoms of thiamin so far. What about toxicity related to excess ingestion of thiamin? Let us find out.

Toxicity

Thiamin toxicity is not a problem because renal clearance of the vitamin is rapid. Since the body stores of thiamin are very low, we must ensure adequate daily intake. What are the requirements for thiamin then? Let us get to know this next.

Recommended Dietary Allowance (RDA)

Thiamin, as it must be clear by now, is needed mainly for the metabolism of carbohydrate, branch-chained amino acids, fat and alcohol. Because the requirements increase as energy expenditure increases, thiamin requirements are expressed as ratios to food energy. Look at Table 8.1, which presents the ICMR and the FAO/WHO 2004 recommendations for thiamin requirement for different age and physiological groups.

Table 8.1: ICMR and FAO/WHO recommended dietary intakes for thiamin by groups

Recommended Thiamin Intake			
Group	ICMR (mg/day)	Group	FAO/WHO 2004 RNI (mg/day)
<i>Man</i> Sedentary work	1.2	<i>Adults (19+ years)</i> Males	1.2
Moderate work	1.4		
Heavy work	1.6		
<i>Women</i> Sedentary work	0.9	<i>Adults (19+ years)</i> Females	1.1
Moderate work	1.1		
Heavy work	1.2		
Pregnancy	+0.2	Pregnancy	1.4
<i>Lactating</i>		Lactation	1.5
0 - 6 months	+0.3		
7 - 12 months	+0.2		
<i>Infants</i> 0 - 6 months	55 mcg /kg	<i>Infants</i> 0 - 6 months	0.2
6 - 12 months	50 mcg /kg	7 - 12 months	0.3
<i>Children</i> 1 - 3 years	0.6	<i>Children</i> 1 - 3 years	0.5
4 - 6 years	0.9	4 - 6 years	0.6
7 - 9 years	1.0	7 - 9 years	0.9
<i>Adolescent</i>		<i>Adolescents</i>	1.0
Boys 10 - 12 years	1.1	Males (10 - 18 years)	1.2
Girls 10 - 12 years	1.0	Females (10 - 18 years)	1.1
Boys 13 - 15 years	1.2		
Girls 13 - 15 years	1.0		
Boys 16 - 18 years	1.3		
Girls 16 - 18 years	1.0		

RNI: Recommended Nutrient Intake.

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

Because thiamin facilitates energy utilization, its requirements have traditionally been expressed on the basis of energy intake, which can vary depending on activity levels. Therefore, the RDA as recommended by ICMR for adults is 0.5 mg/1000 Kcal, however, an intake of no less than 1 mg/day is advised. The individual intake for thiamin, hence for an adult man is 1.2 mg/day and adult woman is 0.9 mg/day as indicated in Table 8.1. You may have noticed that the requirements increase in case of pregnancy and lactation owing to increased energy demands.

How do we get to know then about the thiamin status in our body? The criteria for assessment are highlighted next,

Criteria for Assessment of Thiamin Status

Thiamin status has been assessed by measuring urinary thiamin excretion under basal conditions or after thiamin loading, transketolase activity, and free and phosphorylated forms in blood or serum. Let us understand these assessment criterias.

- 1) *Determination of Erythrocyte Transketolase (ETK) Activity:* The enzyme transketolase requires TPP for its activity, for the metabolism of pentose phosphate sugars as described under the functions earlier. ETK activity is expressed as *basal* (without TPP) activity (ETKA) or as *the difference between stimulated and basal activity as a percentage of basal activity*. In thiamin deficiency, the enzyme activity is reduced (decreased ETKA) and a small part of the added pentose phosphate disappears. When TPP is added, ETK activity is increased. The percent increase in ETK activity due to added TPP, as indicated in the Table 8.2, is an index of the degree of thiamin deficiency.

Table 8.2: Determination of erythrocyte **transketolase** activity

Thiamin Status	Increased due to Added TPP(%)
Normal	0- 15
Marginal deficiency	15 - 25
Moderate deficiency	> 25

- 2) *Urinary Excretion Test:* The urinary excretion of thiamin is an index of recent dietary intake of the vitamin. Hence, determination of thiamin in urine samples, 4 hours after the administration of a 5 mg dose of the vitamin, for a period of 24 hours, is a reliable index of thiamin status. *An excretion value <20 nczg is indicative of thiamin deficiency.*
- 3) *Estimation of blood thiamin levels:* The levels of free thiamin and its phosphoesters in whole blood and erythrocytes is measured using high-performance liquid chromatography methods. This measure gives a good indication of thiamin status.

We end our study of thiamin now. The interaction of thiamin with other nutrients is another aspect we would like to review in this unit. This aspect is covered separately in section 8.10 at the end of this unit, where the interaction of all the water-soluble vitamins me taken up in general.

So now we move on to the next water soluble vitamin i.e. riboflavin.

8.4 RIBOFLAVIN

Riboflavin (vitamin B₂), a water-soluble vitamin, was discovered in milk as a pigment possessing a yellow green fluorescence as early as in 1879. However, its role in our body was identified much later. The name 'riboflavin' was given to this vitamin in view of the similarity of a part of its structure to that of the sugar ribose and because of its relation to the general group of flavins.

Riboflavin and its coenzyme derivatives are *isoalloxazines* i.e. they contain a pteridine ring with a benzene ring fused on to it. The side chain is a C5 polyhydroxy group, a derivative of ribitol, a pentahydroxy compound, as illustrated in Figure 8.1(b). You would recall studying in the Nutritional Biochemistry Course that riboflavin has two major coenzyme derivatives, namely *flavin mononucleotide* (FMN) and *flavin adenine dinucleotide* (FAD) which is formed by the combination of FMN with one molecule of adenosine triphosphate (ATP).

Let us begin our study of riboflavin by recapitulating our knowledge about food sources of riboflavin.

Food Sources

The food sources of riboflavin include:

- *Rich sources:* Liver, dried yeast, egg powder, milk powder.
- *Good sources:* Whole cereals, millets, pulses, green leafy vegetables, oilseeds and nuts, meat, fish, eggs and milk.
- *Fair sources:* Milled cereals and cereal products, roots and tubers, other vegetables and fruits.

Figure 8.3 illustrates some important sources of riboflavin. Look up the Table given at the end of the unit which summarizes the rich, good and fair sources of all water soluble vitamins.

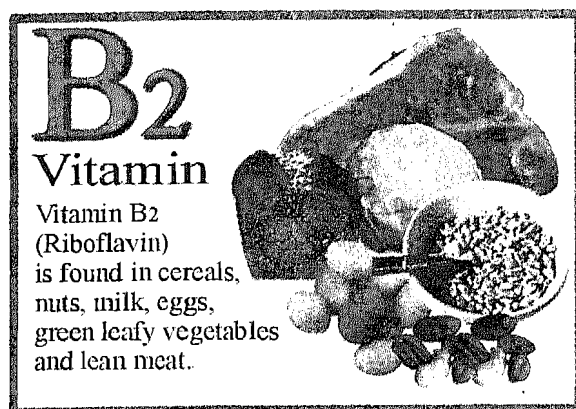


Figure 8.3: Foods rich in riboflavin

Absorption, Storage and Elimination

Riboflavin is absorbed from the small intestine through the portal vein and is passed to all tissues via general circulation. Absorption occurs in the upper part of the gastrointestinal tract by specialized transport involving a phosphorylation-dephosphorylation mechanism more than by diffusion. This process is sodium-dependent and involves an ATPase active transport system. Delaying intestinal transit time may result in an increase in the total amount of riboflavin absorbed from the intestine. Intestinal uptake is increased in cellular riboflavin deficiency and decreased with a high riboflavin status. Riboflavin is also synthesized by intestinal bacteria and then absorbed by the colon. This riboflavin produced by bacterial synthesis also contributes to overall riboflavin nutrition. Transport of riboflavin involves loose binding to albumin and tight binding to a number of globulins—several classes of immunoglobulins (IgA, IgG and IgM). Riboflavin is not stored in the body. A major part is excreted in urine and the rest is broken down in the tissues.

Next, let us learn what is the role of riboflavin in our body.

Functions

The role of riboflavin in human metabolic processes and in maintaining good health is highlighted herewith.

- 1) *Precursor of co-enzymes:* The major function of riboflavin is to serve as the precursor of the coenzymes FMN and FAD and of the covalently bound flavins. These coenzymes are widely distributed in metabolism. The role of FMN and FAD as part of enzymes or as coenzymes are shown in Table 8.3.

Table 8.3: Role of FMN and FAD

Enzyme	Coenzyme	Reaction
NADH dehydrogenase	FMN	$\text{NADH} \rightarrow \text{NAD}$
Succinate dehydrogenase	FAD, Fe	$\text{Succinate} \rightarrow \text{Fumarate}$
L-Glycero-P-dehydrogenase	FAD	$\text{Glycero-P} \rightarrow \text{Dihydroxyacetone-P}$
Choline dehydrogenase	FAD	$\text{Choline} \rightarrow \text{Betaine aldehyde}$
Acyl-CoA-dehydrogenase	FAD (ETK)	$\text{Acyl-CoA} \rightarrow \text{Dehydroacyl-CoA}$
Sarcosine dehydrogenase	Fe, Flavin (ETK)	$\text{Sarcosine} \rightarrow \text{Glycine} + \text{HCHO}$
Dimethylglycine dehydrogenase	Flavin (ETK) Fe	$\text{Dimethylglycine} \rightarrow \text{Sarcosine} + \text{HCHO}$
Aerobic dehydrogenases		
L-amino acid oxidase	FMN	$\text{Amino acid} \rightarrow \text{Keto acid} + \text{NH}_3 + \text{H}_2\text{O}_2$
D-amino acid oxidase	FAD	$\text{Amino acid} \rightarrow \text{Keto acid} + \text{NH}_3 + \text{H}_2\text{O}_2$
Xanthine oxidase	FAD	$\text{Xanthine} \rightarrow \text{Hypoxanthine} + \text{H}_2\text{O}_2$ $\text{Hypoxanthine} \rightarrow \text{Uric acid} + \text{H}_2\text{O}_2$

- 2) *Role in respiratory chain:* Riboflavin catalyzes numerous oxidation–reduction reactions. Conversion of riboflavin to flavin mononucleotide (FMN) and then to the predominant flavin, flavin adenine dinucleotide (FAD), occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. The flavocoenzymes (FMN and FAD) participate in oxidation–reduction reactions in metabolic pathways and in energy production via the respiratory chain.
- 3) *Drug and lipid metabolism:* Flavoproteins catalyze dehydrogenation reactions, as well as, hydroxylations, oxidative decarboxylations, deoxygenations and reduction of oxygen to hydrogen peroxide.
- 4) *Antioxidant activity:* Flavoproteins also have powerful antioxidant activity from their role as precursors to FMN and FAD. Among the FAD-requiring enzymes glutathione reductase, is involved in the glutathione redox cycle and provides a major protective role against lipid peroxides.
- 5) *Protective role:* Riboflavin protects the ectodermal tissues and prevents lesions of the skin, eye and nervous system. Riboflavin ameliorates cardiac damage and also has antimalarial effects.
- 6) *Regulatory functions:* Riboflavin is concerned with the regulatory functions of some hormones involved in carbohydrate metabolism.
- 7) *Other functions:* Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B₆ which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the coenzyme pyridoxal 5'-phosphate (PLP). Riboflavin deficiency slows down the uptake of pyridoxine and decreases the conversion of pyridoxine to its metabolites.

Before we move on to the study about the deficiency and toxicity of riboflavin, let us get to know about the bioavailability of riboflavin from the diet.

Bioavailability

Riboflavin availability is sodium-dependent. Prolonged contact of dietary riboflavin with the absorptive surface of the intestinal mucosal cells increases the bioavailability of riboflavin. Intestinal uptake is increased in cellular riboflavin deficiency and decreased with a high riboflavin status. Diets high in psyllium gum decrease absorption whereas antacids, as well as, the mere presence of food increase absorption.

Metals such as copper, zinc and iron; drugs, caffeine and saccharin and vitamins such as nicotinamide and ascorbic acid, tryptophan and urea form chelates and complexes with riboflavin and FMN and thus affect bioavailability.

Pregnancy induces the formation of proteins which bind flavins. Bioavailability of riboflavin in foods, mostly as digestible flavocoenzymes, is excellent at nearly 95%, but absorption of the free vitamin is limited to about 27 mg per single meal or dose in an adult.

Deficiency

Riboflavin deficiency results in the condition of *hypo-* or *ariboflavinosis*, with sore throat, hyperaemia (condition in which the blood collects in a part of the body), oedema of the pharyngeal and oral mucous membranes, cheilosis (cracking of the corner of the mouth), angular stomatitis (inflammation at the corner of the mouth), glossitis (inflammation or the infection of the tongue), seborrheic dermatitis and normochromic, normocytic anaemia associated with pure red cell cytoplasia of the bone marrow. As riboflavin deficiency almost invariably occurs in combination with a deficiency of other B-complex vitamins, some of the symptoms (e.g. glossitis and dermatitis) may result from other complicating deficiencies.

The major cause of *hyporiboflavinosis* is inadequate dietary intake as a result of limited food supply, which is sometimes exacerbated by poor food storage or

processing. Children in developing countries like ours will commonly demonstrate clinical signs of riboflavin deficiency during periods of the year when gastrointestinal infections are prevalent. Decreased assimilation of riboflavin also results from abnormal digestion, such as that which occurs with lactose intolerance. This condition is highest in African and Asian populations and can lead to a decreased intake of milk, as well as, an abnormal absorption of the vitamin. Absorption of riboflavin is also affected in some other conditions, for example, tropical sprue, celiac disease, malignancy and resection of the small bowel and decreased gastrointestinal passage time. In relatively rare cases, the cause of deficiency is inborn errors, in which the genetic defect is in the formation of a flavoprotein (e.g. acyl-coenzyme A [CoA] dehydrogenases). Also, at-risk are infants receiving phototherapy for neonatal jaundice and perhaps those with inadequate thyroid hormone. Some cases of riboflavin deficiency have been observed in south-east Asian school children infected with hookworm.

Next, let us get to know of toxicity of riboflavin.

Toxicity

Riboflavin toxicity is not a problem because of the limited intestinal absorption of this vitamin.

So then what are the requirements for riboflavin to maintain optimum health? Read the next sub-section and find out.

Recommended Dietary Allowance (RDA)

Several nutritional and physiological factors govern riboflavin requirements. Negative nitrogen balance reduces riboflavin requirements and excretion. Physical activity reduces urinary riboflavin excretion. Hence, the dietary requirement is increased by exercise and increased physical activity.

Table 8.4: ICMR and FAO/WHO recommended dietary intakes for riboflavin by groups

Recommended Riboflavin Intake (mg/day)					
Group		ICMR	Group	FAO/WHO 2004 RNI	
<i>Man</i>	Sedentary work	1.4	<i>Adults</i> (19+ years) Males	1.3	
	Moderate work	1.6			
	Heavy work	1.9			
<i>Woman</i>	Sedentary work	1.1	<i>Adults</i> (19+ years) Females	1.1	
	Moderate work	1.3			
	Heavy work	1.5			
<i>Pregnancy</i>		+0.2	<i>Pregnancy</i>	1.4	
<i>Lactation</i>			<i>Lactation</i>	1.6	
	0 - 6 months	+0.3			
	7 - 12 months	+0.2			
<i>Infants</i>	0 - 6 months	60 mcg/kg	<i>Infants</i>	0 - 6 months	0.3
	6 - 12 months	65 mcg/kg		7 - 12 months	0.4
<i>Children</i>	1 - 3 years	0.7	<i>Children</i>	1 - 3 years	0.5
	4 - 6 years	1.0		4 - 6 years	0.6
	7 - 9 years	1.2		7 - 9 years	0.9
<i>Adolescent</i>			<i>Adolescents</i>		
	Boys 10 - 12 years	1.2		Males (10 - 18 years)	1.3
	Girls 10 - 12 years	1.3		Females (10 - 18 years)	1.0
	Boys 13 - 15 years	1.5		Boys 16 - 18 years	1.6
	Girls 13 - 15 years	1.2	Girls 16 - 18 years	1.2	

RNI: Recommended Nutrient Intake.

Source: Recommended Dietary Allowances for Vitamins, NJN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

Therefore, the RDA for individual intake follows the similar pattern as for thiamin. RDI for riboflavin for adults are 0.6 mg /1000 Kcal, i.e. for adult males, the requirements are 1.4 mg/day while for adult females, it is 1.1 mg/day and increase up to 1.3-1.4 mg/day during pregnancy and lactation. Compare these Indian requirement with the FAO/WHO 2004 recommendations presented in Table 8.4. How do the requirements compare?

Next, we shall review the criteria used for assessing the riboflavin status.

Criteria for Assessment of Riboflavin Status

Riboflavin status can be assessed by measuring urinary excretion of the vitamin in fasting, random, and 24-hour specimens or by load return tests (amounts measured after a specific amount of riboflavin is given orally); measuring erythrocyte glutathione reductase activity coefficient or erythrocyte flavin concentration.

Let us get to know about these methods.

- 1) **Urinary excretion test:** Urinary excretion of riboflavin is determined at different levels of intake. Under conditions of adequate riboflavin intake (approximately 1.3 mg/day for adults), an estimated 120 mg (320 nmol) total riboflavin or 80 mg/g of creatinine is excreted daily. The levels indicative of deficiency are given in Table 8.5.

Table 8.5: Urinary excretion of riboflavin levels indicative of deficiency

Condition	Urinary Excretion Levels mcg/day
Normal	120-150
Deficiency	50
Sub clinical deficiency	30 - 120

- 2) **Riboflavin content of RBC:** The erythrocyte glutathione reductase assay, with an activity coefficient (AC) expressing the ratio of activities in the presence and absence of added FAD, continues to be used as a main functional indicator of riboflavin status, but some limitations in the technique have been noted. Addition of FAD to an erythrocyte haemolysate records greater increase in deficient than in repleted subjects.

Suggested guidelines for the interpretation of such enzyme ACs are as follows: less than 1.2, *acceptable*; 1.2-1.4, *low*; greater, than 1.4, *deficient*.

Finally let us look at the interaction of riboflavin with other nutrients.

Interaction with other Nutrients

Riboflavin, as already discussed under the functions, interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B₆, which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the coenzyme pyridoxal 5'-phosphate (PLP). Riboflavin deficiency slows down the uptake of pyridoxine and decreased the conversion of pyridoxine to its metabolites.

A lower fat-carbohydrate ratio may decrease the riboflavin requirements of the elderly.

With a discussion on riboflavin, we would like to take a break here and try to answer the questions given in check your progress exercise 1 and recapitulate what we have learnt so far.

Check Your Progress Exercise 1

- 1) State whether the following statements are true or false. Also correct the false statements.
- a) Thiamin gives the yellow green fluorescence.
.....
 - b) Intestinal uptake of riboflavin is decreased with high riboflavin status.
.....
 - c) The thiamin containing coenzyme to pyruvate oxidase is TPP.
.....
 - d) Thiamin helps convert fat into energy.
.....
 - e) TPP is also known as co-carboxylase.
.....

2) What are the salient features in the processes of absorption, storage and elimination of thiamin?

.....
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.....
.....
.....

3) List any five functions of riboflavin.

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.....
.....

4) List any five factors that affect the bioavailability of riboflavin.

.....
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.....
.....

5) Write notes on thiamin deficiency.

.....
.....
.....
.....

6) Bring out the significance of determination of ETK activity.
.....
.....
.....
.....

7) Describe any one method for assesment of riboflavin.
.....
.....
.....
.....

8) Trace the interaction of macronutrients with thiamin.
.....
.....
.....
.....

Now that our understanding about thiamin and riboflavin is somewhat complete, let us move on next to the other water-soluble vitamins. We continue our study with niacin.

8.5 NIACIN

Niacin is chemically synonymous with *nicotinic acid* although the term is also used for its amide (nicotinamide). Nicotinamide is required for the synthesis of the active forms of niacin i.e. *nicotinamide adenine dinucleotide* (NAD) and its phosphate *nicotinamide adenine dinucleotide phosphate* (NADP), which functions as a cofactor for various coenzymes in our body. Nicotinic acid was first isolated from rice polishings and shown to be a component of coenzyme I and II and several transporting enzymes in the tissues. The structure of niacin is given in Figure 8.1(c).

Look up the structure now and then move on to study about the food sources and metabolism of niacin in our body.

Food Sources

Niacin is widely distributed in plant and animal foods mainly as the pyridine nucleotides NAD and NADP. The food sources of niacin are highlighted herewith:

- *Rich sources:* Dried yeast, rice polishings, peanuts, liver.
- *Good sources:* Whole cereals legumes, meat and fish.
- *Fair sources:* Milled cereals, maize, roots and tubers, other vegetables, milk and eggs.

As you may have seen that whole cereals are good sources of niacin, but the removal of the bran in the milling of wheat reduces the niacin content of white wheat flour to a low level. Niacin is readily soluble in water, but it is resistant to heat, oxidation and alkalis. It is, in fact, one of the most stable vitamins.

Figure 8.4 illustrates some important sources of niacin.

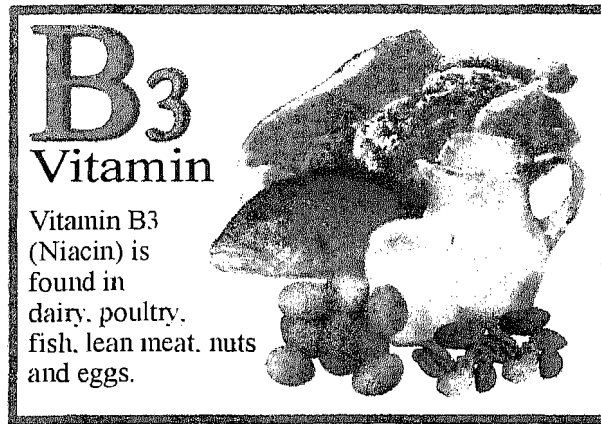


Figure 8.4: Foods rich in niacin

Absorption, Storage and Elimination

Nicotinic acid and nicotinamide are rapidly absorbed from the intestine rather than the stomach. At low concentrations, absorption in the small intestine occurs as Na^+ dependent facilitated diffusion but at higher concentrations, passive diffusion predominates. Nicotinamide is the major form in the blood stream and arises from the enzymatic hydrolysis of NAD in the intestinal mucosa and liver. NAD and NADP, the main dietary forms of niacin are hydrolyzed by enzymes in the intestinal mucosa to release nicotinamide. The intestinal mucosa is rich in niacin conversion enzymes such as glycohydrolase.

Nicotinamide is released from NAD in the liver and intestines by *glycohydrolases* and transported into tissues as needed. Tissues take up both forms by simple diffusion and erythrocytes by facilitated transport. Niacin is methylated in the liver to N¹-methyl nicotinamide (NMN) which is excreted in the urine along with the oxidation products of NMN. The pattern of niacin products excreted depends on the amount and the form of niacin ingested and the niacin status of the individual. Excess niacin is excreted in the urine primarily as N¹-methylnicotinamide and N¹-methyl-2-pyridone-5-carboxamide.

Having studied the metabolic fate of niacin, next let us get to know about the role of niacin in our body.

Functions

The functions of nicotinic acid are as follows:

- 1) *Protective role*: Nicotinic acid is vital to the normal functioning of the skin, intestinal tract and nervous system. It protects the tissues from pellagraic lesions.
- 2) *Coenzyme activity*: Nicotinamide exists within the redox-active coenzymes, nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP), which function in dehydrogenase-reductase systems requiring transfer of a hydride ion. NAD is also required for non-redox adenosine diphosphate-ribose transfer reactions involved in DNA repair and calcium mobilization. NAD functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as glyceraldehyde-3-phosphate, lactate, alcohol, 3-hydroxybutyrate and pyruvate. NADP functions in reductive biosynthesis such as fatty acid and steroid synthesis and in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

The role of NAD(P) is summarized in Table 8.6.

Table 8.6 : Functions of NAD(P)

Enzymes	Coenzyme	Reaction
Anaerobic <i>dehydrogenases</i>		
Alcohol dehydrogenase	NAD, Zn	Alcohols+ Aldehydes
Aldehyde dehydrogenase	NAD	Aldehydes — Acids
L-Glycerophosphate dehydrogenase	NAD	Glycerophosphate → Dihydroxyacetone Phosphate
Hydroxybutyrate dehydrogenase	NAD	Hydroxybutyrate → Acetoacetate
Lactate dehydrogenase	NAD-NADP	Lactate → Pyruvate
Malate dehydrogenase	NAD (NADP)	Malate → Oxaloacetate
Glucose dehydrogenase	NAD (NADP)	Glucose — Gluconate
L-Glutamate dehydrogenase	NAD (NADP), Zn	Glutamate → α-Ketoglutarate+NH ₃
Isocitrate dehydrogenase	NAD, NADP	Isocitrate → α-Ketoglutarate
Hydroxysteroid dehydrogenase	NAD, NADP	Hydroxy steroid → Ketosteroid
Glucose-6-P-dehydrogenase	NADP	Glucose-6-P → Gluconate 6-P
Gluconate-6P-dehydrogenase	NADP	Gluconate-6-P → Ribulose-5-P+CO ₂

- 3) **Metal** chelating ability: This explains its biological interactions with essential trace metals. It is a part of the proposed glucose tolerance factor, an organochromium complex that may potentiate insulin response in man.

Other than functions, another important aspect related to niacin is its **bioavailability** in our body. This aspect is covered next.

Bioavailability

We have already learnt earlier that niacin is provided in the diet primarily as the pyridine nucleotides-NAD and NADP. In addition to its synthesis from dietary niacin, NAD may also be synthesized in the liver from the dietary amino acid, **tryptophan**. The synthesis of niacin from tryptophan also depends on enzymes that require vitamin B₆ and riboflavin, as well as, an enzyme containing **haeme** (iron). On an average, 1 **mg** of niacin can be synthesized from the ingestion of 60 mg of tryptophan. Hence, the **recommended** allowance for niacin is expressed as mg NE (niacin equivalents) where 1 mg NE = 1mg niacin or 60 mg tryptophan.

There are several dietary drug and disease factors that reduce the conversion of **tryptophan** to niacin, such as the use of oral contraceptives.

Next, let us learn about the symptoms of niacin deficiency and toxicity.

Deficiency and Toxicity

Niacin (nicotinic acid) deficiency classically results in **pellagra** (refer to Figure 8.5), which is a chronic wasting disease associated with a characteristic **erythematous dermatitis** that is bilateral and symmetrical, a dementia after mental changes including insomnia and apathy preceding an overt **encephalopathy**, and diarrhoea resulting from inflammation of the intestinal mucous surfaces. The disease is, therefore, characterized by 3 D's- dermatitis, diarrhoea and dementia. The effects of the deficiency on various organs and organ systems are discussed below:

- 1) **Digestive System:** The predominant symptoms are glossitis and **diarrhoea**. Glossitis, cheilosis and stomatitis may vary from mild redness, soreness and smoothness of the tongue and mouth to extreme inflammation with fiery red mucosa and tongue, ulceration and secondary infection of the tongue and buccal mucosa, Nausea and vomiting are seen in most cases. Diarrhoea may range from a few to several loose stools a **day** with blood and mucous. If untreated, death occurs:

- 2) **Skin:** Dermatitis is the characteristic feature of the disease. It is symmetrical in distribution. In early stages, a bright red erythema resembling sunburn occurs over the exposed parts of the body. The common sites are the face, neck and on the extremities such as the back of the fingers, hands, wrists and elbows, the forearms, dorsum of the feet, knees and ankles and the neck. In the beginning, the skin is red and slightly swollen, as illustrated in Figure 8.5. The lesion may worsen by the formation of vesicles and bullae with cracking of the skin. Secondary infection is always present. With improvement, the skin becomes dry, less red and the surface desquamates. The dermatitis is precipitated with exposure to sunlight.
- 3) **Nervous System:** Delirium is the most common mental disturbance in acute pellagra. Dementia is more frequently seen in the chronic cases. Milder mental disturbances consisting of irritability, peripheral neuritis, paralysis, change in disposition, depression, inability to concentrate and poor memory are more common in the mild cases. The symptoms of postero-lateral tract degeneration, ataxia, spasticity and the involvement of the bladder and rectal sphincters are seen in chronic cases.

At present, pellagra occurs endemically in poorer areas of Africa, China and India. Its cause has been mainly attributed to a deficiency of niacin; however, its biochemical interrelationship with riboflavin and vitamin B₆, which are needed for the conversion of L-tryptophan to niacin equivalents (NEs), suggests that insufficiencies of these vitamins may also contribute to pellagra. Pellagra-like syndromes occurring in the absence of a dietary niacin deficiency are also attributable to disturbances in tryptophan metabolism (e.g. Hartnup disease with impaired absorption of the amino acid and carcinoid syndrome where the major catabolic pathway routes to 5-hydroxytryptophan are blocked). Pellagra also occurs in people with chronic alcoholism. Cases of niacin deficiency have been found in people suffering from Crohn's disease (inflammatory disease of GI tract).

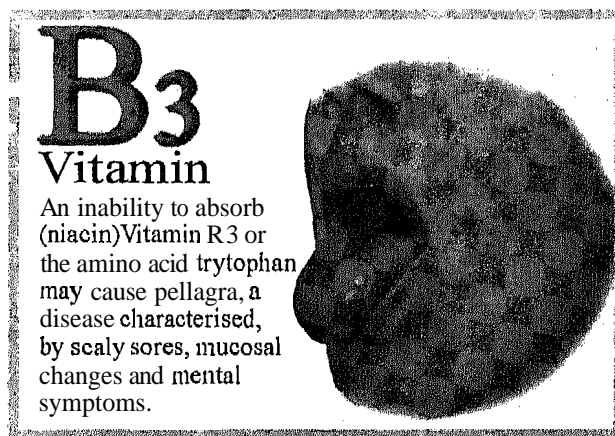


Figure 8.5: Deficiency symptoms of niacin

Toxicity

Although therapeutically useful in lowering serum cholesterol, administration of chronic high oral doses of nicotinic acid can lead to hepatotoxicity, as well as, dermatologic manifestations. An upper limit (UL) of 35 mg/day as proposed by the United States Food and Nutrition Board has been adopted by the FAO/WHO 2004 Consultation on Vitamin and Mineral Requirements.

Having studied the deficiency and toxicity aspects linked with niacin, let us next get to know about the requirements of niacin.

Recommended Dietary Allowance (RDA)

There are various factors on which niacin requirements depend. These are energy utilization, body size and dietary tryptophan. The efficiency of conversion of tryptophan

to niacin is affected by various factors including the amount of tryptophan and niacin ingested, protein and energy intake, as well as, vitamin B₆ and B₂ status.

The ICMR and the FAO/WHO 2004 recommendations for niacin are presented in Table 8.7. ICMR recommended RDI for individual intake of niacin as niacin equivalents (NE) for adult is 6.6 mg/1000 Kcal i.e. for a sedentary male and sedentary female it is 16 and 12 mg/day, respectively. With pregnancy and lactation, the RDA increases to about 14-16 mg/day.

Table 8.7: ICMR and FAO/WHO recommended dietary intakes for niacin by groups recommended Niacin Intake (mg NEs/day)

Group		ICMR	Group	FAO/WHO 2004 RNI
Man	Sedentary work	16	<i>Adults. (19+ years)</i> Males	16
	Moderate work	18		
	Heavy work	21		
Women	Sedentary work	12	<i>Adults (19+ years)</i> Females	14.0
	Moderate work	14		
	Heavy work	15		
<i>Pregnancy</i>		+2	<i>Pregnancy</i>	18
<i>Lactation</i>			<i>Lactation</i>	17
	0 - 6 months	+4		
	7 - 12 months	+3		
<i>Infants</i>	0 - 6 months	710 µg/kg	<i>Infants</i> 0 - 6 months 7 - 12 months	2.0 ^a
	6 - 12 months	650 µg/kg		4.0
<i>Children</i>	1 - 3 years	8	<i>Children</i> 1 - 3 years 4 - 6 years 7 - 9 years	6.0
	4 - 6 years	11		8.0
	7 - 9 years	13		12.0
<i>Adolescent</i>			<i>Adolescents</i>	
	Boys 10 - 12 years	15	Males (10 - 18 years)	16.0
	Girls 10 - 12 years	13	Females (10 - 18 years)	16.0
	Boys 13 - 15 years	16	Boys 16 - 18 years	17
	Girls 13 - 15 years	14	Girls 16 - 18 years	14

RNI: Recommended Nutrient Intake.

NEs, niacin equivalents.

^a Preformed.

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

Having learnt about the requirements, next we shall learn about how to assess the niacin status of individuals.

Criteria for Assessment of Niacin Status

Niacin status can be monitored by daily urinary excretion of methylated metabolites, especially the ratio of the 2-pyridone to N'-methyl-nicotinamide, erythrocyte pyridine nucleotides, oral dose uptake tests, erythrocyte NAD, and plasma 2-pyridone.

Excretion of N-methyl nicotinamide in urine after an oral niacin load of 20 mg nicotinamide/70 kg body weight over 24 hours is measured and levels of <5.8 mmol/dl represents deficiency and 5.8-17.5 mmol/dl represents low niacin status. *Shibata* and *Matsuo* suggest that the ratio of urinary 2-pyridone to N'-methyl-nicotinamide is as much a measure of protein adequacy as it is a measure of niacin status. The ratio of the 2-pyridone to N'-methyl-nicotinamide also appears to be associated with the clinical symptoms of pellagra, principally the dermatitic condition. In plasma, 2-pyridone levels change in reasonable proportion to niacin intake. As in the case of the erythrocyte pyridine nucleotides (nicotinamide coenzymes), NAD

concentration decreased by 70% whereas NADP remained unchanged in adult males fed diets with only 6 or 10 mg NEs/day.

Interaction with other Nutrients

You may recall reading earlier that tryptophan present in dietary proteins is converted to niacin. There is an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B₆ (as pyridoxal phosphate) and riboflavin (as FAD) are functional. Besides this interaction, do look up the interaction of niacin with other nutrients presented in section 8.10.

Next, we shall review pyridoxine.

8.6 PYRIDOXINE (VITAMIN B₆)

Pyridoxine or vitamin B₆ is one of the B complex vitamins which prevents and cures dermatitis in rats fed on vitamin B₆ deficient diets. Vitamin B₆ comprises a triad of closely related heterocycles that in free form are called pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM). Look up Unit 3 in the Nutritional Biochemistry Course for understanding the structure of these compounds. Here, for your reference, the basic pyridoxine structure is illustrated in Figure 8.1(d). As you would have noticed, pyridoxine contains a pyridine nucleus, two primary alcoholic groups and one phenolic hydroxyl group. Pyridoxal contains an aldehyde group in place of one primary alcoholic group and pyridoxamine contains a primary amide side chain in place of a primary alcoholic group. The natural base forms of the three vitamin B₆ vitamers vary in the substituent at position 4 of 2-methyl 3 hydroxy -5-hydroxy methyl-pyridine.

Let us next review the food sources of pyridoxine.

Food Sources

Raw foods contain more of this vitamin than cooked foods. The food sources include:

- *Rich sources:* Rice polishings, wheat bran, wheat germ, dried yeast, liver.
- *Good sources:* Whole cereals, legumes, nuts and seeds, milk powder, meat, egg, leafy vegetables.
- *Fair sources:* Milled cereals, vegetables and fruits.

Figure 8.6 illustrates some rich sources of pyridoxine.

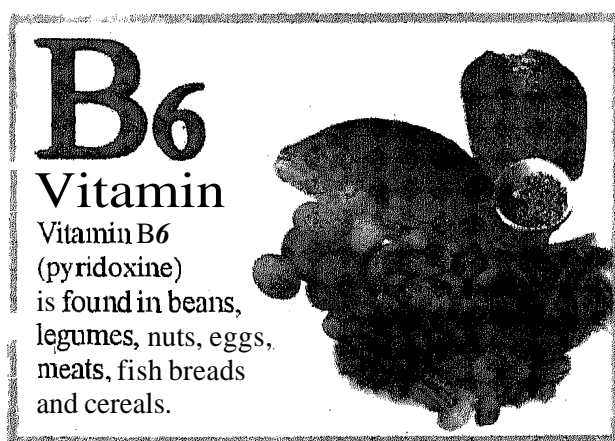


Figure 8.6: Food sources of pyridoxine

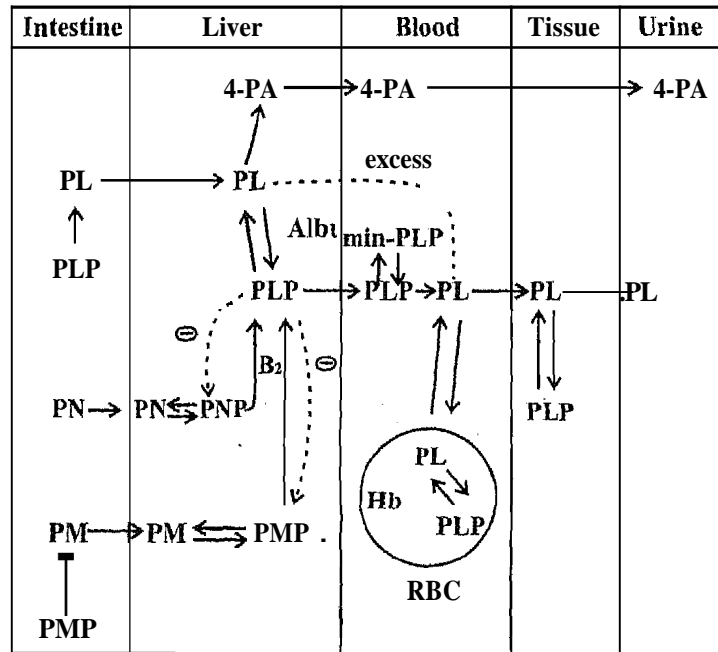
Remember, long storage, canning, roasting or stewing of meat, food-processing techniques, use of alcohol are destructive to this vitamin,

Next, let us get to know what happens to this vitamin once it is ingested.

Absorption, Storage and Elimination

Pyridoxine, pyridoxal and pyridoxamine (along with their phosphorylated forms) occur in plant and animal foods. The phosphorylated B₆ vitamers are dephosphorylated by

membrane bound *alkaline phosphatase* (found at the intestinal brush border) and absorption occurs primarily in the intestine (jejunum) by passive diffusion as illustrated in Figure 8.7.



PL - pyridoxal	PLP - pyridoxal S-phosphate	RBC - red blood cell
PN - pyridoxine	PNP - pyridoxine S-phosphate	O feedback inhibition
PM - pyridoxamine	PMP - pyridoxamine S-phosphate	B ₂ - riboflavin
4 PA - pyridoxic acid		

Figure 8.7: Graphical representation of B₆ absorption and transport

After absorption, each form of the vitamin is again phosphorylated (i.e. PLP, PNP, PMP) and retained. This process is called *metabolic trapping*. Thus, free vitamins enter by passive diffusion, facilitated by metabolic trapping.

The vitamin is transported in blood both in plasma and in red cells, mainly bound to albumin (refer to Figure 8.7) in plasma and haemoglobin in erythrocytes. Eighty percent of vitamin B₆ is present in muscle. Excretion is through the urinary pathway.

Once, the *pyridoxine* is absorbed and utilized by the body, it is used up for the various functions as described next.

Functions

There are three different forms of vitamin B₆, namely pyridoxine, pyridoxamine, and pyridoxal. All three must be phosphorylated and the 5'-phosphates of the first two forms are oxidized to the functional *pyridoxal phosphate* (PLP), which serves as a carbonyl-reactive coenzyme to a number of enzymes involved in the metabolism of amino acids. Such enzymes include aminotransferases, decarboxylases, and dehydratases; δ -aminolevulinatase in haem biosynthesis, and phosphorylase in glycogen breakdown and sphingoid base biosynthesis. Let us study these functions in greater details.

- 1) **Formation of amines:** Pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP) are vital for the formation of several amines that are functional in nervous tissues (e.g. epinephrine, nor epinephrine, serotonin and α - amino butyrate) for the biosynthesis of haem, formation of sphingolipids and phosphorylation of glycogen. A synopsis of the cellular process involving pyridoxine are given in Figure 8.8

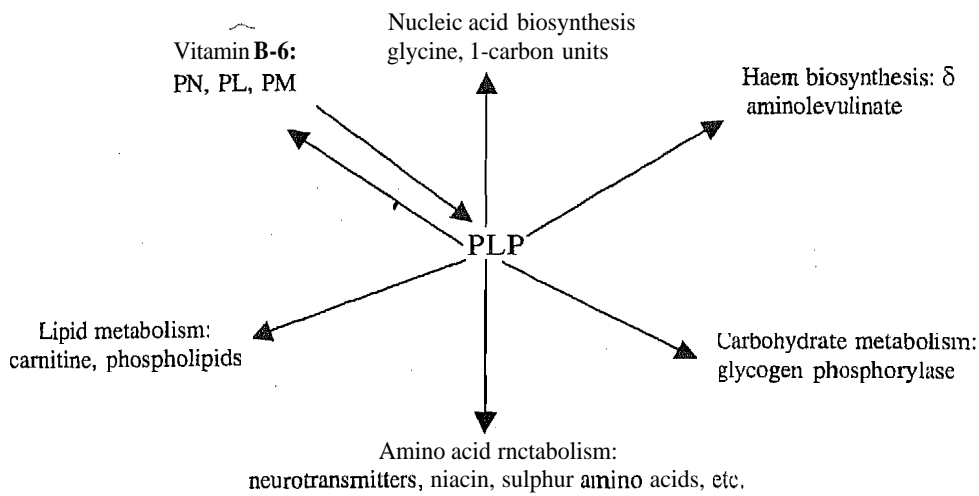


Figure 8.8: Cellular process involving pyridoxine

- 2) *Growth purposes:* Pyridoxal 5-phosphate (PLP) is essential for growth of infants and prevents degeneration of the nerves.
- 3) *Coenzyme activity:* Pyridoxal phosphate acts as a coenzyme in the following set of reactions.
 - a) *Transaminase system:* The two important transaminase systems are:

Glutamic acid + Oxaloacetic acid \rightleftharpoons α -ketoglutarate + Aspartate

Alanine + α -ketoglutarate \longrightarrow Glutamic acid + Pyruvic acid
 - b) *Amino acid decarboxylase system:* These enzymes convert the amino acids into the corresponding amines.

Histidine \longrightarrow Histamine + CO₂

Tyrosine \longrightarrow Tyramine + CO₂
 - c) *Conversion of tryptophan to niacin:* Tryptophan is converted into niacin, as you may recall studying earlier. Look at Figure 8.9, which illustrates the steps involved in the conversion of tryptophan to niacin.

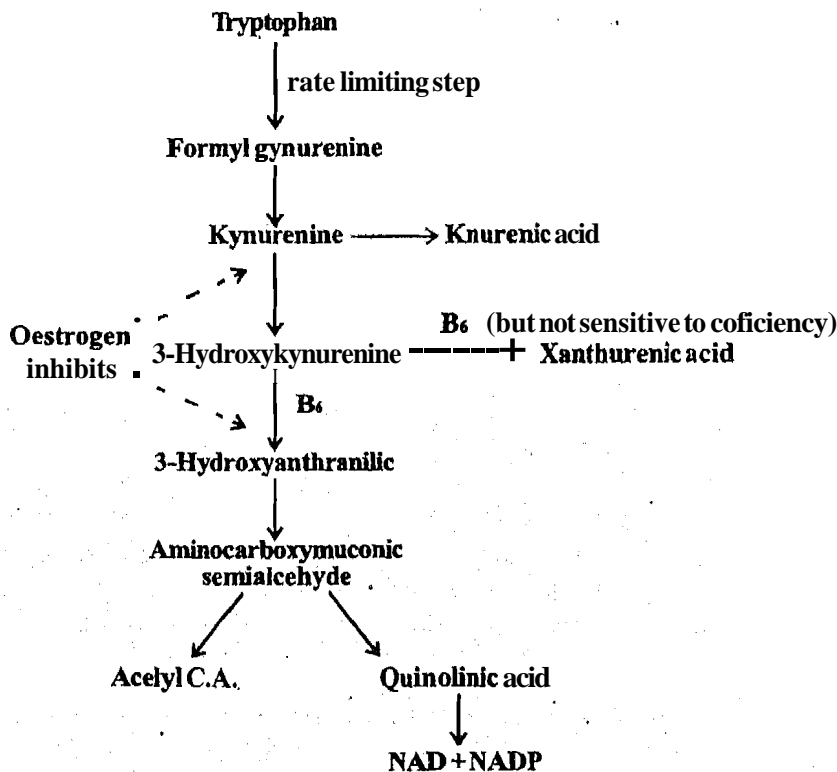


Figure 8.9: Conversion of tryptophan to niacin

Pyridoxine is needed for the conversion of hydroxy kynurenine to hydroxy anthranilic acid. Hence, niacin is not formed in pyridoxine deficiency. Hydroxy kynurenine is converted to xanthurenic acid and excreted in the urine.

- d) *Muscle phosphorylase*: Pyridoxal phosphate is a component of muscle phosphorylase.
- e) *Dehydrases*: These enzymes are vital to the catabolism of threonine, serine and homoserine and contain pyridoxine.



- f) *Racemases*: Racemases convert D-amino acids to their L-forms.



- 4) *Synthesis of Porphyrin*: Pyridoxal phosphate is required for the synthesis of 6-amino levulinic acid, an important intermediate in the synthesis of porphyrin and haem nuclei.
- 5) *Neurohormones*: Pyridoxal phosphate is essential for the formation of several neurohormones such as serotonin, α -amino butyric acid and epinephrine.
- 6) *Anti-atherosclerotic effect*: Vitamin B₆ deficiency precipitates hypercholesterolemia and atherosclerosis. However, the exact role of pyridoxine in this process is not clear.
- 7) *Immune bodies*: Vitamin B₆ deficiency is associated with impairment in both humoral and cell mediated immunity.
- 8) *Coenzyme A synthesis*: Pyridoxine is involved in the synthesis of coenzyme A from pantothenic acid. In pyridoxine deficiency, coenzyme A level in the liver is restored.

Having read about the functions, next let us get to know about bioavailability of pyridoxine.

Bioavailability

A recent review by Gregory confirms that bioavailability of vitamin B₆ in a mixed diet is about 75%, with approximately 8% of this total contributed by pyridoxine α -D-glucoside, which is about half as effectively utilized as free B₆ vitamers or their phosphates. The amine and aldehyde forms of vitamin B₆ are probably about 10% less effective than pyridoxine. Despite the involvement of PLP with many enzymes affecting amino acid metabolism, there seems to be only a slight effect of dietary proteins on vitamin B₆ status. Several studies have reported decrease in indicators of vitamin B₆ status in women receiving oral contraceptives, but this probably reflects hormonal stimulation of tryptophan catabolism rather than any deficiency of vitamin B₆ per se.

The deficiency and toxicity symptoms are described next.

Deficiency and Toxicity

A deficiency of vitamin B₆ alone is uncommon because it usually occurs in association with a deficit in other B-complex vitamins. Early biochemical changes include decreased levels of plasma pyridoxal 5'-phosphate (PLP) and urinary 4-pyridoxic acid. These are followed by decrease in synthesis of *transaminases* (aminotransferases) and other enzymes of amino acid metabolism such that there is an increased presence of xanthurenate in the urine and a decreased glutamate conversion to the anti-neurotransmitter α -aminobutyrate. Hypovitaminosis B₆ may often occur with riboflavin deficiency, because riboflavin is needed for the formation of the coenzyme PLP.

Infants are especially susceptible to insufficient intakes, which can lead to epileptic form convulsions. Skin changes include dermatitis with *cheilosis* and *glossitis*. Moreover, there is usually a decrease in circulating lymphocytes and sometimes a normochromic, microcytic, or sideroblastic anaemia as well. The sensitivity of such

systems as sulphur amino acid metabolism to vitamin B₆ availability is reflected in homocysteinaemia. A decrease in the metabolism of glutamate in the brain, which is found in vitamin B₆ insufficiency, reflects a nervous system dysfunction. As is the case with other micronutrient deficiencies, vitamin B₆ deficiency results in an impairment of the immune system. Of current concern is the pandemic-like occurrence of low vitamin B₆ intakes in many people who eat poorly (e.g. people with eating disorders). Vitamin B₆ deficiency has also been observed in south-east Asian school children (infected with hookworm), elderly Europeans (Dutch), and in some individuals with hyperhomocysteinaemia or who are on chronic haemodialysis. Several medical conditions can also affect vitamin B₆ metabolism and thus lead to deficiency symptoms.

Toxicity

Though toxicity related to pyridoxine intake are rare, but use of high doses of pyridoxine for the treatment of pre-menstrual syndrome, carpal tunnel syndrome (compression of the median nerve at the wrist resulting in numbness, tingling, weakness in the hand and fingers), and some neurologic diseases has resulted in neurotoxicity. A upper limit (UL) of 100 mg/day as proposed by the United States Food and Nutrition Board has been adopted by the FAO/WHO 2004 Consultation on vitamin and mineral requirements.

So then what are the recommendations for pyridoxine intake? Let us find out.

Recommended Dietary Allowance (RDA)

Average requirements for pyridoxine vary with age, sex and physiological conditions such as protein status, pregnancy and lactation.

The ICMR recommendations for individual intake of pyridoxine for adult males and female, is the same – 2.0 mg/day, as can be seen in Table 8.8. During increased demands of the body, i.e. pregnancy and lactation, the recommended level of intake is 2.5 mg/day. These Indian recommendations are much higher than those recommended by the FAO/WHO 2004.

Table 8.8: ICMR, FAO/WHO recommended nutrient intake of pyridoxine by groups

Recommended Pyridoxine Intake (mg/day)			
Group	ICMR	Group	FAO/WHO 2004 RNI
Man Sedentary work	2.0	<i>Adults</i> Males	13
Moderate work		(19 - 50 years)	
Heavy work		(51+ years)	
Woman Sedentary work	2.0	<i>Adults</i> Females	13
Moderate work		(19 - 50 years)	
Heavy work		(51+ years)	
Pregnancy	2.5	<i>Pregnancy</i>	1.9
Lactation	2.5	<i>Lactation</i>	2.0
<i>Infants</i> 0 - 6 months	0.1	<i>Infants</i> 0 - 6 months	0.1
7 - 12 months	0.4	7-12 months	0.3
<i>Children</i> 1 - 3 years	0.9	<i>Children</i> 1 - 3 years	0.5
4 - 6 years	0.9	4 - 6 years	0.6
7 - 9 years	1.6	7 - 9 years	1.0
<i>Adolescent</i>		<i>Adolescents</i>	
Boys 10 - 12 years	1.6	Males (10-18 years)	12
Girls 10 - 12 years		Females (10-18 years)	13
Boys 13 - 15 years	2.0		
Girls 13 - 15 years			
Boys 16 - 18 years	2.0		
Girls 16 - 18 years			

RNI: Recommended Nutrient Intake.

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements, in Human Nutrition, FAO/WHO (2004).

Let us next learn about the criteria for assessment of pyridoxine status.

Criteria for Assessment of Pyridoxine Status

Vitamin B₆ status is most appropriately evaluated by using a combination of indicators, namely plasma PLP concentration, urinary excretion, erythrocyte aminotransferases activity coefficients, tryptophan catabolites, erythrocyte and whole blood PLP concentration, and plasma homocysteine concentration, including those considered as direct indicators (e.g. **vitamer** concentration in cells or fluids) and those considered to be indirect or functional indicators (e.g. erythrocyte aminotransferase saturation by PLP or tryptophan metabolites).

Plasma PLP may be the best single indicator because it appears to reflect tissue stores. A plasma PLP concentration of 20 mmol/l has been proposed as an index of adequacy based on recent findings. Plasma PLP levels have been reported to fall with age. **Urinary 4-pyridoxic acid level** responds quickly to changes in vitamin B₆ intake and is therefore of questionable value in assessing status. However, a value higher than 3 mmol/day, achieved with an intake of approximately 1mg/day, has been suggested to reflect adequate intake. *Erythrocyte aminotransferases* for aspartate and **alanine** are commonly measured before and after addition of PLP to ascertain amounts of apoenzymes, the proportion of which increases with vitamin B₆ depletion. Values of 1.5-1.6 for the aspartate **aminotransferase** and approximately 1.2 for the **alanine aminotransferase** have been suggested as being adequate. Catabolites from tryptophan and **methionine** have also been used to assess vitamin B₆ status. In a review of the relevant literature, *Leklem* suggested that a 24-hour urinary excretion of less than 65 mmol xanthurenate after a 2g oral dose of tryptophan indicates normal vitamin B₆ status.

Details related to interaction of pyridoxine with other nutrients is presented next.

Interaction with other Nutrients

Pyridoxine is shown to have effects with the **macronutrients**, as well as, other **water-soluble vitamins**. What are these? Let us read and find out.

- **Carbohydrates:** Pyridoxine is involved in glyconeogenesis through its action in **transaminase** reactions. Low levels of pyridoxine impair glucose tolerance. The coenzyme form of vitamin B₆ or **pyridoxal phosphate** or co-decarboxylase is responsible for **all** non oxidative enzymic amino acid transformations and catalyzes reactions such as decarboxylation transamination, racemization, B₆ urinary elimination, amino acid **metabolism**. PLP has a key role in lipid metabolism and vitamin B₆ deficiency lowers body fat, liver lipid levels and impairs degradation of lipids. Administration of riboflavin, **pantothenic acid** and thiamine provide partial protection against seizures in Vitamin B₆ deficient experimental animals.
- **Ascorbic acid:** Vitamin B₆ metabolism increases with **higher** levels of vitamin C intake. Whole blood ascorbic acid levels fell during vitamin B₆ depletion and returned to normal levels, during repletion phase.
- **Leucine:** Excess of the amino acid, **leucine** in the diet antagonizes the function of vitamin B₆ and impairs the conversion of tryptophan to **niacin**.
- **Cyanocobalamin:** Vitamin B₆ deficiency is reported to cause impairment in vitamin B₁₂ absorption in rats.

With this, we end our study of pyridoxine. Next, let us review folate.

8.7 FOLATE.

Folate is a generic term which includes naturally occurring food folate and folic acid in supplements and fortified foods. Pteroyl **monoglutamic acid** and its derivatives are known as the *folic acid* group. Common structural features of the folate family include a **pteridine bicyclic ring system**, para-aminobenzoic acid (PABA) and one or more **glutamic moieties** (refer to Figure 8.1e). The term **folic acid** relates specifically to the fully oxidized monoglutamate form of the vitamin synthesized for commercial use in supplements and fortified foods.

What are the food sources of folate? Let us have a look.

Food Sources

Folate occurs naturally in foods. Although folate is found in a wide variety of foods, it is present in a relatively low density except in liver. Diets that contain adequate amounts of fresh green vegetables (i.e. in excess of three servings per day) will be good folate sources. Folate losses during harvesting, storage, distribution and cooking can be considerable. Similarly, folate derived from animal products is subject to loss during cooking. Some staples, such as white rice are low in folate. Figure 8.10 illustrates some common sources of folate as also highlighted in the classification herewith:

- *Rich sources:* Liver, dried yeast, leafy vegetables, wheat germ and rice polishings.
- *Good sources:* Whole cereals, dried legumes (pulses have twice as much folic acid as cereals), nuts, fresh oranges, green leafy vegetables,
- *Fair sources:* Milled cereals, other vegetables, milk and fruits.

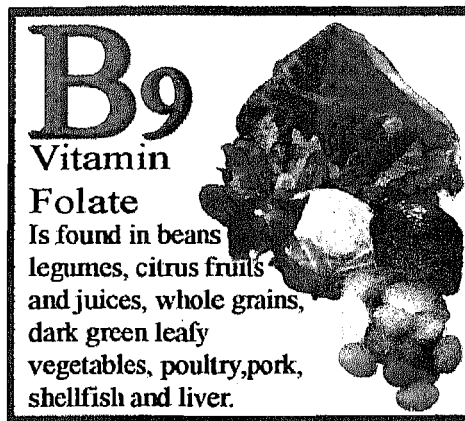


Figure 8.10: Rich food sources of folate

It is important to remember that the natural folates found in foods are in a conjugated form, which reduces their bioavailability by perhaps as much as 50%. In addition, natural folates are much less stable.

The metabolic fate of folate is discussed next.

Absorption, Storage and Elimination

Folic acid is readily absorbed from the small intestines through the portal vein and passed onto the tissues through general circulation. Naturally occurring food folate is converted into the monoglutamate form by the enzyme *pteroylpolyglutamate hydrolase* or *folate conjugase* or *glutamate carboxypeptidase II*, located in the jejunal brush border membrane. After deconjugation, the monoglutamyl folate is transported across the membrane by a pH-dependent carrier-mediated mechanism.

Folic acid once absorbed is acted upon by hepatic *dihydrofolate reductase* to convert to its metabolically active form which is *tetrahydrofolic acid (THF)*. Following absorption, folic acid is largely reduced and methylated in the liver to *N-5 methyltetrahydrofolic acid*, which is the main transporting and storage form of folate in the body. Folate transport across membranes into cells in kidney, placenta and choroid plexus, occurs via membrane-associated folate binding proteins that act as folate receptors and facilitate cellular uptake of folate.

Larger doses of folate may escape metabolism by the liver and appear in the blood mainly as folic acid,

Having studied about the metabolic fate of folate, next let us get to know what role folate plays in our body.

Functions

Folate, also known as folic acid, is essential for good health. Folate requiring reactions include those involved in phases of amino acid metabolism, DNA (purine and pyrimidine) biosynthesis and the formation of the primary methylating agent, S-adenosyl methionine (SAM) as shown in Figure 8.11.

Folate is involved in the de novo synthesis of purines (adenine and guanine), requiring the folate form, 10-formyl tetrahydro folic acid (THF), which is produced from 5, 10-methylene THF reactions catalyzed by the enzyme THF synthetase. The 5,10-methylene THF molecule has several fates, one of which is the reconversion to 5-methyl THF, catalyzed by *methylene tetra hydrofolate reductase* (MTHFR). Thus, folate in its reduced and polyglutamylated forms is essential for the DNA biosynthesis cycle, as shown in Figure 8.11. This conversion (5,10-methylene THF molecule reconversion to 5-methyl THF) forms *methionine* from homocysteine. Folate, specially helps in reducing the risk of heart disease and stroke by lowering the level of the amino acid homocysteine in the blood (by forming methionine). At high levels, homocysteine can damage coronary arteries or make it easier for blood clotting cells to clump together and form a clot. This can increase the risk of heart attack or stroke. This methylation reaction (refer to Figure 8.11) requires the enzyme methionine synthase, cobalamin (vitamin B₁₂) and 5-methyl THF. A methyl group is removed from 5 methyl THF and is sequentially transferred first to cobalamin coenzyme and then to homocysteine forming methionine and reconverting 5-methyl THF to tetrahydrofolate (THF). The dependency of methionine synthase on both folate and cobalamin explains why a single deficiency of either vitamin leads to the same megaloblastic changes in the bone marrow and other tissues, with rapidly dividing cells.

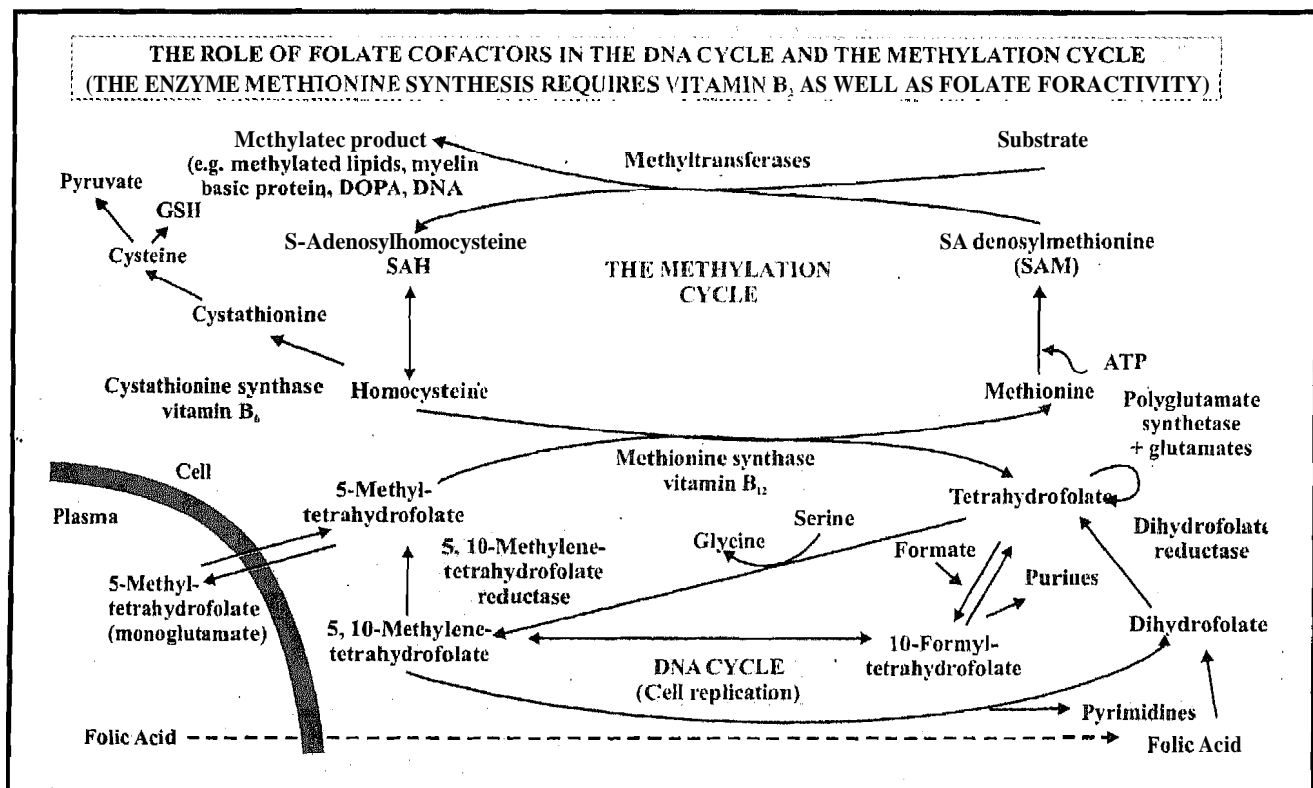


Figure 8.11: The role of folate in the DNA biosynthesis and methylation cycle

Alternatively, 5,10-methylenetetrahydrofolate can be channelled to the *methylation cycle* (refer to Figure 8.11). This cycle has two functions. It ensures that the cell always has an adequate supply of S-adenosylmethionine (SAM), an activated form

of methionine which acts as a methyl donor to a wide range of methyltransferases. The methyltransferases methylate a wide range of substrates including *lipids*, *hormones*, DNA and *proteins*. One particularly important methylation is that of myelin basic protein, which acts as insulation for nerve cells. When the methylation cycle is interrupted, as it is during vitamin B₁₂ deficiency, one of the clinical consequences is the demyelination of nerve cells resulting in a neuropathy which leads to ataxia (lack of coordination), paralysis, and, if untreated, ultimately death. Other important methyltransferase enzymes down-regulate DNA and suppress cell division.

Folate is also important for pregnant women. Low blood levels of folate during pregnancy can cause neural tube defects—*anencephaly* (a defect in the closure of the neural tube) and *spina bifida* (a congenital defect in which the spinal column is imperfectly closed so that part of the meninges or spinal cord protrudes, often resulting in hydrocephalus and other neurological disorders). And people with anaemia or at risk of anaemia need to be sure they consume enough folate as well.

Having read about the functions of folate, now you should be in a better position to appreciate why it is important to have an adequate intake and availability of folate from our diet. Next, let us learn about the requirements and availability of folate.

Bioavailability

Bioavailability of folate from naturally occurring food sources is variable and frequently incomplete, as mentioned earlier in the food sources section. The bioavailability of natural folates is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete, thereby reducing the bioavailability of natural folates by as much as 25-50%. In contrast, synthetic folic acid appears to be highly bioavailable—85% or greater. The low bioavailability and, more importantly, the poor chemical stability of the natural folates have a profound influence on the development of nutrient recommendations. This is particularly true if some of the dietary intake is as stable and bioavailable as the synthetic form, folic acid. Fortification of foods such as breakfast cereals and flour can add significant amounts of folic acid to the diet.

Since folic acid (synthetic) taken with food is 85% bioavailable but food folate is only about 50% bioavailable, folic acid taken with food is 85/50 (i.e. 1.7) times more available. Thus, if a mixture of synthetic folic acid plus food folate has been fed, dietary folate equivalents (DFEs) are calculated as follows to determine the EAR:

$\mu\text{g of DFE provided} = [\mu\text{g of food folate} + (1.7 \times \text{mg of synthetic folic acid})]$.

To be comparable to food folate, only half as much folic acid is needed if taken on an empty stomach, i.e. $1 \mu\text{g of DFE} = 1 \mu\text{g of food folate} = 0.5 \mu\text{g of folic acid taken on an empty stomach} = 0.6 \mu\text{g of folic acid with meals}$.

Alcohol interferes with the absorption of folate and increases excretion of folate by the kidney.

Before we move on to the recommendations for folic acid intake, let us quickly look at the conditions arising when folate is deficient in the diet.

Deficiency

If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles, described above, will be reduced. A decrease in the former will reduce DNA biosynthesis and thereby reduce cell division. Although this will be seen in all dividing cells, the deficiency will be most obvious in cells that rapidly divide, including for example red blood cells, thereby producing *megaloblastic* anaemia characterized by large, abnormally nucleated erythrocytes, as can be seen in Figure 8.12, that accumulate in bone marrow.

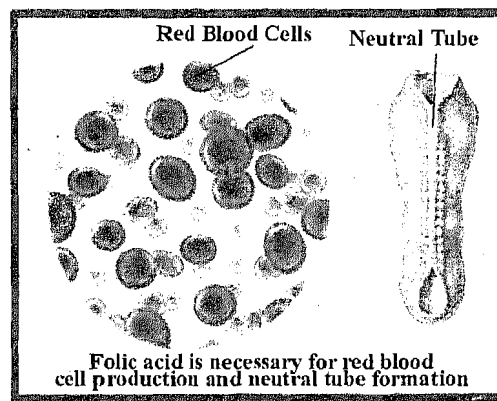


Figure 8.12: Function of folic acid

Taken together, the effects caused by the reduction in the DNA cycle result in an increased susceptibility to infection, a decrease in blood coagulation, and intestinal malabsorption. Folate deficiency will also decrease the flux through the methylation cycle but the DNA cycle may be more sensitive. The most obvious expression of the decrease in the methylation cycle is an elevation in plasma homocysteine. This is due to a decreased availability of new methyl groups provided as 5-methyltetrahydrofolate, necessary for the remethylation of plasma homocysteine. Previously it was believed that a rise in plasma homocysteine was nothing more than a biochemical marker of possible folate deficiency. However, there is an increasing evidence that *plasma homocysteine concentration, if only moderately elevated, is an independent risk factor for cardiovascular disease and stroke*. Interruption of the methylation cycle resulting from impaired folate status or decreased vitamin B₁₂ or vitamin B₆ status may have serious long-term risks.

Pregnant women are at a higher risk of developing folate deficiency because of increased demand for folate. In addition to megaloblastic anaemia, inadequate folate intake is associated with poor pregnancy outcomes. Impaired folate status is associated with increased risk of pre-term delivery, infant low birth weight and foetal growth retardation. An elevated maternal homocysteine concentration leads to increased habitual spontaneous abortion and pregnancy complications (e.g. abruptio placentae or placental infarction with foetal growth retardation and pre eclampsia) which increase the risk of low birth weight and preterm delivery.

Folate deficiency is associated with Neural Tube Defects (NTDs) as highlighted in Figure 8.12. During pregnancy, there is an increased risk of foetal neural tube defects (NTDs), with risk increasing 10-fold as folate status goes from adequate to poor. Between days 21 and 27 post-conception, the neural plate closes to form what will eventually be the spinal cord and cranium. Spina bifida, anencephaly and other similar conditions are collectively called NTDs. They result from improper closure of the spinal cord and cranium, respectively, and are the most common congenital abnormalities associated with folate deficiency.

Multivitamin supplements containing folic acid reduce the risk of NTDs. It is now agreed that a supplement of 400 µg of folic acid taken near the time of conception will prevent most NTDs. The recommendation to prevent recurrence in women with a prior NTD birth remains 4 mg/day.

Folate status is also related to birth defects other than NTDs such as cleft lip and palate, limb deficiencies and conotruncal or the outflow tract defects of the heart.

In addition, evidence also suggests a link between colorectal cancer and dietary folate intake and folate status. Low folate status has been associated with an increased risk of colorectal cancer.

Thus, it is evident that folate is very essential for good health. The next question that comes to mind then is their a risk associated with excessive consumption of folate as well. Read the next sub-section on toxicity and find out.

Toxicity

There is no evidence to suggest that it is possible to consume sufficient natural folate to pose a risk of toxicity. However, this clearly does not apply to folic acid given in supplements or fortified foods. The main concern with fortification of high levels of folic acid is the masking of the diagnosis of pernicious anaemia, because high levels of folic acid correct the anaemia, allowing the neuropathy to progress undiagnosed to a point where it may become irreversible, even upon treatment with vitamin B₁₂. Consumption of large amounts of folic acid might also pose other less well-defined risks. The United States National Academy of Sciences (NAS), after reviewing the literature, has suggested an upper level of 1000 µg. There is probably no great risk of toxicity at a range of intakes between 400 and 1000 µg of folic acid per day, with the exception of some increased difficulty in diagnosing pernicious anaemia.

So then what are the requirements for folate? Let us find out,

Recommended Dietary Allowance (RDA)

Folate requirements are the intake levels necessary to prevent deficiency with clinical symptoms. The requirements are expressed as differences in bioavailability between dietary folate equivalents (DFE) and food folate. One DFE is equal to 1 mcg of food folate. Table 8.9 presents the ICMR and the FAO/WHO recommended nutrient intake for folic acid by groups. The individual requirements of folate for both the sexes recommended by ICMR is 100 µg/day, which increases in conditions of pregnancy and lactation to 400 and 150, respectively.

In 1998, the United States National Academy of Sciences (NAS) exhaustively reviewed the evidence regarding folate intake, status, and health for all age groups, including pregnant and lactating women. On the basis of their review, the NAS calculated estimated average requirements (EARs) and recommended dietary allowances (RDAs), taken to be the EAR plus 2 standard deviations, for folate. The 2004 FAO/WHO Expert Consultation has adopted the RDAs of the NAS as the basis for their RNIs (Table 8.9).

Table 8.9: ICMR and FAO/WHO recommended dietary intakes for folic acid expressed as dietary folate equivalent by groups

Group	ICMR µg/day	Group	FAO/WHO 2004						
			EAR (µg/day)	RNI (µg/day)					
<i>Man</i> Sedentary work Moderate work Heavy work	100	<i>Adults</i> Males (19- 65 years) (65+ years)	320	400					
					<i>Women</i> Sedentary work Moderate work Heavy work	100	<i>Adults</i> Females (19 - 65 years) (65+ years)	320	400
Lactation	150	Lactation	450	500					
<i>Infants</i> 0-6 months 6-12 months	25	<i>Infants</i> 0-6 months ^a 7-12 months	65	80					
					<i>Children</i> 1-3 years 4-6 years 7-9 years	30 40 60	<i>Children</i> 1-3 years 4-6 years 7-9 years	120 160 250	150 200 300
<i>Adolescents</i> Boys 10-12 years Girls 10-12 years Boys 13-15 years Girls 13-15 years Boys 16-18 years Girls 16-18 years	70 100 100	<i>Adolescents</i> (10-18 years)	330	400					

^a Based on a human milk intake of 0.75 l/day.

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

The RNI's suggested for various groups by FAO/WHO in Table 8.9 assume that food folate is the sole source of dietary folate because most societies in developing countries consume folate from naturally-occurring sources. As discussed earlier, natural folates are found in a conjugated form in food, which reduces their bioavailability by perhaps as much as 50%. In addition, natural folates are much less stable. If chemically pure folic acid (pteroylmonoglutamate) is used to provide part of the RNI, by way of fortification or supplementation, the total dietary folate, which contains conjugated forms (pteroylpolyglutamates), could be reduced by an appropriate amount.

Finally, let us find out how can we assess the folate status in the body.

Criteria for Assessment of Folate Status

Red cell folate, continues to be used as an important index of folate status. It is suggested that adequate folate status is reflected in a red cell folate level of greater than 150 µg/L. Indicators of haematologic status such as raised mean corpuscular volume, hypersegmentation of neutrophils, and, eventually, the first stages of anaemia also remain important indicators of reduced folate status.

More recently, the biomarker plasma homocysteine has been identified as a very sensitive indicator of folate status and is added to the list of possible indicators of folate adequacy. Any elevation in plasma homocysteine, even at levels where overt folate deficiency is not an issue, may be undesirable because it is a risk factor for chronic disease. This new information requires the consideration of a folate intake that would reduce plasma homocysteine to a minimum level of less than 7.0 mmol/L.

Before we end our study on folate, let us quickly review the interaction of folate with other nutrients.

Interactions with other Nutrients

The interaction of folate with few nutrients is highlighted herewith:

- **Vitamin C:** Anaemia is observed in vitamin C deficient patients. Normochromic, normocytic or macrocytic or megaloblastic anaemia has been reported. These conditions responded to ascorbic acid therapy alone or along with folic acid.
- **Vitamin B₁₂:** For the conversion of folic acid to folinic acid, vitamin B₁₂ is required. Vitamin B₁₂ deficiency causes a rise in unconjugated folates and a marked depletion of intracellular conjugated folates. One of the vitamin B₁₂-dependent enzymes, methionine synthase, functions in one of the two folate cycles, namely, the methylation cycle, as you may recall reading earlier. Interruption of the cycle reduces the level of S-adenosylmethionine. Disruption of the methylation cycle also causes lack of DNA biosynthesis and anaemia.

Now evaluate your understanding on the topic by answering the check your progress exercise 2.

<p>Check Your Progress Exercise 2</p> <p>1) Fill in the blanks:</p> <p>a) The requirement of pyridoxine during lactation is mg.</p> <p>b)mcg of folate is required during pregnancy.</p> <p>c) Megaloblastic anaemia is associated with the deficiency of</p> <p>d) The three D's of niacin deficiency are , and</p>

2) Bring about the role of pyridoxine in the conversion of tryptophan to niacin.

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3) Give reasons:

a) Nicotinic acid is a part of the glucose tolerance factor.

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b) Commercial folic acid preparations are more effective than dietary food sources.

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.....

4) What is Niacin Equivalent (NE)?

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5) Comment on Neural Tube Defects.

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6) Describe any one method for assessment of the following vitamins:

a) Niacin

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a) Folic acid

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.....

7) Trace the interaction of macronutrients with pyridoxine.

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.....

We will now study about vitamin B₁₂ which is yet another B-group vitamin and finally we shall review the other water soluble vitamin i.e. vitamin C.

8.8 CYANOCOBALAMIN (VITAMIN B₁₂)

Vitamin B₁₂ (cobalamin, cbl) is a unique vitamin in human nutrition, since its malabsorption leads to the fatal syndrome of pernicious and megaloblastic anaemias with demyelinating lesions of the central nervous system. The structure of vitamin B₁₂ is shown in Figure 8.1. As you may have noticed, vitamin B₁₂ is the largest of the B complex vitamins. It consists of a corrin ring made up of four pyroles with cobalt at the centre of the ring. There are several vitamin B₁₂-dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B₁₂ synthesis. This fact has significant implications for the dietary sources and availability of vitamin B₁₂ as highlighted next.

Food Sources

Vitamin B₁₂ is unique among vitamins in the sense that it is mostly found in foods of animal origin but is not generally present in plant products as also is evident from the food sources highlighted herewith and in Figure 8.13.

- *Rich sources:* Liver (goat, sheep, ox, pig).
 - *Good sources:* Meat, fish, egg, kidney, brain.
- Fair sources:* Fresh milk, milk powder and cheese.

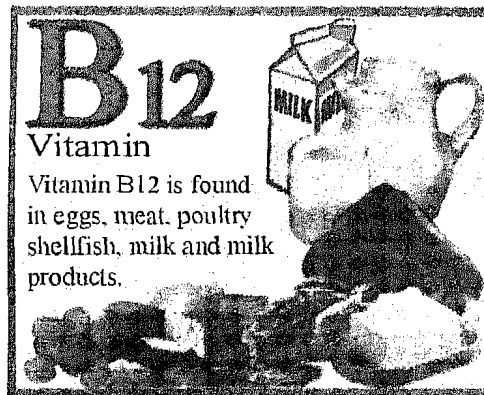


Figure 8.13: Food sources of vitamin B₁₂

Most microorganisms, including bacteria and algae, synthesize vitamin B₁₂, and they constitute the only source of the vitamin. The vitamin B₁₂ synthesized in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals, gastrointestinal fermentation supports the growth of these vitamin B₁₂ synthesizing microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B₁₂ is stored in large concentrations. Products from herbivorous animals, such as milk, meat and eggs, thus constitute important dietary sources of the vitamin.

Humans, therefore, derive dietary vitamin B₁₂ almost exclusively from animal tissues or products (i.e. milk, butter, cheese, eggs, meat, poultry). Considering the fact that this vitamin intake can only be assured through animal food sources, vegetarians, therefore, have to be very cautious about meeting their vitamin B₁₂ requirements and are hence often advised to increase their milk intake or take vitamin B₁₂ as a supplement.

Next, let us learn about the metabolic fate of vitamin B₁₂.

Absorption, Storage and Elimination

Vitamin B₁₂ in food is bound to proteins and is only released by the action of a high concentration of hydrochloric acid present in the stomach. Once released from foods, vitamin B₁₂ absorption involves contact with two proteins, *intrinsic factor* (IF) and *R binder*. The glycoprotein, called R-binders (or haptocoinins), protect

vitamin B₁₂ from chemical denaturation in the stomach, IF is a glycoprotein synthesized by the gastric parietal cells and function in the small intestine.

Although the formation of the vitamin B₁₂–intrinsic factor complex was initially thought to happen in the stomach, it is now clear that this is not the case. At an acidic pH, (in the stomach) the affinity of the intrinsic factor for vitamin B₁₂ is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which in turn causes them to release their vitamin B₁₂. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B₁₂, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B₁₂–intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptor. The absorbed cbl is processed into a complex transcobalamin–II–cbl (TCII-cbl), secreted into portal blood and delivered to the liver and ultimately all tissues.

Vitamin B₁₂ is the only B vitamin our body can store. The average adult body contains 2 to 5 mg of vitamin B₁₂ with 80 percent of this stored in the liver.

What is the role of vitamin B₁₂ in our body? Next, let us find out.

Functions

The manufacture and normal functioning of blood cells requires vitamin B₁₂ as highlighted in Figure 8.14. Vitamin B₁₂ is also essential for metabolism of fats and carbohydrates and the synthesis of proteins. You may also recall studying under the folate vitamin that vitamin B₁₂ is essential for the transport and storage of folate in cells and for conversion to its active form. Rapidly dividing cells, such as those in the epithelium and bone marrow, have the greatest need for vitamin B₁₂.

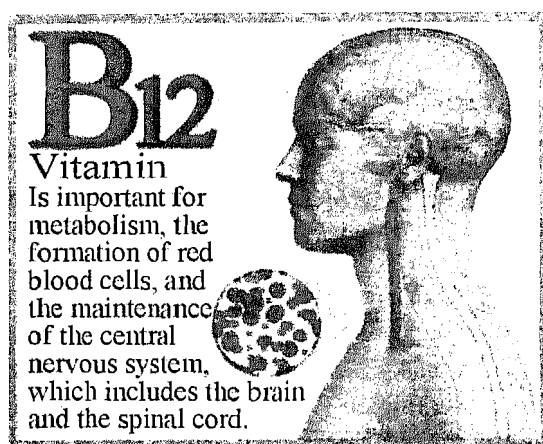


Figure 8.14: Some important functions of vitamin B₁₂

The functions of cyanocobalamin can thus be summarized as follows. It:

- promotes the maturation of erythroid cells,
- acts on other bone marrow elements and increases WBC and platelet count,
- stimulates the appetite and general health of the subject,
- cures neurological symptoms of pernicious anaemia. It is involved in the manufacture of the myelin sheath, a fatty layer which insulates nerves,
- is necessary for the production of nucleic acids, which make up DNA, the genetic material of the cell,
- functions as a coenzyme for the following reactions.:
 - 5'-deoxyadenosyl cyanocobalamin isomerizes glutamic acid to threo- α -methyl aspartate,
 - converts methyl malonyl CoA to succinyl CoA,
 - dehydrates ethylene glycol to acetaldehyde, and
 - converts homocysteine to methionine.

In mammalian cells, there are only two vitamin B₁₂-dependent enzymes. One of these enzymes, *methionine synthase*, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called *methylcobalamin*. The other enzyme, *methylmalonyl coenzyme (CoA) mutase*, uses a form of vitamin B₁₂ that has a 5'-deoxyadenosyl moiety attached to the cobalt and is called *5' - deoxyadenosylcobalamin*, or *coenzyme B₁₂* (as mentioned in the reaction above). In nature, there are two other forms of vitamin B₁₂: *hydroxycobalamin* and *aquacobalamin*, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B₁₂ found in supplements and fortified foods is *cyanocobalamin*, which has cyanide attached to the cobalt. These three forms of vitamin B₁₂ are enzymatically activated to the methyl-or deoxyadenosylcobalamin in all mammalian cells.

Bioavailability

Vitamin B₁₂ is widely available. Availability is more from non-vegetarian foods as described earlier under the food sources section. Atrophic gastritis, loss of intrinsic factor (IF), surgical manipulations of the gastrointestinal tract including total and partial gastrectomy, gastric bypass operations, ileal resections, parasitic infection with fish tapeworm and jejunal bacterial overgrowth cause malabsorption of vitamin B₁₂. Bioavailability decreases with age. A common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid. The absence of acid in the stomach is postulated to prevent the release of protein bound vitamin B₁₂ contained in food but not to interfere with the absorption of the free vitamin B₁₂ found in fortified foods or supplements.

Drugs like nitrous oxide, metformin and stomach acid blockers decrease availability. Other factors that destroy this vitamin are sunlight, alcohol, oestrogen—the female hormone. Calcium and protein-rich foods greatly help the absorption of this vitamin in the intestine. Hence, remember these practical tips discussed above to ensure good vitamin B₁₂ status.

What would be the outcome of lack of this vitamin in our diet. Read the next subsection and find out.

Deficiency

Malabsorption of vitamin B₁₂ can occur at several points during digestion. By far, the most important condition resulting in vitamin B₁₂ malabsorption is the autoimmune disease called *pernicious anaemia* (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, and lose their ability to produce intrinsic factor and secrete hydrochloric acid. In some forms of PA, the parietal cells remain intact but autoantibodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B₁₂. In another less common form of PA, the antibodies allow vitamin B₁₂ to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B₁₂ complex by the ileal receptors. The principal signs and symptoms of pernicious anaemia are as follows:

- 1) *Blood*: The RBC count is low—1.5-2.5 million per mm³ (normal range is 4.5-5.5 million per mm³). The average diameter of the cells is well above normal, about 8.2 μ as compared to a normal diameter of 7.3 μ. Abnormal circulating red cells undergo excessive destruction with a consequent increase in the serum bilirubin content. The haemoglobin content is low (8-9 percent).
- 2) *Bone marrow*: The nucleated red cells of the marrow are greatly increased. The successive nucleated cell stages in erythropoiesis are called stages I, II, III

and IV and in pernicious anaemia, cells of stages I and II constitute 70 percent and of stages III and IV, 30 percent while in normal persons, the case is reverse. The cells of stage I are peculiar and differ from the normal cells and are called megaloblastic. The overacting bone marrow in pernicious anaemia shows megaloblastic hyperplasia.

- 3) Stomach: The cells which secrete acid and enzymes are atrophied. The gastric secretions are devoid of acid, pepsin and intrinsic factor (IF).
- 4) Mouth: Soreness and inflammation of the tongue are commonly observed.
- 5) Nervous system: Parasthesia (numbness and tingling) occurs in fingers and toes. Occasionally, there are objective signs of involvement of the spinal cord (vitamin B₁₂ neuropathy). In advanced cases, demyelination of the white fibres of the spinal cord occurs, affecting first the dorsal column and later, the lateral column.

Historically, PA was considered to be the major cause of vitamin B₁₂ deficiency, more recently, it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic *gastritis*, where there is a progressive reduction, with age, of the ability of the parietal cells to secrete hydrochloric acid.

Vitamin B₁₂ Deficiency in Vegans

Because plants do not synthesize vitamin B₁₂, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B₁₂ deficiency. This is not true of lacto-ovo vegetarians, who consume the vitamin through eggs, milk and other dairy products.

Persons living exclusively on vegetarian diets (vegans) have low serum levels of vitamin B₁₂ and develop specific symptoms such as sore tongue, paraesthesia and signs of degeneration of the long tracts of the spinal cord as a result of low intakes of vitamin B₁₂. Megaloblastic anaemia is not so common when folic acid intake is adequate. The anaemia results from decreased DNA synthesis and failure of the cells to divide properly, coupled with the continued formation of RNA.

So now you would appreciate how important this vitamin is for all of us. What then is the recommended dietary allowance for this vitamin? Let us find out. But before that, we will also review the toxicity aspect of vitamin B₁₂, if any.

Toxicity

Intake of 1000 µg vitamin B₁₂ has never been reported to have any side-effects. Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects. However, there are no established benefits for such amounts. Such high intakes thus represent no benefit in those without malabsorption and should probably be avoided.

Recommended Dietary Allowance (RDA)

Vitamin B₁₂ deficiency is common in true vegans who can be treated with small doses since the daily requirement is only 1.0 µg/day, as you may have noticed in Table 8.10 which presents the TCMR recommendations for vitamin B₁₂ for all age groups. However, the requirements do increase in lactation by 0.5 µg/day.

Table 8.10: ICMR and FAO/WHO recommended dietary intakes for vitamin B₁₂ by group!

Group	ICMR µg/day	Group	FAO/WHO 2004	
			EAR (µg/day)	RNI (µg/day)
Man Sedentary work Moderate work Heavy work	1	Adults Males (19-65 years) (65+ years)	20	24
Woman Sedentary work Moderate work Heavy work	1	Adults Females (19-65 years) (65+ years)	2.0	2.2
Pregnancy	1	Pregnancy	2.2	2.6
Lactation	1.5	Lactation	24	2.8
Infants 0-6 months	0.2	Infants 0-6 months	0.3	0.4
6-12 months		7-12 months	0.6	0.7
Children 1-3 years	0.2-1.0	Children 1-3 years	0.7	0.9
4-6 years		4-6 years	0.1	1.2
7-9 years		7-9 years	1.5	1.8
Adolescent		Adolescents	2.0	2.4
Boys 10-12 years	0.2-1.0	(10-18 years)		
Girls 10-12 years				
Boys 13-15 years	0.2-1.0			
Girls 13-15 years				
Boys 16-18 years	0.2-1.0			
Girls 16-18 years				

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

The FAO/WHO 2004 recommendations also given in Table 8.10 include both the estimated average requirement (EAR) and the recommended nutrient intake (RNI) calculated as the EAR plus 2 SD. FAO/WHO suggests a requirement of 0.7-1.0 µg/day for those without pernicious anaemia. Since vitamin B₁₂ is not completely absorbed from food, an adjustment of 50% has to be added, giving a range of 1.4-2.0 µg/day.

Finally then how do we assess the vitamin status in our body? The next sub-section focuses on this aspect.

Criteria for Assessment of Vitamin B₁₂ Status

Low serum or plasma levels of vitamin B₁₂ should be the first indication of poor status and this could be confirmed by an elevated methylmalonic acid (MMA) which is excreted in urine. Let us get to know more about these measures.

- 1) *Serum vitamin B₁₂ assay:* The vitamin B₁₂ content of serum can be determined. A serum level of <140 µg/ml indicates vitamin B₁₂ deficiency.

Status	Serum level (µg/mL)
Normal	200 - 960
Subnormal	140 - 190

- 2) *Excretion of Methyl malonic acid in urine (Methyl malonic aciduria):* Methyl malonic acid is found only in traces in normal urine (1-2 mg/day). In vitamin B₁₂ deficiency (pernicious anaemia), the excretion of methyl malonic acid in urine increases to about 100 to 200 mg/day due to the absence of vitamin B₁₂ co-enzyme (methyl malonyl CoA isomerase) involved in the conversion methyl malonyl CoA to succinyl CoA. This is a sensitive method for assessing vitamin B₁₂ deficiency.

Before we end our discussion on vitamin B₁₂, we would like to highlight that vitamin B₁₂ interaction with folate or folic acid is very important from the human nutrition point of view. We have already emphasized earlier under the folate section that the vitamin B₁₂-dependent enzymes, *methionine synthase*, functions in one of the two folate cycles, namely, the methylation cycle. Interruption of the cycle reduces the level of S-adenosylmethionine. This occurs in pernicious anaemia and other causes of vitamin B₁₂ deficiency, producing as a result demyelination of the peripheral nerves and the spinal column, giving rise to the clinical condition called subacute combined degeneration. This neuropathy is one of the main presenting conditions in pernicious anaemia. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle also causes a lack of DNA biosynthesis and anaemia.

With this, we end our study of vitamin B₁₂ and also our study of the B-complex vitamins. Finally let us review the other water soluble vitamin i.e. ascorbic acid.

8.9 ASCORBIC ACID (VITAMIN C)

Ascorbic acid was discovered as the anti-scurvy vitamin. Vitamin C (chemical names ascorbic acid and ascorbate) is the six-carbon lactone of α -keto-L-gulonic acid. It is, in fact, a derivative of carbohydrate. It is closely related to monosaccharide sugars in its structure.

Vitamin C is synthesized in the liver in some mammals and in the kidney in birds and reptiles. However, humans are unable to synthesize vitamin C. Hence, when there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease-scurvy.

Having learnt about some basic facts about vitamin C, we move on to the food sources.

Food Sources

Food sources of vitamin C include:

- **Rich sources:** Amla and guava.
- **Good sources:** Drumstick leaves, other leafy vegetables and fruits such as cashew fruit melons, berries, pine apple and tomatoes.
- **Fair sources:** Apples, banana, grapes.

Vitamin C is found in many fruits and vegetables as highlighted in Figure 8.14. Citrus fruits and juices are particularly rich sources of vitamin C but other fruits including honeydew melons, cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, tomatoes, and water melon also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, brussels sprouts, bean sprouts, cauliflower, mustard greens, red and green peppers, peas, and potatoes may be more important sources of vitamin C than fruits, given that the vegetable supply often extends for longer periods during the year than does the fruit supply. Figure 8.15 highlights the foods in the food pyramid which provide you vitamin C.

The vitamin C content of food is strongly influenced by season, transport to market, length of time on the shelf and in storage, cooling practices, and the chlorination of the water used in cooking. Cutting or bruising of produce releases ascorbate oxidase. Blanching techniques inactivate the oxidase enzyme and help to preserve ascorbate, lowering the pH of a food will similarly achieve this, as in the preparation of sauerkraut (pickled cabbage). In contrast, heating and exposure to copper or iron or to mildly alkaline conditions destroys the vitamin, and too much water can leach it from the tissues during cooking.

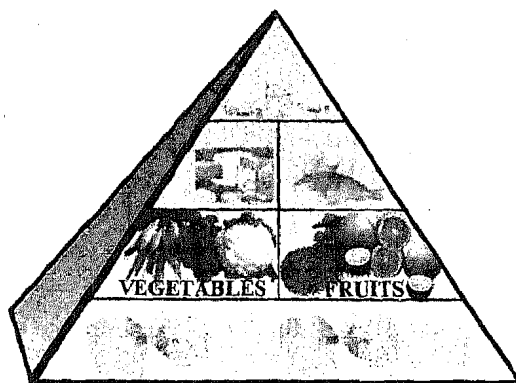


Figure 8.15: Vegetable and fruits- the rich sources of vitamin C

From food sources, we move on to facts about the absorption, storage and elimination of this vitamin from our body.

Absorption, Storage and Elimination

Ascorbic acid is rapidly absorbed from the intestines primarily by active transport. Simple diffusion or carrier-mediated transport may also contribute to a small extent of uptake of the vitamin from the mouth and stomach. Prior to absorption, ascorbate may be oxidized to form dehydroascorbate which is absorbed by passive diffusion or by use of glucose transporters. It passes through the portal vein to the general circulation and to all tissues. Each organ or tissue has an optimal saturation level of ascorbic acid. It is not stored to any appreciable extent in the body. The degree of absorption decreases with increased vitamin intake and varies from 16% to 98%, the average overall absorption being about 80% to 95%. Unabsorbed vitamin C may be metabolized by the intestinal flora. Pectin and zinc are a few substances that impair its absorption. Excess is excreted in urine.

Next, the role of vitamin C is described.

Functions

The vitamin C is involved in several physiological and biochemical functions in the body. *Ascorbate* is the biochemically active form of the vitamin, which has several functions. It is essential for the following:

- 1) ***Enzyme function:*** Vitamin C acts as an electron donor for 11 enzymes. Three of those enzymes are found in fungi but not in humans. Of the eight remaining human enzymes, three participate in collagen hydroxylation and two in carnitine biosynthesis; of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine norepinephrine, one is necessary for amidation of peptide hormones, and one is involved in tyrosine metabolism.
- 2) ***Protective role as an antioxidant:*** Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (i.e. L-ascorbate anion). As a scavenger, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen which could damage DNA, proteins or membrane structures. Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules. The ascorbyl free radical can be converted back to reduced ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate. Dehydroascorbate is unstable but is more fat-soluble than ascorbate and is taken up 10-20 times more rapidly by erythrocytes, where it will be reduced back to ascorbate from the hexose monophosphate shunt.

- 3) *Synthesis of hormones*: Ascorbate is involved in the amidation, thereby conferring stability to hormones such as thyrotropin releasing hormone, adrenocorticotrophic hormone, vasopressin, oxytocin and cholezystokinin.
- 4) *Formation of collagen and inter cellular cement substance*: The vitamin is required in the formation of collagen and in the formation of intercellular cement substances for capillaries, teeth, bones etc. When the vitamin is deficient, these tissues are not formed fully.
- 5) *Absorption of iron and incorporation of plasma iron in ferritin*: A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilize folate in food and in plasma. Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous, Fe²⁺) form. The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase iron absorption exceeds 25 mg and depends largely on the amount of inhibitors, such as phytates and polyphenols, present in the meal.
- 6) *Reduced cancer risk*: Concentrations of vitamin C appear to be high in gastric juice. Vitamin C present in gastric juice may prevent the formation of *N*-nitroso compounds, which are potentially mutagenic. High intakes of vitamin C correlate with reduced gastric cancer risk, but a cause-and-effect relationship has not been established. Epidemiological studies indicate that diets with a high vitamin C content have been associated with lower cancer risk, especially for cancers of the oral cavity, oesophagus, stomach, colon and lung.
- 7) *Hydroxylation of aromatic nuclei*: Ascorbic acid plays a central role in the hydroxylation of deoxycorticosterone, hydroxylation of tryptophan to 5-hydroxytryptophan and phenylalanine to tyrosine etc.
- 8) *Bone formation*: Ascorbate is vital for bone formation. In deficiency, though calcification is unaffected, formation of bone matrix and ground substance is defective. Osteoblasts invading the area of calcification change histologically into fibroblasts. The bone matrix is abnormal, as it lacks ossification.
- 9) *Wound healing*: Ascorbic acid deficiency delays healing of wounds, as collagen formation is affected. The rapid healing of wounds require the formation of strong connective tissue on the scar.
- 10) *Cholesterol metabolism*: Vitamin C plays a protective or curative role in diseases resulting from atherosclerosis through its effect on cholesterol metabolism. It protects low density lipoproteins against oxidation.

From our discussion above, it must be evident what important role vitamin C has in maintaining good health. Next, let us study about its bioavailability and the consequences of lack of vitamin C in our body.

Bioavailability

Ascorbic acid is a crucial constituent of plants which brings about efficient photosynthesis. In humans, its availability is inversely related to the vitamin C status of the individual. More absorption and retention occur to compensate tissue depletion. Humans maintain a body pool of vitamin C 114 $\mu\text{mol/kg}$ (20 mg/kg) and a plasma vitamin C concentrates of 28-40 $\mu\text{mol/L}$ (0.5-0.7 mg/dl). Hence, humans though incapable of manufacturing the vitamin, are adept at conserving it.

Next, let us get to know about the consequences of ascorbic acid deficiency.

Deficiency

Severe ascorbic acid deficiency results in the development of the disease known as scurvy, as highlighted in Figure 8.16, Three important manifestations of scurvy -

gingival changes, pain in the extremities and haemorrhagic manifestations – precede oedema, ulcerations, and ultimately death. The disease occurs in adults and infants.

In infantile scurvy, the changes are mainly at the sites of most active bone growth, characteristic signs are a pseudoparalysis of the limbs caused by extreme pain on movement and haemorrhages under the periosteum, as well as, swelling and haemorrhages of the gums surrounding erupting teeth.

Vitamin C deficiency can be detected from early signs of clinical deficiency, such as the follicular hyperkeratosis, petechial haemorrhages, swollen or bleeding gums, and joint pain, or from the very low concentrations of ascorbate in plasma, blood, or leukocytes. In adults, one of the early principle adverse effects of the collagen-related pathology may be impaired wound healing.

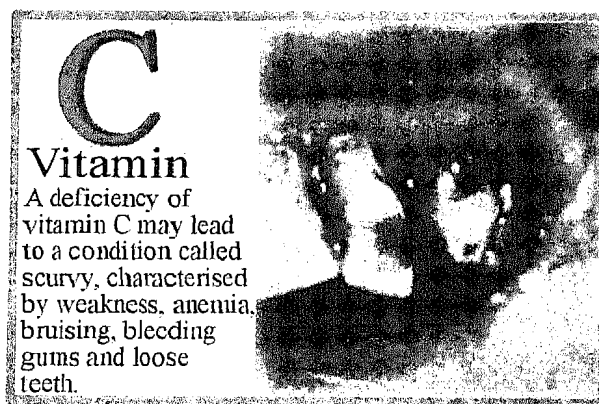


Figure 8.16: Scurvy

Symptoms of scurvy in adults include:

- 1) *General weakness*: The first symptoms are weakness, easy fatigue and listlessness. These are followed quickly by shortness of breath, pain in bones, joints and muscles of the extremities.
- 2) *Swollen and tender joints and haemorrhage in various tissues*: Haemorrhages occur deep in muscle, particularly in calf, thigh, buttocks and forearm, causing pain in surrounding tissues. The most specific sign includes the hyperkeratotic hair follicle with a haemorrhagic halo. Haemorrhages may also occur in joints, causing swelling and pain.
- 3) *Bleeding gums and loose teeth*: As ascorbic acid deficiency advances, the gums become swollen, blue-red, spongy and very friable. They may become infected by bacteria. The teeth loosen in the alveolar bone.

So that was a morbid picture. The populations at risk of vitamin C deficiency are those for whom the fruit and vegetable supply is minimal. Epidemics of scurvy are associated with famine and war, when people are forced to become refugees and food supply is small and irregular.

But, is there a danger also linked with excessive consumption of vitamin C? Let us find out next.

Toxicity

The potential toxicity of excessive doses of supplemental vitamin C relates to intraintestinal events and to the effects of metabolites in the urinary system. Intakes of 2-3 g/day of vitamin C produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people.

Further, oxalate is an end-product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily amounts of vitamin C produce hyperoxaluria.

Vitamin C may also precipitate haemolysis in some people, including those with glucose-6-phosphatedehydrogenase deficiency, paroxysmal nocturnal haemoglobinuria (a disorder characterized by haemolytic anaemia, haemoglobinuria, pallor, bronzing of skin, splenomegaly and hepatomegaly), or other conditions where increased risk of red cell haemolysis may occur or where protection against the removal of the products of iron metabolism may be impaired. On the basis of the above, the FAO/WHO 2004 Consultation agreed that 1g of vitamin C appears to be the advisable upper limit of dietary intake per day.

Let us then learn about the recommended dietary allowances for this important vitamin, next.

Recommended Dietary Allowance (RDA)

Table 8.11 presents the recommendations for vitamin C, as recommended by ICMR and FAO/WHO 2004. As you may have noticed, the ICMR recommendation is 40 mg/day for both adult males and females. The requirements go up by another 40 mg (total 80 mg) in case of lactation.

Table 8.11: ICMR and FAO/WHO recommended dietary intakes for vitamin C by groups

Recommended Vitamin C Intake			
Group	ICMR (mg/day)	Group	FAO/WHO 2004 RNI (mg/day) ^a
<i>Man</i> Sedentary work Moderate work Heavy work	40	<i>Adults</i> Males (19 - 65+ years) (65+ years)	45
<i>Woman</i> Sedentary work Moderate work Heavy work	40	<i>Adults</i> Females (19 - 65+ years) (65+ years)	45
Pregnancy	40	Pregnancy	55
Lactation	80	Lactation	70
<i>Infants</i> 0-6 months	25	<i>Infants</i> 0-6 months	25
6-12 months		7-12 months	30 ^b
<i>Children</i> 1-3 years	40	<i>Children</i> 1-3 years	30 ^b
4-6 years		4-6 years	30 ^b
7-9 years		7-9 years	35 ^b
<i>Adolescent</i>	40	<i>Adolescents</i>	
Boys 10-12 years		Males (10-18 years)	40 ^b
Girls 10-12 years		Females (10-18 years)	40
Boys 13-15 years	40		
Girls 13-15 years			
Boys 16-18 years	40		
Girls 16-18 years			

^a Amount required to half saturate body tissues with vitamin C in 97.5 percent of the population. Larger amounts may often be required to ensure an adequate absorption of non-haem iron.

^b Arbitrary values.

Source: Recommended Dietary Allowances for Vitamins, NIN, ICMR, Hyderabad, India (2002) and Vitamin and Mineral Requirements in Human Nutrition, FAO/WHO (2004).

To ensure that we are in a good vitamin C status, various assessment parameters are available. Let us learn about them.

Criteria for Assessment of Vitamin C Status

The different measures which can be used for assessment include:

- 1) **Ascorbic acid content of white blood cells:** The ascorbic acid content of white blood cells and platelets is a good index of the tissue levels of ascorbic

acid. The levels of ascorbic acid in normal persons range from 24-38 mg/100g. After feeding individuals on vitamin C deficient diet for 121 days, the value becomes zero. Determination of ascorbic acid content of white blood cells thus offers a highly sensitive index of the ascorbic acid nutrition of the individuals.

- 2) **Plasma or serum ascorbic acid levels:** The serum or plasma ascorbic acid levels are used as an index of ascorbic acid nutrition of humans. The normal serum values vary over a wide range from 0.5 to 2.2 mg percent.
- 3) **Urinary excretion:** The urinary excretion of ascorbic acid falls when the dietary intake is low. Since the tissues are not saturated, a greater part of the test dose of ascorbic acid will be retained by the tissues and only a small part will be excreted in urine. In contrast, when the daily intakes are high, a greater part of the test dose will be excreted. A normal saturated person excretes 50 percent of the test dose while a person on a deficient diet excretes only about 35 percent.
- 4) **Intradermal dye test:** The rate of decolouration of a small quantity of the dye, 2,6-dichlorophenol injected intradermally is an index of the ascorbic acid nutrition. In persons saturated with ascorbic acid, the dye is decolourised in 5 minutes. In deficient persons the time taken is 10 minutes or more.
- 5) **Skin capillary fragility test:** One of the earliest signs of ascorbic acid deficiency is the fragility of the capillaries. Capillary fragility is measured by applying negative pressure on a particular area and the number of petechiae (haemorrhagic spots) that have appeared in that area counted. It is low in normal persons (<10 petechiae) and high in deficient persons (10-20 petechiae).

With this, we end our study on ascorbic acid. Now read section 8.10 which covers the important aspect related to the interaction of these water soluble vitamins with other nutrients. This is an important issue hence we have taken this up separately in this unit.

8.10 INTERACTION WITH OTHER NUTRIENTS

Having gone through the discussion above it must be evident to you that nutrients are interdependent and are related to one another. The excess or deficiency of certain vitamins affects the requirements of certain other nutrients. The interaction of the B complex vitamin and vitamin C with other nutrients can be traced under the following headings:

I) Interaction with carbohydrate, fats and proteins

Dietary carbohydrates, fats and proteins require water-soluble vitamins for their metabolism and in turn influence the requirements of these vitamins. Let us review these interactions one by one.

- a) **Thiamin:** Carbohydrates require thiamin for their metabolism since thiamin pyrophosphate (TPP) is a coenzyme of decarboxylases and aldehyde transferases. Thiamin plays a key role in the oxidative decarboxylation of pyruvic acid (in the breakdown of carbohydrate and proteins and α -keto glutarate (in the citric acid cycle). TPP is a coenzyme for transketolase in lipid metabolism.
- b) **Riboflavin:** The two coenzyme forms of riboflavin are FMN and FAD. They are found in a large number of systems which function in the metabolism of carbohydrates, fats and protein. The role of riboflavin is central to energy production. In its role as a precursor to FAD, riboflavin exhibits significant antioxidant activity and protects against lipid peroxides.

- c) *Nicotinic acid*: NAD, NADP and NMN act as constituents of the hydrogen transferring coenzymes in glycolysis, Krebs's cycle and in the oxidation and biosynthesis of lipids. Also, tryptophan present in dietary proteins is converted to niacin. Thus, tryptophan serves as a source of nicotinic acid meets the niacin requirements.
- d) *Pyridoxine*: Pyridoxine is involved in glyconeogenesis through its action in transaminase reactions. Low levels of pyridoxine impair glucose tolerance. The coenzyme form of vitamin B₆ or pyridoxal phosphate is responsible for all non-oxidative enzymic amino acid transformations and catalyzes reactions such as decarboxylation, transamination, racemization. PLP has a key role in lipid metabolism and vitamin B₆ deficiency lowers body fat, liver lipid levels and impairs degradation of lipids.
- e) *Ascorbic acid*: The biologically active form, ascorbate, is a cofactor or co-substrate for eight isolated enzymes involved in hydroxylation of amino acids, metabolism of tyrosine etc. It protects lipids, protein and membrane structures from oxidative damage. It also regulates protein translation and gene transcription. Since ascorbic acid closely resembles glucose, most mammals can synthesize ascorbate from glucose. However, man lacks the terminal enzyme in the biosynthetic pathway and hence must ingest ascorbic acid to survive.
- f) *Leucine*: Excess of the amino acid, leucine in the diet antagonizes the function of vitamin B₆ and impairs the conversion of tryptophan to niacin.

II) Interacton with other vitamins

- a) *Ascorbic acid and other vitamins*: Ascorbic acid synthesis is diminished in thiamin and riboflavin deficiency. Vitamin A also plays an important role in the biosynthesis of ascorbic acid.
- b) *Thiamin and riboflavin*: Thiamin deficiency is accompanied by disturbances in riboflavin metabolism and excretion of riboflavin in urine.
- c) *Vitamin B₆ an other vitamins*: Administration of riboflavin, pantothenic acid and thiamin provide partial protection against seizures in vitamin B₆ deficient experimental animals.
- d) *Niacin and other vitamins*: Livers of rats fed thiamin or riboflavin deficient diets contained smaller amounts of niacin than normal controls. Treatment of pellagra with niacin precipitates symptoms of thiamin and riboflavin deficiencies indicating that niacin deficiency is accompanied by secondary deficiencies of thiamin and riboflavin.
- e) *Vitamin B₆ and C*: Vitamin B₆ metabolism increases with higher levels of vitamin C intake. Whole blood ascorbic acid levels fall during vitamin B₆ depletion and returned to normal levels, during repletion phase.
- f) *Vitamin B₆ and vitamin B₁₂*: Vitamin B₆ deficiency is reported to cause impairment in vitamin B₁₂ absorption in rats.
- g) *Folic acid and vitamin C*: Anaemia is observed in vitamin C deficient patients. Normochromic, normocytic or macrocytic or megaloblastic anaemia has been reported. These conditions responded to ascorbic acid therapy alone or along with folic acid.
- h) *Vitamin E and C*: Both vitamin E and vitamin C are powerful anti oxidants. They protect biological systems against oxidative damage due to free radicals. Their functions are synergistic to each other. Vitamin C synthesis is reduced

in the livers of vitamin E deficient rats and several vitamin E deficiency symptoms resemble those of scurvy.

- i) *Riboflavin and pyridoxine*: Riboflavin deficiency slows down the uptake of pyridoxine and decrease the conversion of pyridoxine to its metabolites.
- j) *Folic acid and vitamin B₁₂*: For the conversion of folic acid to folinic acid vitamin B₁₂ is required. Vitamin B₁₂ deficiency causes a rise in unconjugated folates and a marked depletion of intracellular conjugated folates.

III) Interaction with minerals

- a) *Vitamin C and iron*: Ascorbic acid powerfully enhances absorption of non-haem iron and reverses the inhibiting effect of tea and calcium phosphate. Iron absorption is directly proportional to the quantity of ascorbic acid present. Ascorbic acid forms a chelate with ferric iron at acid pH and renders it soluble for absorption.
- b) *Vitamin C, iron and cadmium*: Toxic levels of dietary cadmium (5-200 ppm) interfere with iron absorption and produces iron deficiency. Supplements of iron and ascorbic acid protect against cadmium toxicity. With low levels of dietary cadmium, supplements of iron and ascorbic acid decreased cadmium uptake in the liver, kidney and small intestines.
- c) *Vitamin C, lead and mercury*: Iron alleviates lead toxicity but ascorbic acid is ineffective. Ascorbic acid alleviates mercury toxicity but iron exacerbates the condition.
- d) *Riboflavin and iron*: Riboflavin deficiency is reported to decrease mobilization of hepatic iron and impair absorption of dietary iron.

Check Your Progress Exercise 3

1) List the food sources of ascorbic acid.

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.....
.....

2) Give reasons for the following statements:

a) Why does a single deficiency of either folic acid or vitamin B₁₂ lead to megaloblastic anaemia?

.....
.....

b) Ascorbate is called an antioxidant vitamin.

.....
.....

3) List the functions of vitamin B₁₂.

.....
.....
.....

4) Describe any one method for assessment of ascorbic acid.

.....

.....

.....

5) Trace the interaction of :

a) Vitamin C and E

.....

.....

b) Vitamin C and iron

.....

.....

8 . LET US SUM UP

Vitamins are vital to the body functions though needed in very small amounts. Water-soluble vitamins comprise of vitamin C and vitamins of the B complex group. In this unit, we learnt about the important functions and food sources of water-soluble vitamins which are summarized herewith. We also learnt about the recommended nutrient intake for each of these vitamins.:

Vitamin	Food Sources	Function
<i>Vitamin of the B-complex group</i> Thiamin or B ₁	Whole grain cereals, pulses, nuts, egg yolk, meat	Role in carbohydrate meabolism in particular.
Riboflavin or B ₂	Green leafy vegetables, milk, eggs, organ meats like liver, kidney	Role in the metabolism of carbohydrates, fats and proteins.
Niacin	Cereals, pulses, milk, nuts & oil seeds, organ meats Fish	Role in the metabolism of carbohydrates, fats and proteins.
Folic acid	Whole grain cereals, leafy vegetables, milk and eggs, organs meats like liver and kidney	Role in the formation of normal red blood cells in the bone marrow.
Vitamin B ₁₂	Animal foods like milk, egg, organ meats	Role in the formation of normal red blood cells in the bone marrow and proper functioning of the digestive tract and nervous system.
<i>Ktamin C</i>	Citrus fruits, amla, guava, capsicum, green leafy vegetables, green chillies	Role in collagen formation and hence in wound healing. Role in absorption of iron and prevention of destruction of other substances.

8.12 GLOSSARY

Angular stomatitis : inflammation of the mucous membrane of the mouth.

Antacids : medicines used to reduce or prevent acid collecting in the stomach.

Apoenzyme	:	a protein that combines with a coenzyme to form an active enzyme.
Cheilosis	:	a disorder of the lips marked by scaling and fissures at the corners of the mouth; caused by riboflavin deficiency.
Coenzymes	:	a small molecule associated with an enzyme that participates in enzymatic catalysis.
Cyanosis	:	bluish colour of the skin due to the insufficient oxygen in the blood.
Delirium	:	a state in which the thoughts, expressions, and actions are wild, irregular.
Dyspnoea	:	difficult respiration.
Dyspepsia	:	a kind of indigestion or a state of the stomach in which its functions are distributed.
Enzyme	:	proteins produced by living organisms and functioning as biochemical catalysts.
Erythema	:	a disease of the skin, in which a diffused inflammation forms rose-coloured patches of variable size.
Erythroid	:	red coloured tissue.
Glossitis	:	inflammation of the tongue.
Haptocorrin	:	a cobalamin- binding protein.
Hyperaesthesia	:	an abnormal increase in sensitivity to sensory stimuli, as of the skin to touch or the ear to sound.
Immunoglobulins	:	group of large glycoproteins that are secreted by plasma cells which function as antibodies in the immune response by binding with antigens incoherent as a consequence of fever or some other disease.
Lesions	:	inflammations.
Ligand	:	a molecule that binds to a receptor protein to form a larger complex.
Megaloblastic anaemia	:	deficiency of RBCs characterized by many large immature and dysfunctional RBCs in the bone marrow associated with pernicious anaemia.
Metabolic trapping	:	phosphorylation and retention of each form of vitamin after the process of absorption.
Neural Tube Defect	:	malformations of the neural tube, during embryogenesis (i.e., formation of embryo).
Neural tube	:	a tube of extradermal tissue in the embryo from which the brain and spinal cord develop.
Niacin equivalents	:	1 mg of niacin or 60 mg of tryptophan.
Opriguria	:	a lower than normal volume of urine.
Ossification	:	the process of forming new bone by which inorganic material is deposited in cartilage or membrane, forming bony tissue.

Oxaluria	:	abnormal excretion of oxalates or oxalic acid in the urine, especially calcium oxalate.
Pernicious anaemia	:	condition caused by vitamin B ₁₂ deficiency and characterized by deficiency of RBCs and spinal cord abnormalities.
Polishings	:	bran layers of a cereal (rice).
Redox system	:	a group of compounds having oxidizing and reducing properties.
Sideroblastic anaemia	:	a form of refractory anaemia caused by small basophilic granules containing ferric iron in the bone marrow.
Tachycardia	:	a very rapid heart beat.
Vitimers	:	different forms of vitamin occurring in free form.

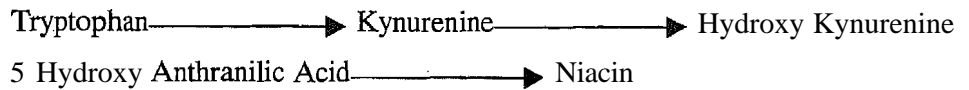
8.13 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

- 1)
 - a) False - Riboflavin gives the yellow green fluorescence.
 - b) True
 - c) True
 - d) False - Thiamin helps convert carbohydrate into energy.
 - e) True
- 2) After a meal, thiamin is found in the free form. Absorption involves two mechanisms—active carrier mediated systems, which involves phosphorylation and passive diffusion. After absorption, a greater part is converted into thiamin pyrophosphate in the liver and intestinal mucosa with the help of thiamin kinase and ATP. A small quantity of thiamin is also converted into thiamin triphosphate. Thiamin is transported in blood as erythrocytes in free and phosphorylated forms and in plasma as free thiamin and thiamin monophosphate.
- 3) Look up section 8.4 and answer on your own.
- 4) The five factors include presence of sodium, intestinal transit time, presence of metal, presence of drugs and presence of globulins.
- 5) Thiamin deficiency causes the disease beriberi in human beings. Look up section 8.2 and briefly discuss the symptoms on your own.
- 6) The enzyme transketolase requires TPP for its activity, for the metabolism of pentose phosphate sugars. In thiamin deficiency, the enzyme activity is reduced and a small part of the added pentose phosphate disappears. When TPP is added, ETK activity is increased. The percent increase in ETK activity is increased which is an index of the degree of thiamin deficiency.
- 7) Look up the sub-section on criteria for assessing the riboflavin status under section 8.3, and answer the question on your own.
- 8) Carbohydrates require thiamin for their metabolism since thiamin pyrophosphate is a coenzyme of decarboxylases and aldehyde transferases. Thiamin plays a key role in the oxidative decarboxylation of pyruvic acid and α -keto glutarate. TPP is a coenzyme for transketolase in lipid metabolism.

Check Your Progress Exercise 2

- 1) a) 2.5
b) 400
c) folic acid
d) dermatitis, dementia, diarrhoea.
- 2) Pyridoxine is needed for the conversion of hydroxy kynurenine to hydroxy anthranilic acid. The conversion is as follows:



- 3) a) Nicotinic acid has a metal chelating ability. This explains its biological interactions with essential trace metals. It is a part of the glucose tolerance factor, an organochromium complex that may potentiate insulin response in man.
b) Bioavailability of food folate, is only 50 percent that of folic acid. Folic acid taken along with food is only 85 percent available i.e., absorption is reduced by 15 percent relative to an equivalent dose of folic acid taken alone while fasting.
- 4) Niacin equivalent (NE) is an expression for recommended allowance for niacin; 1 mg NE = 1 mg niacin or 60 mg tryptophan.
- 5) Folate deficiency is associated with Neural Tube Defects (NTDs). The deficiency is associated with faulty foetal folate transport and neurotoxicity of homocysteine. Homocysteine induces abnormal development in neural tube and neural crest derivatives by acting as an antagonist of the N-methyl-D-aspartate subtype of the glutamate receptor.
- 6) a) Urinary excretion of N-methyl nicotinamide (NMN): Excretion of N-methyl nicotinamide in urine after an oral niacin load of 20 mg nicotinamide/70 kg body weight over 24 hours is measured and levels of 5.8 mmol/dl represents deficiency and 5.8-17.5 mmol/dl represents a low niacin status.
b) Measurement of serum folate: This is the most reliable and widely used method since low folate levels indicate low body stores. The levels indicative of deficiency are as follows:

Serum level (mg/ml)	Status
<3	Deficiency
3 - 6.1	Sub clinical deficiency
6 - 20.1	Normal
>21	Elevated

- 7) Pyridoxine is involved in gluconeogenesis through its action in transaminase reactions. Low levels of pyridoxine impair glucose tolerance. The coenzyme form of vitamin B₆ is responsible for all non-oxidative enzymic amino acid transformations and catalyzes reactions such as decarboxylation, transamination, racemization, elimination in amino acid metabolism. PLP has a key role in lipid metabolism and vitamin B₆ deficiency lowers body fat, liver lipid levels and impairs degradation of lipids.

Check Your Progress Exercise 3

- 1) Amla and guava, citrus fruits, drumstick leaves, other green leafy vegetables, germinated cereals and pulses.

- 2) a) The conversion of homocysteine to methionine requires the enzyme methionine synthase, cobalamin and 5-methyl THF. This dependency of methionine synthase on both folate and cobalamin explains why a single deficiency of either vitamin leads to the same megaloblastic anaemia.
- b) Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (i.e. L-ascorbate anion). As a scavenger, ascorbate has been shown to be effective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen which could damage DNA, proteins or membrane structures.
- 3) Look up section 8.8 for the functions of cyanocobalamin. Now write the answer on your own.
- 4) Look up the assessment of ascorbic acid status sub-section under section 8.9 and answer the question on your own.
- 5) a) Both vitamin E and vitamin C are powerful antioxidants. They protect biological systems against oxidative damage due to free radicals. Vitamin C synthesis is reduced in the livers of vitamin E deficient rats and several vitamin E deficiency symptoms resemble those of scurvy.
- b) Ascorbic acid powerfully enhances absorption of non-haem iron and reverses the inhibiting effect of tea and calcium phosphate. Iron absorption is directly proportional to the quantity of ascorbic acid present. Ascorbic acid forms a chelate with ferric iron at acid pH and renders it soluble for absorption.