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Crystals of **ascorbic acid (vitamin C)**
viewed under polarized light

CHAPTER

34

Water-soluble Vitamins

VITAMIN B COMPLEX

Originally, vitamin B referred to a vitamin whose deficiency causes beriberi in man and polyneuritis in birds. Later, Goldberger's researches on pellagra led to the view that vitamin B consisted of at least 2 factors : a heat-labile *antiberiberi factor* and a comparatively heat-stable *antipellagra factor*. Some called the former factor as vitamin B₁ and the latter as vitamin B₂. But the later researches conducted by Richard Kuhn, Conrad Elvehjem and others have established the fact that vitamin B complex, as represented by yeast, rice bran and liver extracts, contains still other factors. At present, the vitamin B complex is known to consist of a group of at least 13 components usually named as B₁, B₂, B₃ etc. But to prevent confusion, their chemical names are now frequently used. The various members of the vitamin B complex are not related either chemically or physiologically, yet they have many features in common:

- (a) All of them except lipoic acid are water-soluble.
- (b) Most of them, if not all, are components of coenzymes that play vital roles in metabolism (refer Table 34-1).
- (c) Most of these can be obtained from the same source, *i.e.*, liver and yeast.
- (d) Most of them can be synthesized by the intestinal bacteria.

Table 34-1. Coenzyme derivatives of water-soluble vitamins

<i>Vitamin</i>	<i>Coenzyme form</i>
Vitamin B ₁ (Thiamine)	Thiamine pyrophosphate (TPP)
Vitamin B ₂ (Riboflavin)	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)
Vitamin B ₃ (Pantothenic acid)	Coenzyme A (CoA)
Vitamin B ₅ (Niacin)	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)
Vitamin B ₆ (Pyridoxine)	Pyridoxal phosphate (PALP), Pyridoxamine phosphate (PAMP)
Vitamin B ₇ (Biotin)	Biocytin
Vitamin B ₉ (Folic acid)	Tetrahydrofolic acid (THFA)
Vitamin B ₁₂ (Cyanocobalamin)	Deoxyadenosyl cobalamin
Vitamin C (Ascorbic acid)	Not known

VITAMIN B₁

A. History. Thiamine was the first member of the vitamin B group to be identified and hence given the name vitamin B₁. Thiamine was first isolated by Jansen (1949) in Holland and Adolf Windaus in Germany. On account of its curing action against beriberi, it is commonly known as **antiberiberi factor**. It is also known as **antineuritic factor** or heat-labile factor. In Europe, it is also designated **aneurin**.

Thiamine is also spelt as thiamin (Frank B. Armstrong, 1989 ; Albert L. Lehninger, David L. Nelson and Michael M. Cox, 1993)

B. Occurrence. *Thiamine is found practically in all plant and animal foods.* Cereals, heart, liver and kidney are excellent sources of it. In cereals, the outer layers of seeds are especially rich in thiamine (Fig. 34-1). In yeasts and animal tissues, however, it is present mainly as its coenzyme, thiamine pyrophosphate (TPP). Milk also contains thiamine, although in relatively low amounts. The milling of wheat flour lowers the thiamine content considerably, sometimes to the extent of even 80%. Consequently, wheat flour is usually enriched with thiamine at many places. Furthermore, improper cooking (*esp.*, when the water in which foods are cooked is discarded) loses thiamine content. This is because of the solubility of thiamine in water. Therefore, it is desirable to use the “cook water” for soups and sauces. Thiamine is easily destroyed by heat in neutral or alkaline media. Because the covering of the grains of cereals contains most of the vitamin, polishing reduces its availability. Canning processes are, however, not particularly destructive.

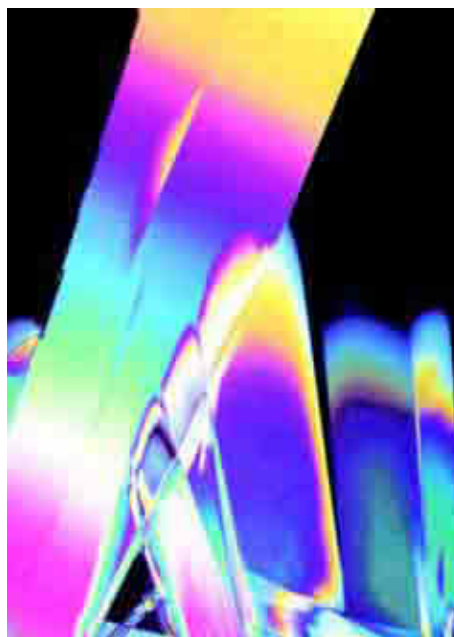


Fig. 34-1. Crystals of thiamine (vitamin B₁)

C. Structure. The chemical structure of thiamine (Fig. 34-2) was determined in 1935 by Thiamine pyrophosphate (TPP) is a cofactor in a number of enzymes.

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Robert R. Williams and his associates in the United States and its chemical synthesis was achieved soon thereafter. Thiamine ($C_{12}H_{17}N_4OS$) is 2,5-dimethyl-6-aminopyrimidine bonded through a methylene linkage to 4-methyl-5-hydroxyethyl-thiazole. Thus, pyrimidine and thiazole are the two moieties present in its molecule. The pyrimidine is unique in that it is the only natural pyrimidine containing an alkyl group at C_2 . Also, *with the possible exception of penicillin, thiamine is the only natural compound which contains a thiazole group*. It is interesting to note that plants can use a mixture of pyrimidine and thiazole compounds in place of thiamine itself. On the other hand, all the animals except pigeon require the complete vitamin.

The monies that **Robert R. Williams** received from his patents on the chemical synthesis of thiamine were used to establish the Williams-Waterman Fund for the Combat of Dietary Disease, which supported nutritional research and field programmes to abolish malnutrition.

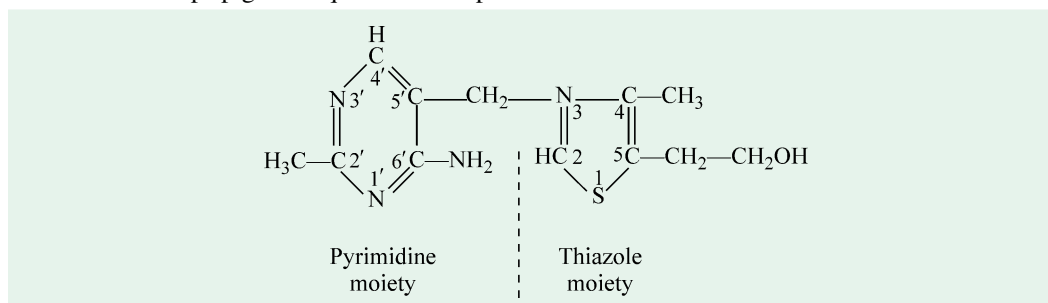


Fig. 34-2. Vitamin B₁ or thiamine or aneurin

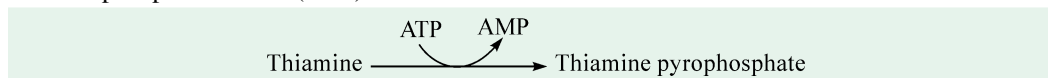
D. Properties. Thiamine is a white crystalline substance, readily soluble in water, slightly so in ethyl alcohol but insoluble in ether and chloroform. Its odour resembles that of a yeast. The aqueous solution is optically inactive. Thiamine is destroyed at elevated temperature, unless the pH is low. It can stand short boiling up to 100°C. Hence, it is only partly lost in cooking or canning processes. Long boiling or boiling with alkali destroys it. But it is stable in acid medium. On oxidation, it produces *thiochrome*, which gives fluorescence.

E. Metabolism. The requirement of this vitamin is increased under high metabolic conditions such as fever, increased muscular activity, pregnancy and lactation and also under surgery and stress. A correlation also exists between the type of food taken and the vitamin B₁ requirement. Fats and proteins reduce while carbohydrates increase the amount of this vitamin required in the daily diet. Thiamine absorption decreases with gastrointestinal or liver disease.

Raw seafoods (*e.g.*, fishes and molluscs) contain an enzyme, *thiaminase* which destroys thiamine in the body. People consuming such foods may, therefore, reveal symptoms of thiamine deficiency. Thiaminase cleaves the thiamine molecule between the pyrimidine and thiazole rings.

If thiamine is administered in human body, a part of it is excreted or recovered in the urine and a part is converted to pyrimin by the enzyme, thiaminase. Besides thiaminase, certain flavonoids of nonenzymic nature also work against thiamine. These have been shown to be present in ferns and certain higher plants.

Thiamine is phosphorylated with ATP to form thiamine pyrophosphate (TPP), which is also called diphosphothiamine (DPT).



TPP, in association with lipoic acid, forms the prosthetic group, *cocarcboxylase* for the enzyme *carboxylase*. TPP participates in many reactions, such as decarboxylation of α -keto acids, notably pyruvic and α -ketoglutaric and transketolation.

F. Deficiency. Vitamin B₁ deficiency leads to polyneuritis in animals and beriberi in human beings.

Polyneuritis in birds renders them unable to fly, walk or even stand. Rats develop, among other symptoms, a bradycardia (slowing of the heart rate).

Beriberi (*beri*^{singhalese} = weakness, which here means *I cannot*, symbolizing the incapacitated condition created by thiamine deficiency) has been and continues to be a serious health problem in Far East where polished or refined rice (rice from which husk has been removed) is eaten. The rice has a rather low content of thiamine. The problem is aggravated if the rice is polished because the husk contains nearly all the thiamine of rice.

Beriberi is also occasionally seen in alcoholics who are severely malnourished. Even before the concept of vitamins was developed, beriberi was described as a deficiency disease. It is a disease of the nervous system and is characterized by polyneuritis (degeneration of the peripheral nerves) leading to partial paralysis of the extremities, muscular atrophy, cardiovascular changes and gastrointestinal disorders.

At first, there is fatigue, apathy, irritability, depression, drowsiness, anorexia (loss of appetite), insomnia (sleeplessness), nausea and abdominal discomfort. This is followed by symptoms like peripheral neuritis with tingling, burning paresthesias of the toes and feet; decreased tendon reflexes; loss of vibration sense; tenderness and cramping of leg muscles; congestive heart failure and psychic disturbances. There may be ptosis of the eyelids and atrophy of the optic nerve. Hoarseness due to paralysis of the laryngeal nerve is a typical sign. Muscular atrophy and tenderness of the nerve trunks are followed by ataxia, loss of coordination, and loss of deep sensation. Paralytic symptoms are more common in adults than in children. Finally, the major symptoms may follow one of the following 3 courses (and accordingly beriberi is of 3 types) :

- (a) Symptoms involving nervous system, causing *dry beriberi* : In it, the child may appear plump but is pale, flabby, listless and dyspneic; the heart beat is rapid and the liver enlarged.
- (b) Symptoms associated with edema and effusions, leading to *wet beriberi* : In it, the child is undernourished, pale and edematous and has dyspnea, vomiting and tachycardia. The skin appears waxy. The urine may contain albumin and casts.
- (c) Symptoms involving heart, resulting in *acute pernicious beriberi* : In it, the lesions may be found principally in the heart, peripheral nerves, subcutaneous tissue and serous cavities. The heart is enlarged, especially to the right and there is fatty degeneration of the myocardium. Generally edema or edema of the legs, serous effusions, and venous engorgement may be seen. Lesions in the brain include vascular dilatation and hemorrhage. Finally, death ensues due to heart failure.

Often the symptoms characteristic of more than one of these 3 types of beriberi appear simultaneously in individuals causing *mixed beriberi*. Although beriberi is caused due to thiamine avitaminosis, it is usually associated with deficiencies of other vitamins. This is true of all vitamin B complex-deficiency conditions in man.

G. Human requirements. The daily recommended dietary allowances are 1.2–1.4 mg for men and 1.0 mg for women. Pregnant and lactating mothers, however, require up to 1.5 mg daily. The thiamine requirement for infants is between 0.2 and 0.5 mg daily.

CLINICAL IMPLICATIONS

BERIBERI

Jacobus Bonitus, a Dutch physician, had first described beriberi, in 1630, as follows : "A certain very troublesome affliction, which attacks men, is called by the inhabitants Beri-beri (which means sheep). I believe those, whom this same disease attacks, with their knees shaking and the legs raised up, walk like sheep. It is a kind of paralysis, or rather Tremor : for it penetrates the motion and sensation of the hands and feet indeed sometimes of the whole body"

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H. Treatment : If beriberi occurs in breast-fed infant, both the mother and child should be treated with thiamine. In such cases, the daily dose for adults is 50 mg and for children 10 mg or more. Oral administration is effective until gastrointestinal disturbances prevent absorption. Thiamine should be instilled intramuscularly or intravenously to children with cardiac failure.

VITAMIN B₂

A. History. Riboflavin or vitamin B₂ was first isolated in 1879 from milk whey which is an essential dietary factor for rats. Since it was first isolated from milk, vitamin B₂ is also known as **lactoflavin**. Originally, it was also known as **ovoflavin** (from eggs) and **hepatoflavin** (from liver). Its synthesis was done by **Richard Kuhn** and **Paul Karrer**. It is popularly called as the “**yellow enzyme**” because of its colour.

B. Occurrence. In nature, it occurs almost exclusively as a constituent of one of the two flavin coenzymes, namely, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Milk, cheese, eggs, liver, kidney, heart and brewer's yeast are excellent sources of this vitamin. Cow's milk contains about 5 times as much riboflavin as human milk. Leafy vegetables are good sources. They are usually richer in riboflavin than they are in thiamine. Fruits and most root vegetables contain moderate quantities. Whole grains, cereals and milled flour contain low riboflavin content. The riboflavin contents in cereals, however, increase strikingly during germination. The ordinary cooking processes do not affect the riboflavin content of the food. Roasted or boiled meat retains about 75% of the vitamin. *It is only very rarely that vitamin B₂ is present in free or uncombined state as in retina and spleen.* Fermentation residues from alcohol manufacture probably offer the richest large supplies.

C. Structure. Riboflavin (C₁₇H₂₀N₄O₆) belongs to a class of water-soluble pigments called *lyochromes*. A molecule of thiamine (Fig. 34-3) consists of a sugar alcohol, D-ribitol, attached to a chromogenic dimethyl isoalloxazine ring at position number 9.

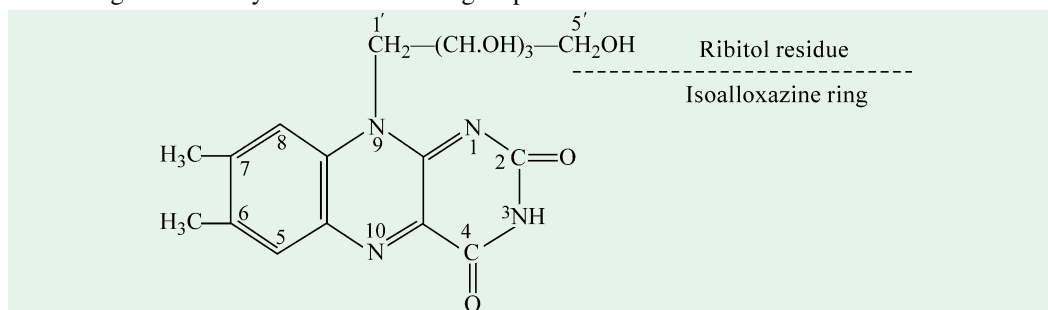


Fig. 34-3. Vitamin B₂ or riboflavin or lactoflavin

[6, 7-dimethyl-9-(1'-D-ribityl) isoalloxazine]

D. Properties. Riboflavin is a bright orange-yellow crystalline powder. It is soluble in water and ethanol but insoluble in ether and chloroform. It is stable to heat and acids but is easily decomposed by alkalis and exposure to light. The aqueous solution exhibits yellow-green

RICHARD KUHN (LT, 1900-1967)

Kuhn, a Austria-born German chemist, received his doctorate in 1922 on enzymes. He was awarded **Nobel Prize in Chemistry in 1938** for his work on carotenoids and vitamins but its acceptance was forbidden by the German government. After the war, he received the gold medal and diploma of the prize. His main contributions include: the separation of carotenoids (α and β) from carrots and thus isolated the third isomer (γ carotene), and the structure of lycopene.



fluorescence. It stands ordinary cooking and canning. On exposure to light, the ribityl residue splits off, forming a compound *lumiflavin* in alkaline solution and *lumichrome* in acidic or neutral solution.

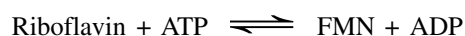
E. Metabolism. Riboflavin is synthesized by all green plants, most bacteria, yeasts and moulds. *Ashbya gossypii*, an yeast, produces it in such large amounts that riboflavin crystals are formed in the culture medium. *Animals have, so far, not been shown to synthesize riboflavin.* In man, the ingested riboflavin is largely passed out as such or as its coenzyme, the FMN.

Experiments with plant tissues have suggested that riboflavin and flavoproteins may play a significant role in phototropic curvature of various plant organs (Galston, 1950).

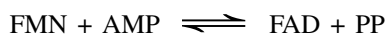
Riboflavin is essential for growth and tissue respiration; it may have a role in light adaptation and is required for conversion of pyridoxine to pyridoxal phosphate.

When riboflavin is phosphorylated in the presence of an enzyme, *flavokinase*, it gets converted to FMN which is essential in the biosynthesis of fats.

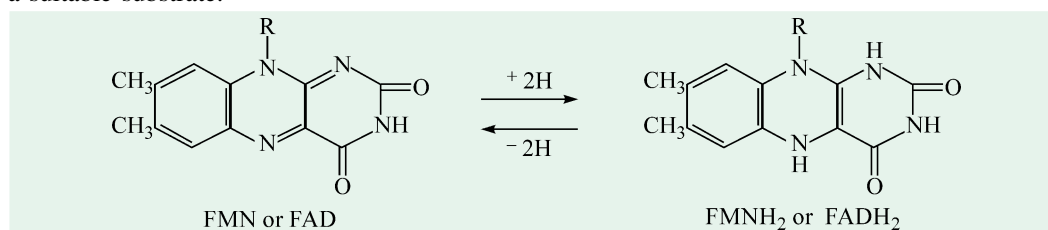
Flavokinase



FMN may undergo a further reaction with ATP, in the presence of an enzyme found in yeast and animal tissues, to produce FAD. It is a chief constituent of electron transport system (ETS). A decrease in the amount of FAD, therefore, would severely hamper the efficiency of ETS.



The coenzymes undergo reversible oxidation-reduction in the presence of their enzymes and a suitable substrate.



The flavoenzymes play a key role in cell metabolism. They function in accepting hydrogen atoms from reduced pyridine nucleotides. They have been shown to participate in the enzymic oxidation of glucose, fatty acids, amino acids and purines.

F. Deficiency. Riboflavin deficiency is usually caused by inadequate intake. Faulty absorption may contribute in patients with biliary atresia or hepatitis or in those receiving probenecid, phenothiazine or oral contraceptives. Phototherapy destroys riboflavin content. It is interesting to note that riboflavin deficiency without deficiency of other member of the B complex is rare.

Persons deficient in vitamin B₂ show cheilosis (fissuring at the corners of the mouth and lips), glossitis (inflammation of the tongue), keratitis, conjunctivitis photophobia, lacrimation, **corneal vascularization** (bloodshot eyes) and seborrheic dermatitis. But these symptoms are not specific to ariboflavinosis since similar symptoms may also develop in the absence of nicotinic acid and iron. **Cheilosis** (= perle'che) begins with pallor at the angles of the mouth, following by thinning and maceration of the epithelium. Superficial fissures often covered by yellow crusts develop in the angles of the mouth and extend radially into the skin for distances upto 2 cm. In **glossitis**, the tongue is smooth, and loss of papillary structure occurs. A normocytic and normochronic anemia with bone marrow hyperplasia is common.

However, patients suffering from pellagra and beriberi are usually also deficient in riboflavin content.

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G. Human requirements. The minimum daily requirement of riboflavin varies from 0.6 to 1.7 mg for children and adults. During pregnancy and lactation, the women require up to 2.0 mg daily.

H. Treatment : Ariboflavinosis may be prevented by a diet that contains adequate amounts of milk, eggs, leafy vegetables, and lean meats. Treatment consists in oral administration of 3-10 mg of riboflavin daily. If no response occurs within a few days, intramuscular injections of 2 mg of riboflavin in saline solution may be administered 3 times in a day.

VITAMIN B₃

A. History. This was first isolated by **Roger J. Williams** in 1938 from yeast and liver concentrates. On account of its wide distribution, he named it as pantothenic acid (*pantos*^G = everywhere). The coenzyme form of this vitamin (coenzyme A or CoA-SH) was isolated and its structure determined by **Fritz A. Lipmann**. The chemical synthesis of this coenzyme was, however, described by **Khorana** in 1959. This vitamin is sometimes called as **filtrate factor** or the **yeast factor**.

Roger J. Williams was the brother of Robert R. Williams, who established the structure of vitamin B₁, thiamine. Although both shared similar interests in the B-vitamins and nutrition, their research careers were independent of each other and never involved collaboration.

B. Occurrence. Although widespread in nature, yeast, liver and eggs are the richest sources of it. The vegetables (potatoes, sweet potatoes, cabbage, cauliflower, broccoli) and fruits (tomatoes, peanuts) and also the skimmed milk, wheat bran, whole milk and canned salmon are some of the less important sources. In most animal tissues and microorganisms, it occurs as its coenzyme.

C. Structure. Pantothenic acid (C₉H₁₇NO₅) is an amide of pantoic acid (α , γ -dihydroxy- β , β -dimethyl butyric acid) and β -alanine (refer Fig. 34-4).

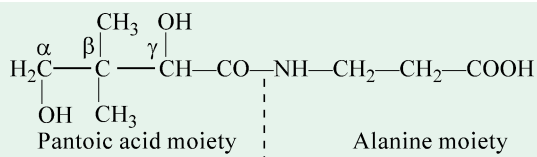


Fig. 34-4. Vitamin B₃ or pantothenic acid
(Pantoyl- β -alanine)

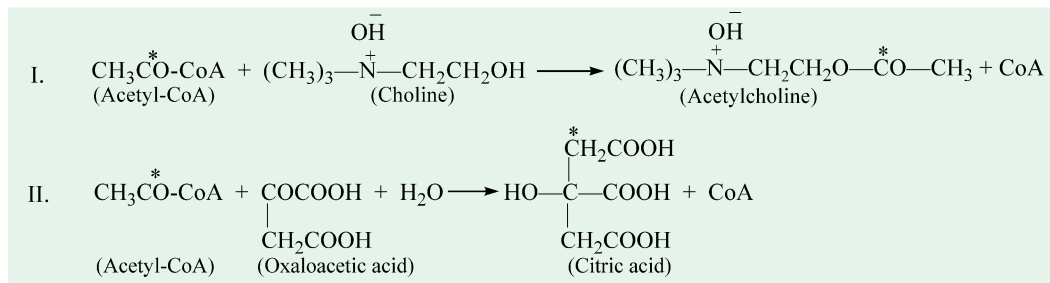
D. Properties. Pantothenic acid is a pale yellow viscous oil, soluble in water and ethyl acetate but insoluble in chloroform. It is stable to oxidizing and reducing agents but is destroyed by heating in an acidic and alkaline medium (*i.e.*, it is heat-labile).

E. Metabolism. Pantothenic acid can be synthesized by green plants and various microorganisms (*Neurospora*, *Escherichia coli*, *Bacteria linens*) but not by mammals. Hence, this must be present in the diet to serve as a starting point for coenzyme A (CoA). Coenzyme A is richly found in the liver and in poor quantities in the adrenals. There may be as much as 400 mg of CoA per kilo of liver. It functions in acetylation reactions. In order to be effective, CoA must be present in the form of acetyl-CoA. It may arise in many ways but the most common way of its production is that CoA, in the presence of ATP, acetate and a suitable enzyme, is converted into acetyl CoA. The overall reaction may be shown in 3 steps :

1. ATP + Enzyme \rightarrow Adenylic acid-Enzyme + Pyrophosphate
2. Adenylic acid-Enzyme + CoA \rightarrow CoA-Enzyme + Adenylic acid
3. CoA-Enzyme + Acetate \rightarrow Acetyl-CoA + Enzyme

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The acetyl groups may be transferred to an acetyl acceptor in the presence of a suitable acceptor. This may occur in two ways : either the acetyl group is attached to the accepting group at the carbonyl end (head reaction) or at the methyl end (tail reaction).



The only known metabolic fate of vitamin B₃ is its participation in the formation of the biologically important coenzyme A. It functions as a thioester of carboxylic acids.

F. Deficiency. A deficiency of pantothenic acid leads to depigmentation of the hair in rats, pigs and dogs and to depigmentation of feathers in chicks. Atrophy of the adrenal cortex with necrosis and hemorrhage may also occur in animals including rat. Corneal changes consisting of vascularization, thickening and opacity may be seen.

In human beings, no definite deficiency syndrome has been ascribed to pantothenic acid, probably because of the ubiquitous nature of this vitamin and because of the fact that a little amount of this vitamin can perhaps be synthesized in the body. Its correlation with **achromotrichia** (premature greying of the hair) has been described in the case of man, sometimes. But it seems too much to hope that grey hair can be averted by attention to diet ; rather it appears we must expect to go grey in spite of this vitamin.

G. Human requirements. The dietary allowance dose has not been officially worked out. Yet, 5—10 mg per day of vitamin B₃ has been suggested.

VITAMIN B₅

A. History. Vitamin B₅ refers to nicotinic acid and was named as **pellagra preventive (PP) factor** by an Austrian-American physician of the U. S. Public Health Service, **Joseph Goldberger** (ca 1920) because of its curing action on pellagra (After Goldberger's death, vitamin B₅ was sometimes called **vitamin G** in his honour). The vitamin role on nicotinic acid was first recognized by **Conrad Elvehjem** and **D. Wayne Woolley** of Wisconsin University in 1937. As this vitamin has a curing action against blacktongue disease in dogs, it is also called as **antiblacktongue factor**. It was first isolated by **Funk** in 1911. Because the name 'nicotinic acid' might mislead some people into thinking that tobacco is nutritious, nicotinic acid has been given the alternative official name *niacin* for public use.

Although **Woolley** was blind since young adulthood, undaunted, he pursued a science career that established him as one of the most prominent biochemists in the United States.

B. Occurrence. Nicotinic acid is widely distributed in nature in plant and animal tissues mainly as its amide called nicotinamide (commercially called **niacinamide**, to avoid any misassociation with the alkaloid nicotine of tobacco). As dietary tryptophan can be converted, in restricted quantities, to niacin in the body, it can partially substitute for niacin, although other sources of vitamin B₅ are necessary. *Niacin is most abundantly found in yeast.* Liver, lean pork, salmon, poultry and red meat are also good sources, but most cereals contain only small amounts of it. Most vegetables and fruits are poor sources of it. Milk and eggs, which contain very little or practically no niacin, are good pellagra-preventive foods because of their high content of

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tryptophan. Since a number of stable vegetable articles of diet are not particularly rich in nicotinamide, the vegetarian's diet may be lacking in this vitamin. Nicotinamide, is present as a constituent in two pyridine nucleotide coenzymes namely NAD and NADP (previously called as DPN and TPN respectively). Since niacin is stable to heating and oxidation, there are only small losses in cooking. Like thiamine, most of vitamin B₅ is lost in the milling process.

C. Structure. Niacin ($C_6H_5O_2N$) is simplest of all the known vitamins. It is a pyridine derivative (Fig. 34-5).

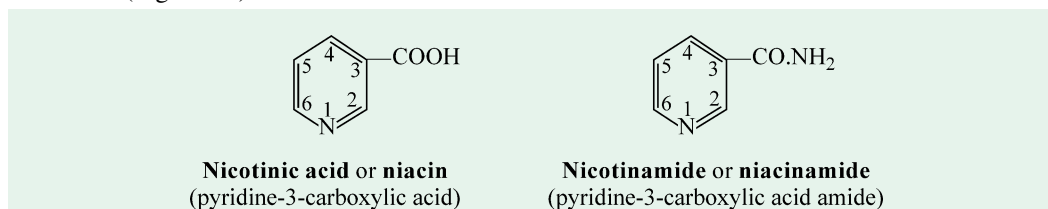
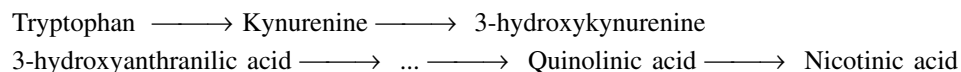


Fig. 34-5. Vitamin B₅ and its amide

D. Properties. Niacin is a white crystalline substance. It is soluble in ethyl alcohol but is less soluble in ether and benzene than nicotinamide. It is heat-stable. Nicotinamide, when pure, occurs as white needle like crystals. It is soluble in water and is stable in air and heat.

E. Metabolism. The conversion of niacin to nicotinamide takes place in the kidney and brain slices and also in the liver slices, if glutathione is present. Nicotinamide is synthesized by amidation of nicotinic acid adenine dinucleotide and subsequent degradation of NAD thus formed.

The niacin in man and other animals is derived from the amino acid tryptophan, which also cures pellagra. The conversion of tryptophan to nicotinic acid in the body takes place through a series of intermediate steps, which are represented below :



Nicotinamide undergoes methylation in mammalian liver to produce N'-methyl nicotinamide which is oxidized to give corresponding 6-pyridone. In many plant seeds nicotinic acid is, however, converted to trigonelline.

The nicotinic acid and its amide both are necessary for the growth of various microorganisms. Pyridine-3-sulfonic acid and its amide (Fig. 34-6) both prevent such growth which can be resumed by the addition of these vitamins. The relationship of these growth inhibitors to the two vitamins is not much different with the relationship of *p*-aminobenzoic acid to sulfanilamide (refer page 390).

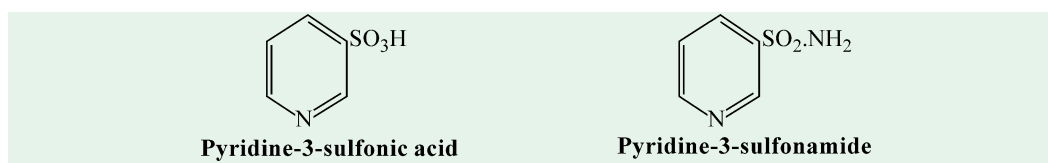


Fig. 34-6. Antagonists of vitamin B₅ and its amide

The two coenzyme forms of this vitamin, NAD and NADP, carry out 2 important functions in the tissues:

- (a) Oxidation of alcohols, aldehydes, amino acids and hydroxy-carboxylic acids.
- (b) Reduction of the flavin coenzymes.

F. Deficiency. A deficiency of niacin causes pellagra in man and blacktongue in dogs. **Pellagra** (of Italian origin, *pellis* = skin; *agra* = rough) is characterized by 3 "Ds", namely **d**ermatitis of the exposed parts, **d**iarrhea and **d**ementia. The early symptoms of pellagra are vague. Anorexia, lassitude, fatigue, burning sensations, numbness and dizziness may be prodromal symptoms. Their manifestation in children who have parasites or chronic disorders may be particularly severe. The most characteristic manifestations are the cutaneous ones, which may

develop abruptly or insidiously and may be elicited by irritants, esp., by intense light. They first appear as symmetric erythema of the exposed surfaces that may resemble sunburn. The lesions are usually sharply demarcated from the healthy skin around them, and their distribution may change very often. The lesions on the hands sometimes have the appearance of a glove (*pellagrous glove*), and similar demarcations are sometimes seen on the foot and leg (*pellagrous boot*) or around the neck (*Casal necklace*). The healed parts of the skin may remain pigmented. The cutaneous lesions are sometimes preceded by stomatitis, glossitis, vomiting or diarrhea. Swelling and redness of the tip of the tongue and its lateral margins may be followed by intense redness of the entire tongue and of the papillae and even ulceration. Nervous symptoms include depression, disorientation, insomnia and delirium. The histologic changes in the nervous system occur relatively late in the disease and consist of patchy areas of demyelination and degeneration of ganglion cells.

The classic symptoms of pellagra are usually not pronounced in infants and children. Anorexia, irritability, anxiety and apathy are common in “*pellagra families*”. They may also have sore tongues and lips and the skin is usually dry and scaly. Diarrhea and constipation may alternate and a moderate secondary anemia may occur. Pellagral children often have symptoms characteristic of other nutritional deficiency diseases. As coffee (*Coffea arabica*) is particularly rich in niacin, the heavy coffee drinkers usually do not develop pellagra. Other factors like thiamine-deficiency also seem to be responsible for this disease. Incredible as it may seem, over 600 deaths were attributed to pellagra in 1948. Pellagra is greatly aggravated in persons kept on a corn diet (as natives of Africa) because corn is very much deficient of tryptophan.

The canine **blacktongue** disease leads to complete loss of appetite. The inner surfaces of the lips and cheeks develop pustules; the pustules may also develop on the thorax and abdomen. Intensive salivation and bloody diarrhea are other symptoms.

G. Human requirements. The recommended daily allowance of nicotinic acid is between 8 and 15 mg for children, between 15 and 20 mg for men and between 13 and 15 mg for women. Pregnant and lactating mothers may require up to 20 mg daily.

H. Treatment : Children respond quickly to antipellagral therapy. A well-balanced diet should be augmented with 50-300 mg/day of niacin; 100 mg may be given intravenously in acute cases or in cases of poor intestinal absorption. The diet should be supplemented with other vitamins, especially with other members of B complex group. Sun exposure should be avoided during the active phase; the skin lesions may be covered by applying soothers. The diet of the cured pellagrins should be supervised continuously to prevent recurrence.

VITAMIN B₆

A. History. The name vitamin B₆ was suggested by Albert Szent-Györgyi (1934) to designate substances, other than thiamine and riboflavin, which cured a dermatitis (acrodynia) in rats. It was, henceforth, also named as **adermin** or **antidermatitis factor**. Vitamin B₆ group includes 3 compounds: pyridoxine, pyridoxal and pyridoxamine. Pyridoxine was first isolated, in 1938, from yeast and liver. Later, Snell (1942) discovered the other two compounds.

B. Occurrence. The B₆ vitamins are widely distributed in nature in plant and animal tissues. They are especially rich in cereals (wheat, rice), peas, turnip greens, brussels sprouts, carrots, potatoes, sweet potatoes, bananas, avocados, watermelons and yeasts. B₆ vitamins are also found in egg yolk, salmon, chicken, fish, beef, pork and liver. Pyridoxine is adequately available in human and cow's milk. Pyridoxal (PAL) and pyridoxamine (PAM) also occur in nature as their coenzymes, namely, pyridoxal phosphate (PALP) and pyridoxamine phosphate (PAMP), respectively.

C. Structure. All the 3 forms of vitamin B₆ (Fig. 34-7) are derivatives of pyridine, C₅H₅N and differ from each other in the nature of substituent at position 4 of the ring. All the 3 forms are readily interconvertible biologically.

D. Properties. Pyridoxine is a white crystalline substance and is soluble in water and alcohol

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and slightly so in fat solvents. It is sensitive to light and ultraviolet irradiation. It is resistant to heat (*i.e.*, heat-stable) in both acidic and alkaline solutions but its two allies pyridoxal and pyridoxamine are destroyed at high temperatures (*i.e.*, heat-labile).

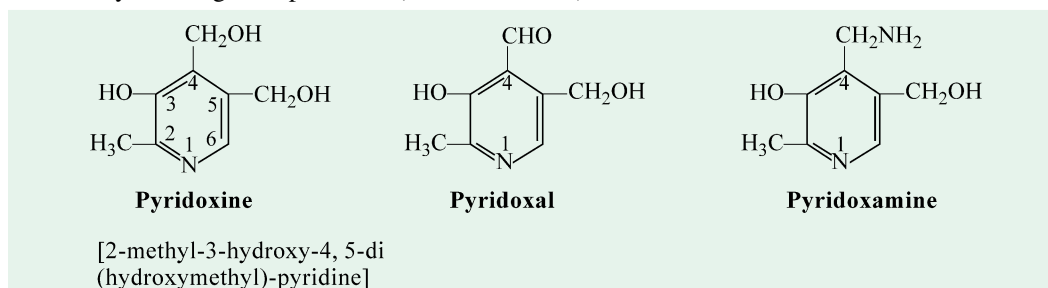


Fig. 34-7. Vitamins of B₆ group

E. Metabolism. The various forms of vitamin B₆ serve as growth factors to a number of bacteria. In addition, the 3 forms (pyridoxine, pyridoxal, pyridoxamine) are converted to pyridoxal-5-phosphate (Fig. 34-8), which acts as a coenzyme in various enzymic reactions involved in amino acid metabolism such as transamination, decarboxylation and racemization and in the metabolism of glycogen and fatty acids. It is also essential in the metabolism of hydroxy amino acids, sulfur-containing amino acids and also tryptophan.

Pyridoxal or its phosphate derivative also possibly acts as a carrier in the active transport of amino acids across cell membranes.

Pyridoxine can be converted to either pyridoxal or pyridoxamine (Fig. 34-8) but neither of them can be changed to pyridoxine. All these three can be detected in the urine after ingestion although 4-pyridoxic acid is the most important excretion product quantitatively. It is for this reason that when administered in the human body, about 90% of pyridoxine is oxidized to pyridoxic acid and excreted in human urine in this form.

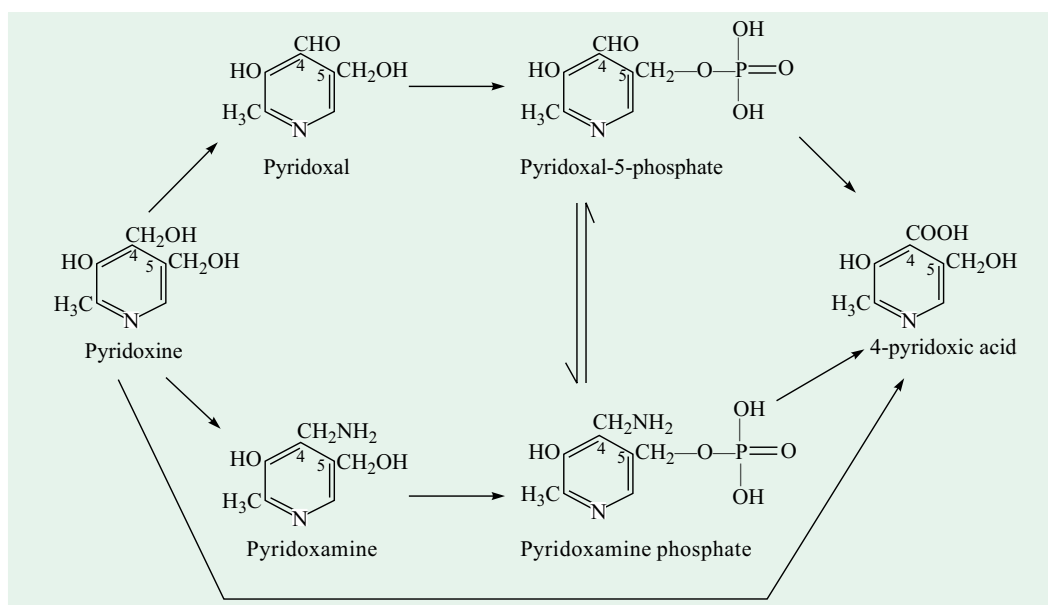


Fig. 34-8. Interrelationship between pyridoxine and its derivatives

B₆ vitamins are also essential for the breakdown of kynurenine. When this does not happen, xanthurenic acid appears in the urine. In addition, adequate functioning of the nervous system depends on pyridoxine, deficiency of which leads to seizures and to peripheral neuropathy.

Pyridoxal phosphate (PALP) is the coenzyme for both glutamic decarboxylase and γ -aminobutyric acid transaminase; each is essential for normal brain metabolism. It participates in active transport of amino acids across cell membranes, chelates metals, and participates in the synthesis of arachidonic acid from linoleic acid. If it is lacking, glycine metabolism may lead to oxaluria.

Normal metabolism of vitamin B₆ in higher animals is inhibited by 4-deoxypyridoxine and isonicotinic acid hydrazide (= isoniazid). *Isoniazid is noted for its curing properties against tuberculosis.*

F. Deficiency. Vitamin B₆ deficiency or apyridoxosis in rats leads to the development of acrodynia, a disease of dermatitis on ears, mouth and tail and accompanied by edema and scaliness of these structures. Dogs and chick develop anemia and nervous lesions in apyridoxosis.

In human infants, vitamin B₆ deficiency results in **convulsions, anemia**, dermatitis and gastrointestinal disorders such as nausea and vomiting. However, this deficiency is rare. Moreover, tryptophan metabolism is also disturbed. In adults, the vitamin B₆ deficiency is normally not found because the intestinal bacteria are capable of synthesizing vitamin B₆.

In B₆-deficient anemia, the RBCs are microcytic and hyperchromic. There are increased serum iron concentrations, saturation of iron-binding protein, hemosiderin deposits in bone marrow and liver, and failure of iron utilization for hemoglobin synthesis.

Diseases with malabsorption, such as celiac syndrome, may contribute to vitamin B₆ deficiency.

A syndrome resembling vitamin B₆ deficiency, as observed in animals, has also been reported in man during the treatment of tuberculosis with high doses of the drug *isoniazid* (Fig. 34-9). Only 2-3% of patients receiving conventional doses (2-3mg/kg) of isoniazid developed neuritis; 40% of patients receiving high doses (20 mg/kg) developed neuropathy. The symptoms were alleviated by the administration of pyridoxine. Thus, 50 mg of pyridoxine per day completely prevented the development of neuritis. It is believed that isoniazid forms a hydrazone complex with pyridoxal, resulting in partial activation of the vitamin. *Isoniazid, thus, is a potent antagonist of vitamin B₆.*

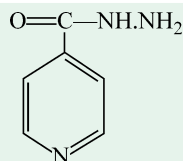


Fig. 34-9. Isoniazid
(Isonicotinic acid hydrazide, INH)

G. Human requirements. The minimum dietary allowance of vitamin B₆ is between 0.2 and 1.2 mg for infants and children and around 2.0 mg for men and women per day. During pregnancy and lactation, the recommended daily dose is 2.5 mg. Pyridoxine antagonists, such as isoniazid used in the treatment of tuberculosis, increase the requirements for pyridoxine as do pregnancy and drugs such as penicillamine, hydralazine and the oral progesterone-estrogen contraceptives.

H. Treatment : Balanced diets usually contain enough pyridoxine so that deficiency is rare. For convulsions due to pyridoxine deficiency, 100 mg of vitamin should be given intramuscularly. Excessive intake may cause sensory neuropathy.

VITAMIN B₇

A. History. In 1935, Fritz Kögl, a Dutch biochemist, isolated in crystalline form from 250 kg of dried egg yolks about 1 mg of a 'bios' factor (growth promoting factor) necessary for yeast and named it as "biotin". Four years later, Szent-Györgyi *et al* conclusively proved that biotin is synonymous to the "**antiegg white injury factor**" which is responsible for the cure of egg white injury, induced in rats and other animals by feeding them with raw egg white. The raw egg white contains a biotin-antagonist protein, *avidin*, which combines with biotin in a firm linkage to form a compound that cannot be absorbed by the intestine and is therefore, excreted. It is also called

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as **coenzyme R** because it is a growth factor for the nitrogen-fixing bacterium, *Rhizobium*.

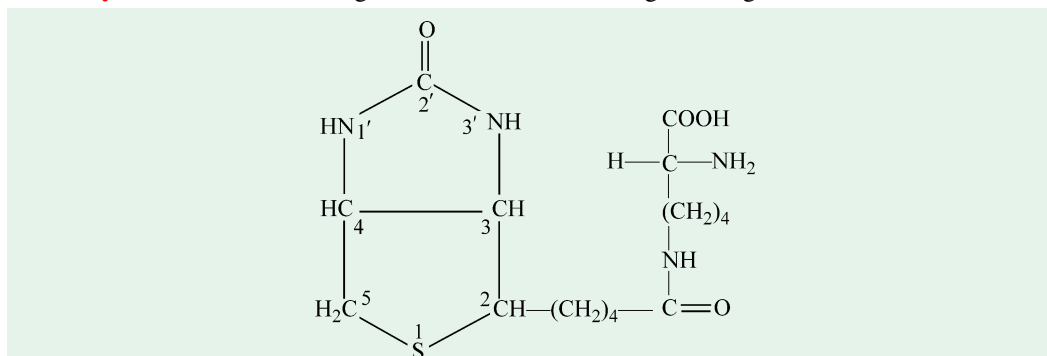


Fig. 34-10. Biocytin or biotinyllysine
(ϵ -N-biotinyl-L-lysine)

B. Occurrence. Biotin has a wide range of distribution both in the animal and the vegetable kingdoms. Yeast, liver, kidney, milk and molasses are among the richest sources ; peanuts and eggs have lesser amounts. Biotin occurs in nature usually in combined state as biocytin (Fig. 34-10). It is a bound form of biotin, linked as a peptide with the amino acid lysine.

C. Structure. The structure of biotin ($C_{10}H_{16}O_3N_2S$) was worked out by Vincent du Vigneaud in 1942. Biotin (Fig. 34-11) has an unusual structure and consists of a fused imidazole and thiophene ring with a fatty acid side chain. Two forms of biotin can exist, *allobiotin* and *epibiotin*. Biotin is optically active. Only the (+) biotin is active; the DL-biotin is half as active as the naturally occurring biotin. The oxybiotin, in which S atom of biotin is replaced with an O atom, has some activity. *Biotin and thiamine are the only sulfur-containing vitamins isolated to date.*

D. Properties. Biotin crystallizes as long needles. It is soluble in water and ethyl alcohol but is insoluble in chloroform and ether. It is heat-stable and is resistant to both acids and alkalis. It has a melting point of 230°C.

E. Metabolism. This vitamin serves as a prosthetic group for many enzymes. These biotin-containing enzymes catalyze the fixation of CO_2 into organic molecules, thus bringing about carboxylation. The carbon dioxide is carried as a carboxyl group attached to one of the ureidonitrogen atoms of biotin, forming *N-carboxybiotin complex* (Fig. 34-12). They also bring about synthesis of fatty acids such as oleic acid.

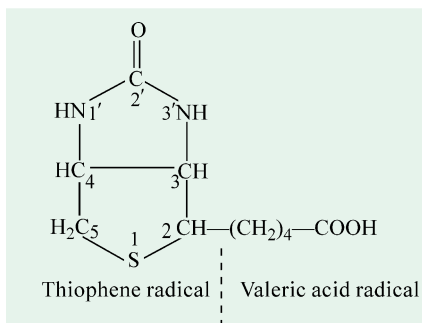


Fig. 34-11. Vitamin B₇ or biotin or vitamin H
(2'-keto-3, 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid)

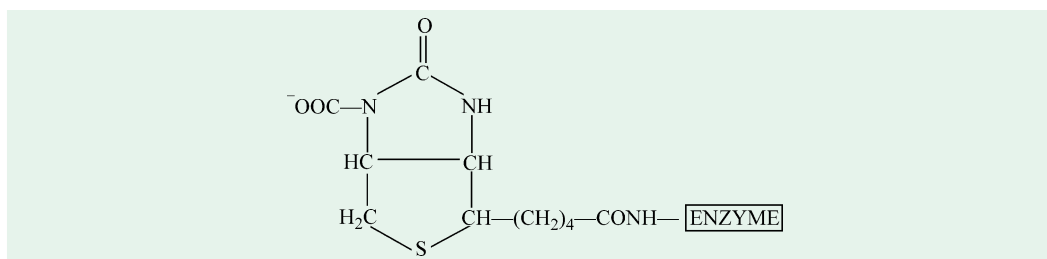
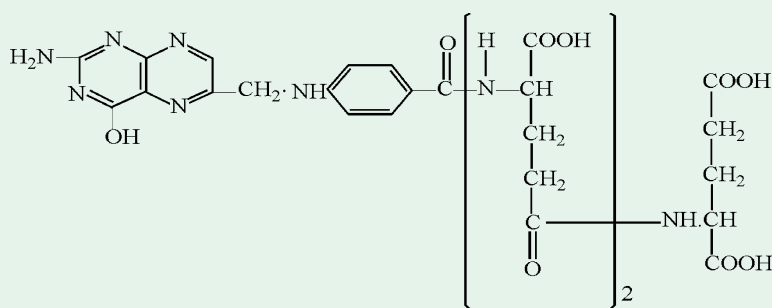
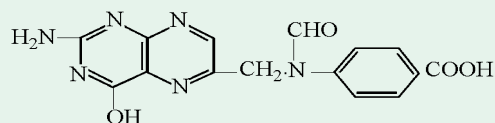


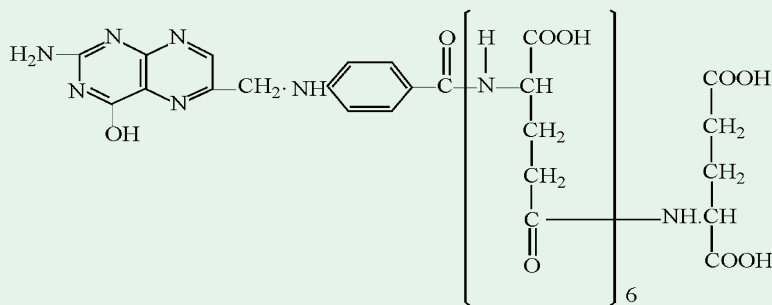
Fig. 34-12. N-carboxybiotin complex



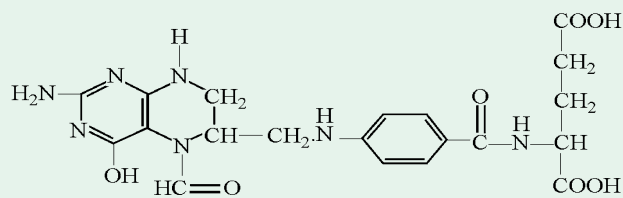
Fermentation *Lactobacillus casei* factor
(pteroyl- γ -glutamyl- γ -glutamylglutamic acid)



***Streptococcus lactis* R (SLR) factor or rhizopterin**
(N¹⁰-formylpterioic acid)



B_c conjugate
(pteroylhexaglutamylglutamic acid)



Citrivorum factor, CF or folic acid
(5-formyl-5,6,7,8-tetrahydropteroylglutamic acid or N⁵-formyl-FH₄)

Fig. 34-13. The four structural homoogues of vitamin B₉

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F. Deficiency. In most animals including man, intestinal bacteria synthesize appreciable amounts of biotin. It is because of this reason that biotin-deficiency in human beings, fed on biotin-free diets, cannot be produced. However, biotin-deficiency may be induced by sterilization of intestine and by feeding with raw egg white. Avidin, the egg white protein, inactivates biotin by eliminating it from an otherwise complete diet. Such a deficiency in man leads to **dermatitis**, loss of hair, decrease in weight and edema. The lesions on skin appear with changes in posture and gait. These disorders may lead to death. Heating egg white destroys the avidin and prevents the so-called egg white injury.

Brawny dermatitis, somnolence, hallucinations, and hyperesthesia with accumulation of organic acids are common. Other neurologic signs and defective immunity may occur.

G. Human requirements. The intestinal bacteria synthesize biotin in such appreciable amounts that the amount excreted in urine exceeds the intake. That is why the RDA for this vitamin has not been established. However, about 10 mg per day of biotin is sufficient for an adult.

H. Treatment : Parenteral solutions should contain biotin. Deficient patients respond to oral administration of 10 mg.

VITAMIN B₉

A. History. Day, for the first time, showed the existence of this nutritional factor by demonstrating that yeast extract could cure cytopenia, a disease experimentally induced in monkeys. The potent factor was obtained from spinach leaf and this led to its nomenclature as folic acid, FA (*folium*^L = leaf). The official name of this vitamin is folacin. This is also known as **liver Lactobacillus casei factor** as it was isolated from liver and was shown as necessary for the growth of lactic acid bacteria. Hogan called this as vitamin Bc.

However, a number of other compounds or factors (Fig. 34-13), having similar or different biochemical functions but closely related to folic acid, were isolated from different sources. These are *fermentation Lactobacillus casei factor*, *Streptococcus lactis R (SLR) factor*, *Bc conjugate and citrivorum factor*, *CF*.

B. Occurrence. Folic acid (Fig. 34-14) and its derivatives (tri- and hepta-glutamyl peptides) are widely distributed in biological world. A few important sources are liver, kidney, tuna fish, salmon, yeast, wheat, dates and spinach. Root vegetables, sweet potatoes, rice, corn, tomatoes, bananas, pork and lamb contain little folic acid. With improper cooking, folacin contents are destroyed, like thiamine.

C. Structure. A molecule of folic acid (Fig. 34-15) consists of 3 units : glutamic acid, *p*-aminobenzoic acid and a derivative of the heterocyclic fused-ring compound pterin. Its molecular formula is C₁₉H₁₉O₆N₇.

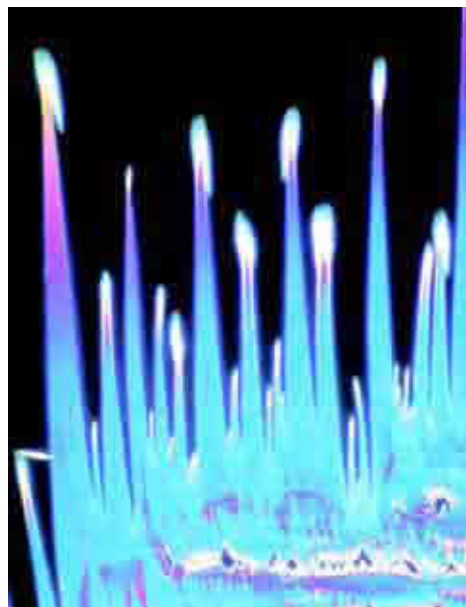


Fig. 34-14. Folic acid crystal

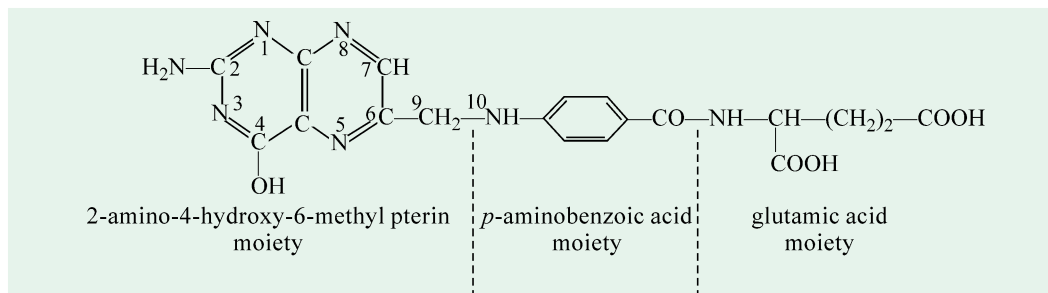
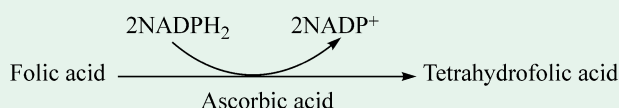


Fig. 34-15. Vitamin B₉ or folic acid or folacin or vitamin M
(pteroyl-L-glutamic acid, PGA)

The various vitamins of B₉ group differ from each other in the number of glutamic acid groups present; the additional glutamic acid group being conjugated in peptide linkages. For example, folic acid contains one, fermentation Lactobacillus casei factor three and Bc conjugate seven glutamic acid groups. The conjugates (*i.e.*, compounds having more than one glutamic acid groups in the molecule) are ineffective for some species as these species do not possess the enzyme *conjugase* which is necessary for the release of free vitamin. Citrivorum factor, however, differs from other vitamins of B₉ group in the structure of one of the rings of the pterin moiety.

D. Properties. Folic acid is a yellow crystalline substance, slightly soluble in water but insoluble in fat solvents. It is stable to heat in alkaline or neutral solutions only. It is inactivated by sunlight.

E. Metabolism. The reduction products of folic acid act as coenzymes. An enzyme, *folic reductase*, reduces folic acid to dihydrofolic acid (DHFA or FH₂), the latter compound is further



reduced by *dihydrofolic reductase* to 5,6,7,8-tetrahydrofolic acid (THFA or FH₄). The formation of FH₄ from FA is associated with the oxidation of NADPH or NADH and requires the presence of ascorbic acid.

The structure of FH₄ appears in Fig. 34-16.

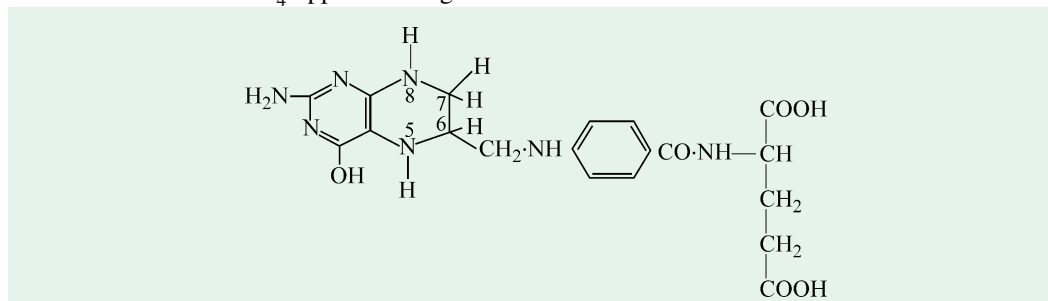


Fig. 34-16. Tetrahydrofolic acid, THFA or FH₄
(5, 6, 7,8-tetrahydropteroylglutamic acid)

The vitamins of B₉ group are involved in *one-carbon* metabolism in a way similar to the *two-carbon* metabolism in which CoA is involved. THFA acts as an acceptor of a one-carbon unit either from formate (in which case 5,6,7,8-tetrahydrofolic acid is formed) or from formaldehyde (in which case 5-hydroxymethyl-5,6,7,8-tetrahydrofolic acid is formed). THFA is also involved in

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the transfer of the methyl group and in the utilization of single carbons (formate) in the synthesis of serine, methionine, thymine, purines, choline and inosinic acid.

Folic acid, in conjunction with ascorbic acid, also appears to be related to tyrosine metabolism.

Citriovorum factor (CF) is 5 formyl derivative of tetrahydrofolic acid and is so named because it supports the growth of *Leuconostoc citriovorum*. CF is about one thousand times more potent biologically than folic acid. Its chemical name is folinic acid. During the conversion of FA to CF, vitamin B₁₂ and ascorbic acid are also required. Citriovorum factor (and also folic acid to a lesser degree) are concerned in the production of an agent that stimulates the formation of normal RBCs.

It is interesting to note that the bacteria, which require PABA for growth, also utilize FA with almost equal ease.

Recent studies have shown that folic acid provides protection against Alzheimer's disease.

Folic acid is, however, essential for lactation in rats and hatchability of eggs in chicks, turkeys and guinea pigs. Rats, dogs and probably man do not need folic acid because the intestinal bacteria synthesize sufficient quantity of this vitamin.

F. Deficiency. In chicks, a lack of this factor leads to **anemia**. Rats develop **achromotrichia** (failure in normal pigmentation of the hair). The monkeys show **macrocytic anemia** (anemia characterized by the presence of giant RBCs), leukopenia, diarrhea and **edema** (retention of water by skin tissues).

On a worldwide basis, deficiency of folic acid is believed to be the most common form of vitamin undernutrition. In man, the folic acid deficiency leads to **megaloblastic anemia, glossitis** and gastrointestinal disorders. Pregnant women and infants are also particularly vulnerable. Folic acid deficiency is a major feature of **tropical sprue**, in which there is a general deficiency in absorption of many nutrients from the small intestine.

Folic acid has been successfully used in the treatment of certain macrocytic anemias such as those developed in sprue and anemias of pellagra, pregnancy and infancy. However, the long-held hope that it would cure pernicious anemia (caused by avitaminosis B₁₂) has not been held true since it fails to cure the neurological lesions of the disease.

G. Human requirements. The daily dietary allowance of folic acid is 0.1 mg for infants, 0.2 mg for children and 0.4 mg for adult men and women. Pregnant mothers may, however, require up to 0.8 mg per day.

VITAMIN B₁₂

A. History. In 1926, two American physicians, George Minot and George William Murphy discovered that patients suffering from pernicious anemia could be cured by feeding them with about half a pound of liver a day. This landmark in medicine brought them Nobel Prize in 1934. In 1929, Castle suggested that gastric juice contained a factor (intrinsic factor) that, together with a factor present in the food (extrinsic factor), is responsible for the cure of pernicious anemia. This **anti-pernicious anemia factor (APA factor)** was, later, isolated in crystalline form in 1948 independently by E. Lester Smith in England and by Edward Rickes and Karl Folkers in the United States. It was then named as vitamin B₁₂ or cyanocobalamin. It is the last B-vitamin to be isolated and is also known as *Factor X* or *L.L.D. factor*. The coenzyme form of this vitamin (deoxyadenosyl cobalamin or cobamide coenzyme) was first isolated by Barker of California. Coenzyme B₁₂ has been called a "*biologic Grignard reagent*".

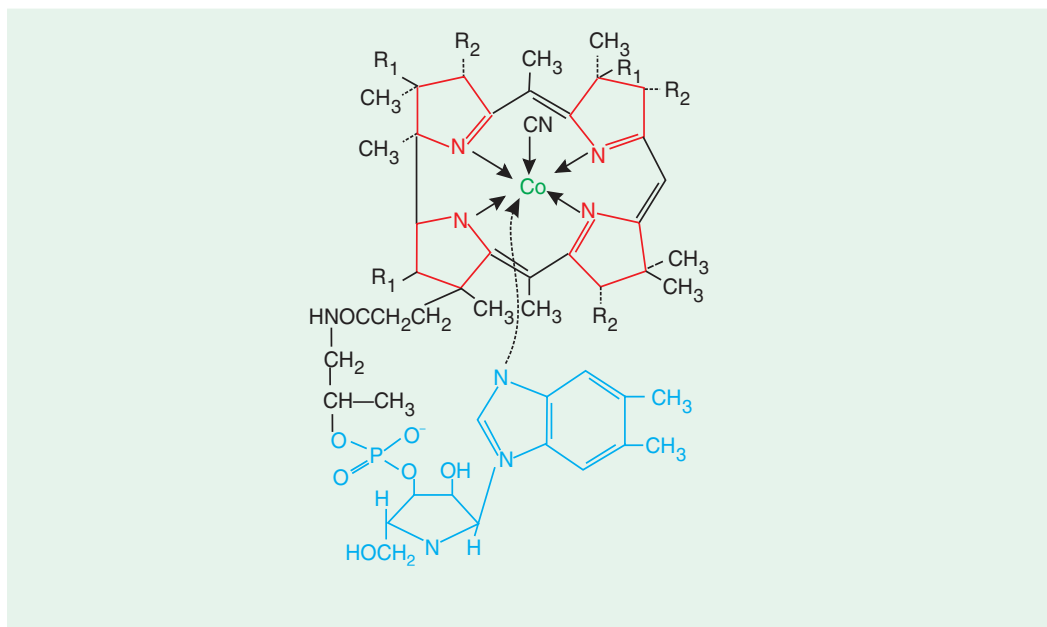
Cyanocobalamin is also spelt as cyanocobalamine (Albert L. Lehninger, 1984)

B. Occurrence. *Vitamin B₁₂ has been found only in animals* ; the chief source is liver, although it is also present in milk, meat, eggs, fish, oysters and clams. Animal tissues contain it in varying amounts as shown in Table 34-2.

Table 34-2. Amount of vitamin B₁₂ in animal tissues

S. N.	Source of Vitamin B ₁₂	Amount Present (in millionth gm per 100 gm of fresh weight)
1.	Liver (Ox)	134
2.	Liver (Herring)	34
3.	Meat (Fish)	25
4.	Meat (Beef)	8
5.	Cheese	3.6
6.	Egg yolk	1.3

Under certain dietary conditions, vitamin B₁₂ may be synthesized by the intestinal microorganisms. *In general, cyanocobalamin is not present in plant foods except in Spirulina, a blue-green alga.* However, it occurs in foods bound to proteins and is apparently split off by proteolytic enzymes.

Fig. 34-17. Structure of vitamin B₁₂ or cyanocobalamin

Animals and plants are unable to synthesize this vitamin. *Cyanocobalamin is unique in that it appears to be synthesized only by microorganisms especially anaerobic bacteria.* However, a process of producing vitamin B₁₂ from waste products has been developed, in 1977, by the department of Chemical Engineering of the Indian Institute of Technology, Chennai.

C. Structure. The structure of vitamin B₁₂ (Fig. 34-17), one of the most complex known, has been established, in 1957, by Dorothy Crowfoot Hodgkin (Nobel Laureate, 1964). Cyanocobalamin (C₆₃H₈₈O₁₄N₁₄P Co) is a pigment alike to the tetrapyrrole ring structure of the porphyrins, *e.g.*, chlorophyll and haem. A unique feature of this vitamin (and other related compounds) is the presence, in its molecule, of an atom of a heavy metal cobalt in the trivalent state. *No other cobalt-containing organic compound has been found in nature.* The cobalt atom is centrally-situated and

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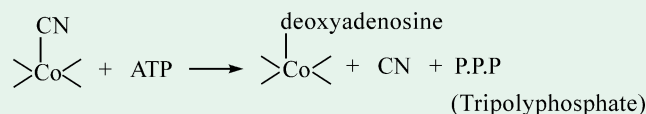
is surrounded by a macrocyclic structure of 4 reduced pyrrole rings (A, B, C and D) collectively called as corrin. It may be noted from the structural formula that the 6 coordinate valences of the cobalt atom (Co^{2+}) are satisfied by the 4 nitrogens of the reduced tetrapyrrole, a nitrogen atom of 5, 6-dimethylbenzimidazole and a cyanide ion. Two of the pyrrole rings, namely A and D, are directly linked to each other and the corrin has lower degree of unsaturation with only 6 double bonds. The other two pyrrole rings, namely B and C, are joined through a single methene carbon. Another distinct feature of the vitamin B_{12} molecule is the presence of a loop of the isopropanol, phosphate, ribose and 5,6-dimethyl-benzimidazole in that order, the end of the loop being attached to the central cobalt atom.

Many compounds with vitamin B_{12} activity have been isolated from natural sources. Cyanocobalamin is the most common form and is sometimes also written as vitamin B_{12a} . In other forms, cyanide ion is replaced by other ions, e.g., by hydroxyl ion in hydroxocobalamin (also designated as vitamin B_{12b}), by nitrite ion in nitrocobalamin (also designated as vitamin B_{12c}) etc. The latter two, B_{12b} and B_{12c} can be converted to vitamin B_{12a} by treatment with cyanide.

The structure of *vitamin B_{12} coenzyme* (= 5'-deoxyadenosyl cobalamin) is similar to that of cobalamin except that here the CN group is replaced by adenosine and the linking with cobalt atom taking place at 5' carbon atom of the ribose of adenosine (Fig. 34–18). *Vitamin B_{12} coenzyme is the only known example of a carbon-metal bond in a biomolecule.*

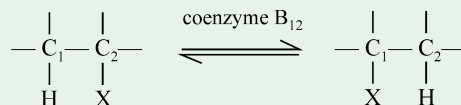
D. Properties. Vitamin B_{12} (molecular weight, ca 1,500) is a deep red crystalline substance. It is soluble in water, alcohol and acetone but not in chloroform. It is levorotatory. It is stable to heat in neutral solutions but is destroyed by heat in acidic or alkaline solutions.

E. Metabolism. Vitamin B_{12} is converted to coenzyme B_{12} by extracts from microorganisms supplemented with ATP.

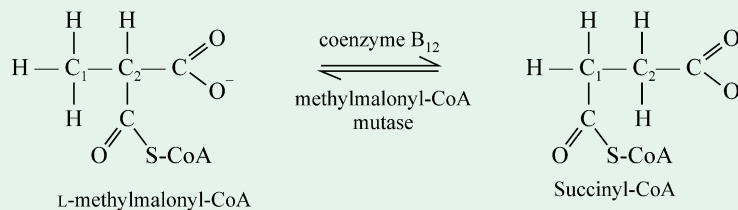


Coenzyme B_{12} is associated with many biochemical reactions :

- (a) *1,2 shift of a hydrogen atom* : Coenzyme B_{12} catalyzes 1,2 shift of a hydrogen atom from one carbon atom of the substrate to the next with a concomitant 2,1 (reverse) shift of some other group, e.g., hydroxyl, alkyl etc.



Conversion of methylmalonyl-CoA to succinyl-CoA is an example of 1,2-shift.



- (b) *Carrier of a methyl group* : Coenzyme B_{12} also serves as a carrier of a methyl group, obtained from N^5 -methyltetrahydrofolate, to the appropriate acceptor molecule. In the reaction, a methyl group occupies the 5-deoxyadenosyl coordination position of coenzyme

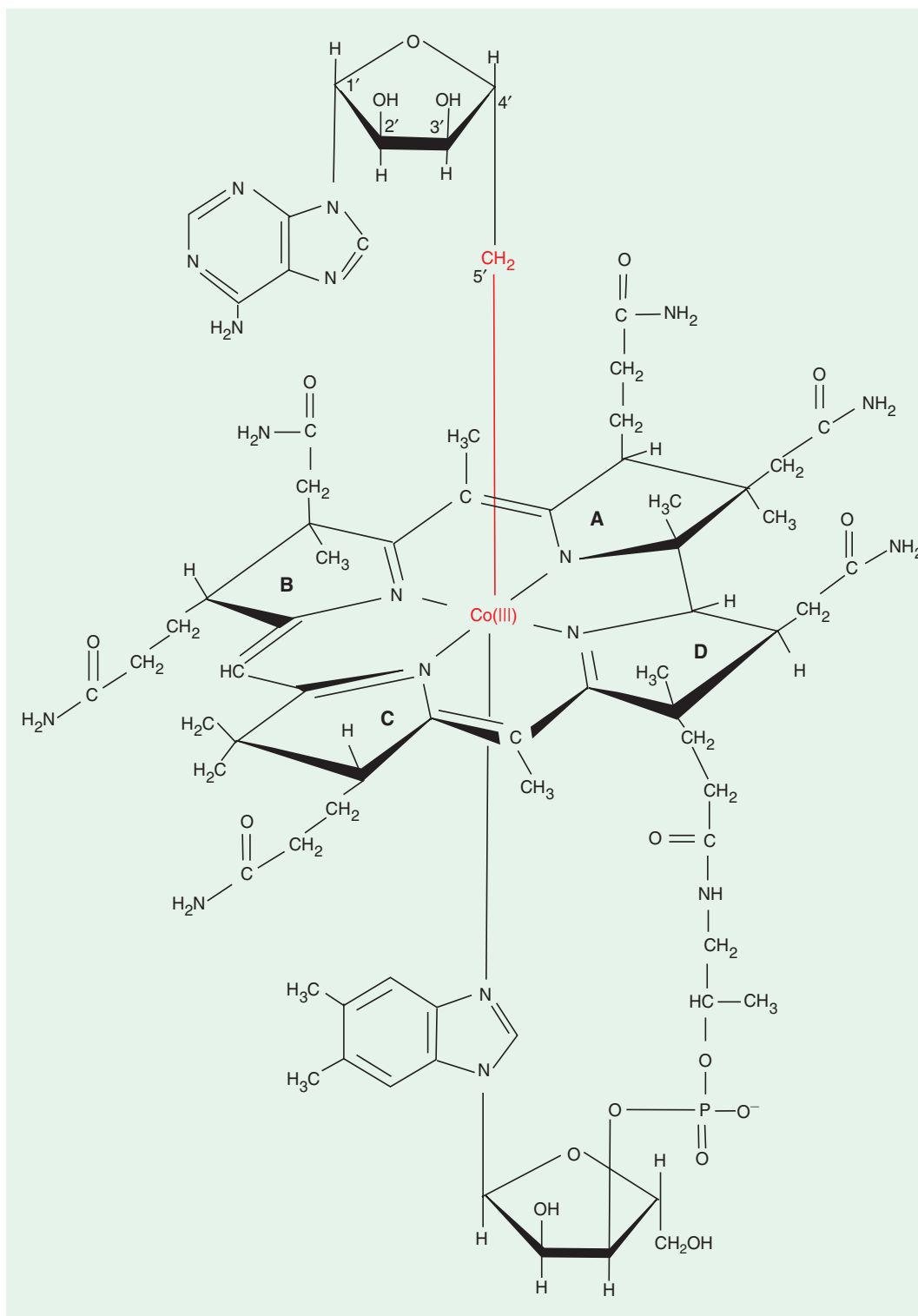
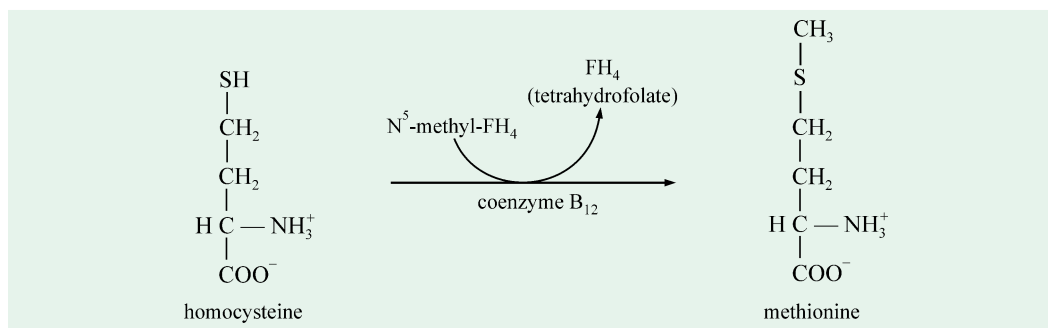


Fig. 34-18. Structure of 5'-deoxyadenosylcobalamin (coenzyme B₁₂)

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B₁₂. Methylation of homocysteine to produce methionine is an example of such reaction.



- (c) *Isomerization of dicarboxylic acids* : Coenzyme B₁₂ is associated with isomerization of dicarboxylic acids, e.g., glutamic acid into β-methyl-aspartic acid.
- (d) *Dismutation of vicinal diols* : Coenzyme 12 also catalyzes dismutation of vicinal diols to the corresponding aldehydes, e.g., propane-1,2-diol into propionaldehyde.

Vitamin B₁₂ is also needed for the biosynthesis of methyl groups from 1-carbon precursors and for the synthesis of thymidine and other deoxyribosides. It also functions in protein synthesis and in the activation of SH enzymes. Cyanocobalamin also affects myelin formation.

F. Deficiency. A nutritional deficiency of this vitamin is usually not observed on account of its ubiquitous (= widespread) nature in foodstuffs. Thus, most cases of deficiency stem from failure to absorb the vitamin. However, deficiency may be observed in individuals who abstain from all animal products including milk and eggs, *i.e.*, those who are strict vegetarians.

The rare **disease juvenile (or congenital) pernicious anemia** springs up due to an inability to secrete gastric intrinsic factors. The symptoms of this disease become prominent at 9 months to 10 years of age. As the anemia becomes severe, irritability, anorexia and listlessness occur. The tongue is smooth, red and painful. Neurologic symptoms include ataxia, paresthesias, hyporeflexia, clonus, Babinski responses and coma. Consanguinity is common in parents of affected children and suggests Mendelian recessive inheritance. The juvenile disease differs from the typical disease in adults in that the stomach secretes acid normally and is histologically normal. This typical deficiency disease, **adult pernicious anemia** (= anemia caused by failure of erythrocyte formation), is characterized by R.B.Cs. becoming abnormally large and fewer in number (1–3 million per cubic millimeter instead of the normal 4–5 million). The patient weakens, loses its weight and the nervous system is also gradually affected because there occurs demyelination of the large nerve fibres of the spinal cord. All these changes ultimately lead to death.

G. Human requirements. The recommended daily allowance of vitamin B₁₂ is 2 to 4 μg for children and 5 μg for men and women. Pregnant and lactating mothers require 8 μg and 6 μg daily.

H. Treatment : The excessive secretion of methylmalonic acid in the urine is a reliable and sensitive index of vitamin B₁₂ deficiency. The physiologic need for vitamin B₁₂ is 1-5 μg/24 hr, and hematologic responses have been observed with these small doses. If there is evidence of neurologic involvement, 1 mg should be injected intramuscularly daily for a minimum of 2 weeks.

CLINICAL IMPLICATIONS

Pernicious Anemia

Pernicious anemia was described in 1855 by **Dr. Thomas Addison** and called pernicious because it is a fatal disease. The disease pernicious anemia affects those of Northern European ancestry including American blacks but not Africans. The major systems affected by this disease are hematopoietic and central nervous systems. There is no secretion of HCl in the stomach, and there is an increased incidence of carcinoma of the stomach.

Maintenance therapy is necessary throughout patient's life; monthly intramuscular administration of 1 mg of vitamin B₁₂ is sufficient.

VITAMIN C

A. History. No other vitamin, with the possible exception of vitamin E, is as generally misunderstood as is vitamin C. It is ironic that the oldest therapeutically-used vitamin, furnished in 1750s in the form the lemons to British sailors to prevent scurvy, is still a subject of controversy. However, in 1928, **Albert G. Szent-Györgyi** isolated this crystalline vitamin from the paprika plant and named it **hexuronic acid**. Later in 1932, C. Glen King and W.A. Waugh in United States isolated this from lemon juice. It was synthesized by Reichstein in 1933. It is also called **cevitamin**. It has a curing action against scurvy and hence popularly called as **antiscorbutic factor**.

ALBERT VON SZENT-GYÖRGYI

(LT, 1893-1986)

Albert G. Szent-Gyorgi, a Hungarian biochemist, was one of the many distinguished biochemists, originally trained in medicine. His first research paper dealt with hemorrhoids. Later, he went into physiology, then biochemistry, and finally physical chemistry. He was awarded the prestigious **1937-Nobel Prize in Physiology or Medicine** for his researches on vitamins C. He was a prolific writer and wrote on many topics of Biology. His concept about research may be expressed in his own words, "The basic texture of research consists of dreams into which the threads of reasoning, measurement, and calculation are woven." Reflecting on his scientific career, he stated he has only one regret : "I started science on the wrong end." One of his famous quotes reads as follows :



"Discovery consists in seeing what everybody else has seen and thinking what nobody else has thought."

B. Occurrence. *In general, ascorbic acid is not as widely distributed as other vitamins.* Among plants, it is present in all fresh fruits and vegetables. *The richest source of vitamin C, known upto date, is the acerola fruit (Malpighia punctifolia).* The fruit yields 1,000–4,000 mg of ascorbic acid per 100 g of edible matter. Citrus fruits (such as orange, lemon, lime), gooseberry, pineapple, guavas, tomatoes, melons, raw cabbage and green pepper are also rich sources of it. New potatoes contain relatively large amounts. Dried legumes and cereals contain very little vitamin C. Dry seeds, in general, are devoid of it but during their sprouting, the vitamin appears. Woody tissues also lack it. Vitamin C is synthesized by most mammals, but not by primates (such as apes, man) and guinea pigs which acquire it from their diets. In animals, the vitamin occurs in tissues and various glands or organs such as liver, adrenals, thymus, corpus luteum etc. Meat contains relatively low concentration. Human milk is 3 to 4 times richer in vitamin C contents than cow's milk. *Vitamin C is, however, absent from fish, fats and oils.* It is also not present in or required by microorganisms. Since this vitamin is a good reducing agent, it is lost under oxidizing conditions like aeration and heating. Thus, many cooked and canned foods contain little ascorbic acid. It is also found in the combined form as *ascorbigen*. The adrenal and lenses have particularly high contents of vitamin C.

The infant is usually born with adequate stores of ascorbic acid if the mother's intake has been adequate; the ascorbic acid content of cord blood plasma is 2-4 times greater than that of maternal plasma. Under these conditions, the breast milk contains ca 4-7 mg/dL of ascorbic acid and is an adequate source of ascorbic acid.

The vitamin C content of some food items are presented in Table 34–3.

Table 34–3 Vitamin C content of some fruits and vegetables

Fruit/Vegetable	Vitamin C (mg/100 g)
Amla	600 – 700
Guava	200 – 300
Lime	65
Papaya	55 – 60
Lemon	40 – 50
Orange	30
Tomato	25 – 30
Drumstick leaves	200 – 250
Bathua leaves	200 – 250
Radish leaves	80
Fenugreek leaves	50
Palak leaves	30

C. Structure. The structure of ascorbic acid ($C_6H_8O_6$) was established mainly by Haworth. It is a derivative of a hexose called L-gulose. Chemically, it is 1-threo-2,4,5, 6-pentoxihexen-2-carboxylic acid lactone (refer Fig. 34–20). Although ascorbic acid is a small molecule when compared with DNA, RNA or proteins, its metabolic impact is no less considerable. The dienolic group consisting of hydroxyls on C2 and C3 with a double bond between them invests the ascorbic acid molecule with redox property.

D. Properties. Ascorbic acid is a colourless and odourless crystalline substance, slightly sour in taste and optically active. *Only the L-isomer has antiscorbutic properties.* It is soluble in water and alcohol but practically insoluble in chloroform, solvent ether and light petroleum. It is readily oxidized, particularly in the presence of copper and iron but not of aluminium. It is for this reason that the foods cooked in copper utensils lose ascorbic acid quickly. This vitamin is also rapidly destroyed by alkalis but is fairly stable in weak acid solutions. Therefore, baking soda has a deleterious effect but steam cooking destroys very little amount of ascorbic acid. Drying of vegetables and also their storage results in a loss of their ascorbic acid. However, freezing has no detrimental effect on this vitamin. Citrus fruit juices and tomato juice may be canned with but little loss of ascorbic acid. On account of its easily oxidizable nature, the ascorbic acid is a powerful reducing agent.

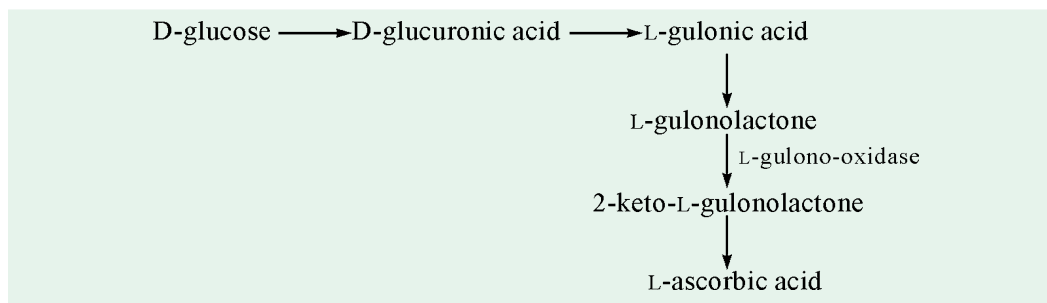


Fig. 34–19. Biosynthesis of vitamin C

E. Metabolism. Higher plants (like pea) and all known mammals except man, the primates

and guinea pig can synthesize ascorbic acid from L-gulonolactone. The rat, for example, is resistant to scurvy. In animals, the liver and adrenals (cortical portion) are the main sites of synthesis. The biosynthesis of ascorbic acid in animals takes place according to the scheme as depicted in Fig. 34–19. The scheme also probably applies to the plants. *Man lacks the enzyme L-gulono-oxidase and as such is incapable of synthesizing ascorbic acid.*

Ascorbic acid can be readily oxidized (Fig. 34–20) to dehydroascorbic acid in the presence of metal ions. Dehydroascorbic acid is a much more powerful electron donor than even ascorbic acid by virtue of its unpaired electron. It is, in fact, the free radical form of ascorbic acid. Dehydroascorbic acid can be reduced, in the presence of H₂S or cysteine, back to ascorbic acid. The reduced form (*i.e.*, L-ascorbic acid) predominates in the plasma and also apparently in tissues at a ratio of about 15 : 1 of the oxidized form (*i.e.*, dehydro-L-ascorbic acid). Both of these are biologically active and are equally potent in carrying out their metabolic functions. When dehydro-L-ascorbic acid is hydrated, 2,3-diketo-L-gulonic acid is formed which is biologically inactive and cannot be converted back to either of the active forms in the body. Since the hydration reaction takes place automatically in the neutral medium, the oxidation of ascorbic acid, in other words, means its biologic inactivation. These reactions have been shown to occur *in vivo* in man and guinea pigs.

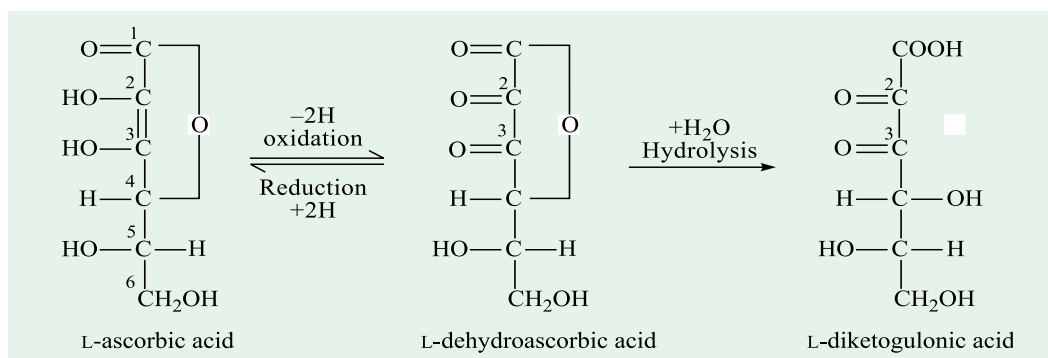


Fig. 34–20. Metabolism of vitamin C

Ascorbic acid functions in a number of enzymatic activities. A major function of ascorbic acid is the formation of tissue collagen or '*intracellular cement substance*'. In fact, ascorbic acid appears to be essential to the activity of the enzyme *collagen proline hydroxylase*, which catalyzes the conversion of proline to hydroxyproline. Hydroxyproline (Hyp) is found exclusively in collagen and is vital in maintaining the tertiary structure of this major vertebrate protein, *i.e.*, collagen.

Recent researches have established the role of ascorbic acid in the conversion of folic acid to a physiologically active form, tetrahydrofolic acid.

Ascorbic acid also plays a key role in tyrosine metabolism. One of the steps in tyrosine metabolism is the oxidation of *p*-hydrophenylpyruvic acid to homogentisic acid. The vitamin C protects the enzyme *p*-hydrophenylpyruvic acid oxidase from inhibition by excess substrate.

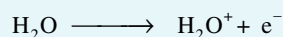
Ascorbic acid is also involved in electron transport in the microsomal fraction. However, *in none of the biological oxidation systems, ascorbic acid has been shown to act as a specific coenzyme.*

Vitamin C is found concentrated in certain parts of human body such as brain and the white blood cells. This body "pool" amounts to roughly 1,500 mg for a man and slightly more for a woman, which is normally enough for about a month's need. However, illness or stress can substantially decrease the body's vitamin C reserves, as also can smoking, drinking, and a variety of drugs such as aspirin. Vitamin C plays an important role in our body's wondrous immune

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system. It may enhance the body's production of interferon, prostaglandins, T-lymphocytes and immunoglobulins-weapons of the body's self-defence arsenal. However, huge doses of ascorbic acid can leach calcium and other needed minerals out of the body. It can act as a diuretic and laxative.

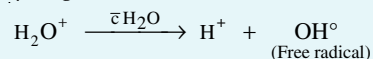
Free radicals are natural by-products of metabolism and are involved in the body's defence against microorganisms. But, if in excess, they damage body cells and tissues and, thus, play a role in degenerative disorders such as heart ailments and cancer. Free radicals are highly reactive, unstable molecules that normally attack cellular proteins, lipids and even DNA. It may be an atom or groups of atoms containing an unpaired electron. Usually free radicals are formed in radiation as intermediates between final chemical products an ion pairs. During an ionizing radiation, an electron is ejected from a water molecule as :



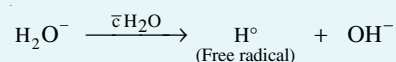
This high energy electron may be picked up by another water molecule as :



In this way, an ion pair, H_2O^+ and H_2O^- , are formed. Each ion then may, in the presence of another water molecule, form a hydrogen ion and a free radical as :



or as :



The H^+ and OH^- will combine to form water. The H° and OH° free radicals are very reactive. In fact, many of them react to form H_2O_2 . In cells containing catalase and peroxidases, hydrogen peroxide formation may not be significant. In the absence of such enzymes, hydrogen peroxide formation in cells may be important biologically. Free radicals may also react with oxygen to enhance the effect of radiation. Free radicals may be formed from nearly any cellular component which ionizes to contribute to the indirect effect of radiation. They are also formed in the body as a result of exposure to smoking, pollution and sunlight.

The fact that in old age the deficiency of vitamin C is frequently observed point to a possible role of vitamin C as an anti-ageing agent. Free radicals are the major cause of ageing. These are oxygen molecules which lose an electron in the course of circulating through the blood-stream. These highly reactive molecules try to regain chemical stability by 'snatching' electrons from other molecules, a process which causes much damage. While normal metabolic processes produce some free radicals, their number increases by tissue injuries from infection, toxins, reduced blood flow, excessive exercise and environmental hazards like radiation, heat, and cold. And *the antioxidants like vitamins A, C and E neutralize these free radicals by 'donating' electrons.*

Vitamin C (and also vitamins A and E) have long been known to be beneficial for the skin. While vitamins A and E work by exfoliating the skin's surface cells, vitamin C works from the inside by boosting collagen production and repair. It also inhibits the excess production of melanin ($\text{C}_{17}\text{H}_{98}\text{O}_{33}\text{N}_{14}\text{S}$) which leads to a tan and hyperpigmentation. Some dermatologists encourage the use of vitamin C as an anti-inflammatory agent as its topical application speeds up the skin's healing process, reducing redness and irritation caused by sun exposure.

Ascorbic acid plays an important role in germination, growth, metabolism and flowering of plants (Chenoy JJ, 1962, 1967-1973). During germination, embryo axis has higher ascorbic acid content as well as higher rate of ascorbic acid utilization compared with those of the endosperm or the cotyledons. Ascorbic acid stimulates amylase, protease and RNAase activity and RNA content in various crops including gram (*Cicer arietinum*). Free radical content of the endosperm/

cotyledon has been shown to be higher during the initial stages of germination as compared to that in the embryo axis, suggesting an active participation of free radicals in the process of energy flow for transport of metabolites from storage organ to the embryo axis. Increase in free radical content of the embryo axis, at later stages of germination is highly suggestive of the important role of free radical in the biosynthesis of macromolecules and other cell constituents for seedling growth. Further, it was established that the ascorbic acid turnover is appreciably higher during the reproductive phase of differentiation in many thermophobes (wheat, barley, oat), as well as in many thermophytes (maize, sesame). During the period of reproductive differentiation, the free radical content is enhanced.

F. Deficiency : Avitaminosis C leads to scurvy, which may occur at any stage but is rare in the newborn infant. The majority of cases appear in infants 6-24 months (mo) of age. Breast-fed infants are protected as the breast milk contains adequate amounts of vitamin C. Clinical manifestations require time to develop. However, after a variable period of vitamin C depletion, contain vague symptoms of irritability, tachypnea (very rapid respiration), digestive disturbances and loss of appetite appear. The main symptoms which later develop are listed below :

1. *Tender bones* : There is evidence of general tenderness, esp., noticeable in the legs when the infant is picked up or when the diaper is changed. The legs assume the typical “frog-like position”, in which the hips and knees are semiflexed with the feet rotated outward. This may be mistaken for paralysis and is hence aptly called **pseudo-paralysis**.
2. *Edematous swellings* : These develop along the shafts of the legs and in some cases a subperiosteal hemorrhage can be palpated at the end of the femur.
3. *Petechial hemorrhages* : The capillaries become brittle and burst, thus giving rise to red and purple spots (or *petechiae*) over the body. Petechiae may be seen in the skin and mucous membranes. Hematuria, melena, and orbital or subdural hemorrhages may be found.
4. *Bleeding gums* : Changes in the gums, most noticeable when the teeth are erupted, are

CLINICAL IMPLICATIONS

SCURVY

A vivid description of scurvy, a dietary deficiency disease, was given by **Jacques Cartier** in 1536, when it afflicted his men when they were exploring the Saint Lawrence River :

“Some did lose all their strength, and could not stand on their feet ... Others also had their skins spotted with spots of blood of a purple colour: then did it ascend up to their ankles, knees, thighs, shoulders, arms and necks. Their mouths became stinking, their gums so rotten, that all the flesh did fall off, even to the roots of the teeth, which did also almost all fall out”.



Fig. 34–21. Bleeding gums in a scorbutic patient

CLINICAL IMPLICATIONS

SCURVY

The need for fruits like oranges, lemons and limes was first discovered in the seventeenth century when sailors on long sea voyages developed scurvy after many months of living on dried and stale meat.

But it was not until the 1940s that anyone showed that the substance in fruit which prevented these symptoms was vitamin C. In 1940, a young American surgeon, **John Crandon**, experimented on himself to investigate the effects of a diet deficient in ascorbic acid. After 3 months of deficiency, a skin cut healed normally, but after 6 months another cut would not heal at all. We now know that vitamin C deficiency leads eventually (but not immediately) to a severe reduction in the body's ability to produce collagen and intercellular 'cement'. Any damage to the skin or other tissues cannot therefore be repaired.

Today, some scientists think that some vitamins, including vitamin C may help protect the body against cancer. There is no hard evidence for this, but many people take vitamin supplements, just in case.

characterized by bluish-purple, spongy swellings of the mucous membrane, usually over the upper incisors (Fig. 34-21).

5. *Scorbutic rosary* : The costochondral junctions become prominent and appear sharp and angular, giving rise to a beaded structure, called scorbutic rosary. The angulation of the scorbutic beads is usually sharper than that of the rachitic rosary because it is produced by a separation of epiphyses of ribs and backward displacement of sternum rather than by widening of the softened epiphyses as occurs in rickets, where the prominence of the costochondral junction is dome-shaped and semicircular.
6. *Delayed wound healing*. The wound healing is delayed or, in many cases, even does not occur because of the failure of the cells to deposit collagen fibrils. The healed wounds may even break down.
7. *Cessation of bone growth* : The bones cease to grow. The cells of growing epiphyses continue to proliferate but no new matrix is laid down between the cells. Consequently, bones fracture easily at the point of growth because they fail to ossify. Moreover, when an already ossified bone fractures in a scorbutic individual, the osteoblasts cannot secrete a new matrix for the deposition of new bone. With the result, the fractured bone does not heal.
8. *"Sicca" syndrome* : Swollen joints and follicular hyperkeratosis may develop, as well as the "sicca" syndrome of Sjögren, which is usually associated with collagen disorders and includes xerostomia, keratoconjunctivitis sicca, and enlargement of the salivary glands.
9. *Anemia* : Anemia may reflect inability to utilize iron or impaired folic acid metabolism.
10. *Pyrexia* : Low-grade fever is usually present in scorbutic children.

Infants 6–12 months of age, who are fed on processed milk only, are very susceptible to this disease (infantile scurvy). Adult cases appear less frequently. Elderly bachelors and widowers who have to cook their own foods are especially prone to the development of vitamin C deficiency—a syndrome termed '*bachelor scurvy*'. Food faddists may also develop scurvy if their diet lacks fruits and vegetables.

In 1975, Prof. Olaf Skinsnes of the University of Hawaii, Honolulu, has succeeded in obtaining almost pure cultures of leprosy bacillus that afflicts human beings. This work has, however, raised the possibility of a vaccine against this dreaded scourge. As an important sidelight of his work, it appears that vitamin C tends to slow down the growth of the bacilli by inhibiting enzyme action. Vitamin C may, thus, have a minor but an important role to play in leprosy treatment.

G. Human requirements. Since vitamin C is continuously oxidized in the body, the daily requirement of this vitamin is rather high. The recommended daily dose for children is 40 mg and for men and women, 50–60 mg. Formula-fed infants should, however, receive even lower doses,

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i.e., 30 mg of ascorbic acid daily. Lactating mothers should take higher doses, *i.e.*, 100 mg daily. According to a report published by the British Nutrition Foundation, the RDA for vitamin C on which most nutritionists base diets, is far too low. The 30 mg daily that is usually recommended in most countries is way below the United States at 60 mg, Germany at 75 mg and Russia at 100 mg. Ante diluvian though it sounds, the figure of 30 mg is based on the amount of vitamin C needed to prevent that scourge of seafarers, scurvy. The Nutrition Foundation believes that a more contemporary approach to vitamin C would be to consider at what level it actually promotes good health. Besides, vitamin C requirement in humans may vary with the time of day and time of year. Early autumn, for example, is when most people's vitamin C levels are at their lowest.

H. Treatment : Scurvy is prevented by a diet rich in ascorbic acid; citrus fruits and juices are excellent sources. The administration of orange juice or tomato juice daily will quickly produce healing but ascorbic acid is preferable. The daily therapeutic dose is 100-200 mg or more, orally or parenterally.

CHOLINE

A. History. Choline is an essential component of the diet of animals and is, therefore, usually included among the vitamins. Best, for the first time, pointed out the role of choline in nutrition. He also showed that choline prevented the development of fatty livers in depancreatized dogs.

B. Occurrence. Choline is widely distributed. The richest source is egg yolk. Liver, kidney, meats, cereals and many vegetables such as beans and peanuts are other good sources. It is an important constituent of lecithins.

A **quaternary ammonium compound** is that in which N bears four organic substituents.

C. Structure. Choline ($C_5H_{15}O_2N$) is a quaternary ammonium compound, where out of the 4 H atoms, one is replaced by hydroxy ethyl group and the other three by 3 methyl groups (Fig. 34-22).

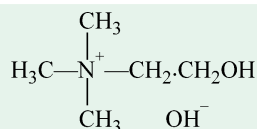


Fig. 34-22. Choline

(trimethylhydroxyethylammonium hydroxide)

D. Properties. Choline is water soluble and has very strong basic properties.

E. Metabolism. Choline can be synthesized in the body by methylation of ethanolamine and, therefore, strictly speaking, this is not a vitamin.

Choline may function in many ways :

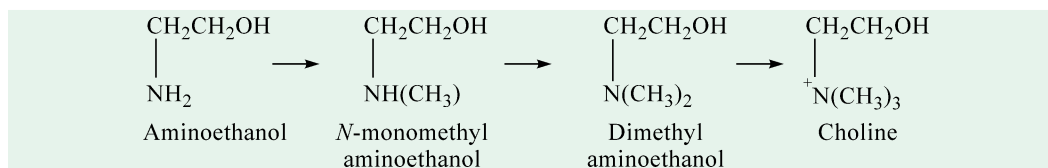
- It is an important constituent of phospholipids like lecithin.
- It undergoes esterification with acetyl-CoA to form acetyl-choline. This is an endergonic reaction, the energy being derived from ATP. Acetylcholine is responsible for the transmission of nerve impulses in the central nervous system (CNS).



- It acts as an important methyl group donor in intermediary metabolism.
- It is an important lipotropic agent and participates in the mobilization of fat from the liver. Its absence, henceforth, causes accumulation of fat in the hepatic tissues.

Using mutants of *Neurospora*, Horowitz has shown that the inability of the fungus to synthesize choline is due to a deficiency in the formation of an intermediate compound, N-monomethyl aminoethanol. The synthesis involves the following steps :

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F. Deficiency. In the deficiency of choline, puppies develop **anorexia**, hens do not lay eggs and mice do not lactate normally. A low-choline diet also develops hemorrhages of the kidneys and eyes, in addition to **fatty livers**, in young rats.

No definite symptoms of choline deficiency have been established in man. However, alcoholic cirrhosis of the liver in man is largely a result of dietary deficiency of many lipotropic agents, of which choline is an important example.

G. Human requirements. In the case of choline, the dietary requirement for human beings has not been established.

INOSITOL

A. History. Woolley (1940) discovered that the mice, when fed on a synthetic diet containing all the known vitamins even, failed to grow and their hair growth was arrested. The addition of pantothenic acid, the absence of which may also cause hair changes, however, proved futile. Neither biotin nor *p*-aminobenzoic acid could also cure them. The curative effects were, however, obtained by the addition of phytin (obtained from cereal grain) or inositol (isolated from liver). Thus, it was established as a vitamin of B group. This is also called as mouse **antialopecia factor**.

Phytin is a mixed calcium and magnesium salt of inositol hexaphosphate (= phytic acid), which is found in plants.

B. Occurrence. Inositol is found in muscles (hence, its nomenclature as **muscle sugar**), liver, kidneys, brain, erythrocytes and tissues of the eye. Among plants, it occurs in fruits, vegetables, whole grains and nuts. Milk and yeast contain appreciable quantities. Inositol is found in nature in at least 4 forms : free inositol, phytin, phosphatidylinositol and a nondialyzable complex. Inositol containing phosphatide or phosphoinositide (= lipositol of Woolley) has been isolated in pure form from soyabeans and is also known to be present in brain and spinal cord.

C. Structure. Inositol, $\text{C}_6\text{H}_{12}\text{O}_6$ or better $\text{C}_6\text{H}_6(\text{OH})_6$, is a carbocyclic hexahydric alcohol. It has 9 possible stereoisomers, of which only one myoinositol (Fig. 34-23), found in muscles, is biologically active and happens to be a symmetric, optically inactive meso-form.

Myoinositol was formerly called **mesoinositol**. This nomenclature is, however, vague since 7 of the 9 isomers are meso-forms.

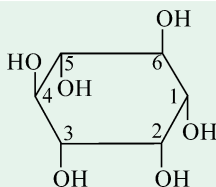


Fig. 34-23. Myoinositol or mesoinositol
(hexahydroxycyclohexane)

D. Properties. Although not a sugar, inositol is sweet in taste. This is, in fact, a common property to many polyatomic alcohols including glycerol. Inositol is soluble in water.

E. Metabolism. Inositol as phosphoinositide helps in transport processes in cells.

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Inositol stimulates the growth of many microorganisms such as *Saccharomyces cerevisiae* and *Neurospora*.

It also acts as a lipotropic agent and prevents the formation of fatty livers.

Possibly, it is an intermediate between carbohydrates and aromatic substances.

F. Deficiency. Inositol deficiency results in **retarded growth** and a peculiar hairlessness in mice. Lack of inositol also causes insufficient lactation in experimental animals. Deficiency of inositol, however, does not occur in man.

G. Human requirements. The amount of inositol needed by man is not known.

PARA-AMINO BENZOIC ACID

A. History. Ansbacher demonstrated that depigmentation of the hair (achromotrichia) in mouse could be cured by feeding rice polishings or by adding *p*-aminobenzoic acid (PABA) to the diet. PABA also appeared essential for the growth of rat and chick and also for bacterial multiplication.

B. Occurrence. PABA is widely distributed in nature. The good sources are liver, yeast, rice bran and whole wheat. PABA occurs in conjugated form as a part of folic acid and its derivatives (see page 1003).

C. Structure. The structural formula of *p*-aminobenzoic acid appears on page 390.

D. Properties. PABA is a white crystalline substance. It is sparingly soluble in cold water but freely soluble in hot water and alcohol.

E. Metabolism. Woods, for the first time, observed that PABA blocks the bacteriostatic properties of sulfanilamide (refer page 390). PABA is synthesized from shikimic acid *via* chorismic acid.

F. Deficiency. The deficiency of PABA affects adversely the growth and the maintenance of a normal fur coat in rats.

G. Human requirements. There is, at present, no evidence that PABA is an essential dietary factor for man.

ALPHA-LIPOIC ACID

A. History. Lipoic acid is a relatively newly-discovered factor. This factor supports the growth of a number of bacteria and protozoa. It has been variously called as **pyruvate oxidation factor (POF)** or **acetate replacement factor** or **protogen**. Lester J. Reed *et al* (1951) isolated this factor in crystalline form from the insoluble residue of liver. When first isolated, lipoic acid was believed to be a B-vitamin because of its coenzyme function. However, the current opinion is in favour of treating lipoic acid as a *pseudovitamin* since it is synthesized by most animals.

Such small quantities of **lipoic acid** are present in tissues that 10 tons of water-insoluble liver residue were used by Reed *et al* to obtain about 30 mg of the biomolecule.

B. Occurrence. It is found in many biologic materials including yeast and liver.

C. Structure. α -lipoic acid ($C_8H_{14}O_2S_2$) is a cyclic disulfide, derived from 6, 8-dimercapto-*n*-caprylic acid (Fig. 34-24).

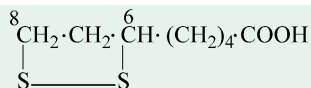


Fig. 34-24. α -lipoic acid or thioctic acid
(6,8-dithiooctanoic acid)

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D. Properties. Lipoic acid is an exception of the vitamins of B series in that *it is fat-soluble rather than water-soluble*.

E. Metabolism. Lipoic acid acts as a catalytic agent for the oxidative decarboxylation of pyruvic acid and α -ketoglutaric acid by certain microorganisms. It is probably a coenzyme or part of a coenzyme, called as lipothiamide pyrophosphate (LTPP), for this reaction.

F. Deficiency. A deficiency of lipoic acid usually does not occur since it is synthesized by most animals.

G. Human requirements. α -lipoic acid is not an essential factor of the diet.

CARNITINE

A. History. Carnitine has long been known as a constituent of meat extractives. Its vitamin nature was first recognized when it was shown to be an essential food factor of certain insects such as the yellow mealworm, *Tenebrio molitor*. It was, however, first isolated from muscles.

B. Occurrence. Carnitine is widely distributed in most tissues including plants, animals and microorganisms. It occurs in the free state and also bound to lipid.

C. Structure. Carnitine (Fig. 34–25) is a betaine (pronounced as ‘bay-tah-een’).

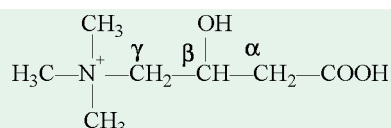
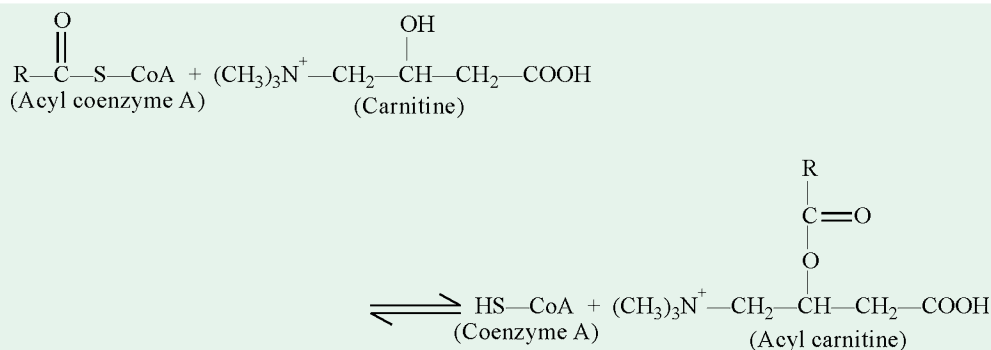


Fig. 34–25. Carnitine

(β -hydroxy- γ -butyrobetaine)

D. Metabolism. Fatty acids are activated on the outer mitochondrial membrane whereas they are oxidized in the mitochondrial matrix. Since the inner mitochondrial membrane is impermeable to long-chain acyl CoA molecules, a special transport mechanism is needed for them. It has been suggested that *carnitine acts as a carrier of the activated long-chain fatty acids across the inner mitochondrial membrane*. The acyl group is transferred from the S atom of acyl CoA to the OH group of carnitine to produce acyl carnitine, which traverses the inner mitochondrial membrane. In the mitochondrial matrix, the acyl group from acyl carnitine is transferred back to CoA so as to regenerate acyl CoA and free the carnitine. As the value of K is near 1, the O-acyl bond of carnitine is a high-energy bond. These transacylation reactions are reversible and are catalyzed by *fatty acyl CoA : carnitine fatty acid transferase*.



The postulated mechanism for the role of carnitine has been outlined in Fig. 34–26.

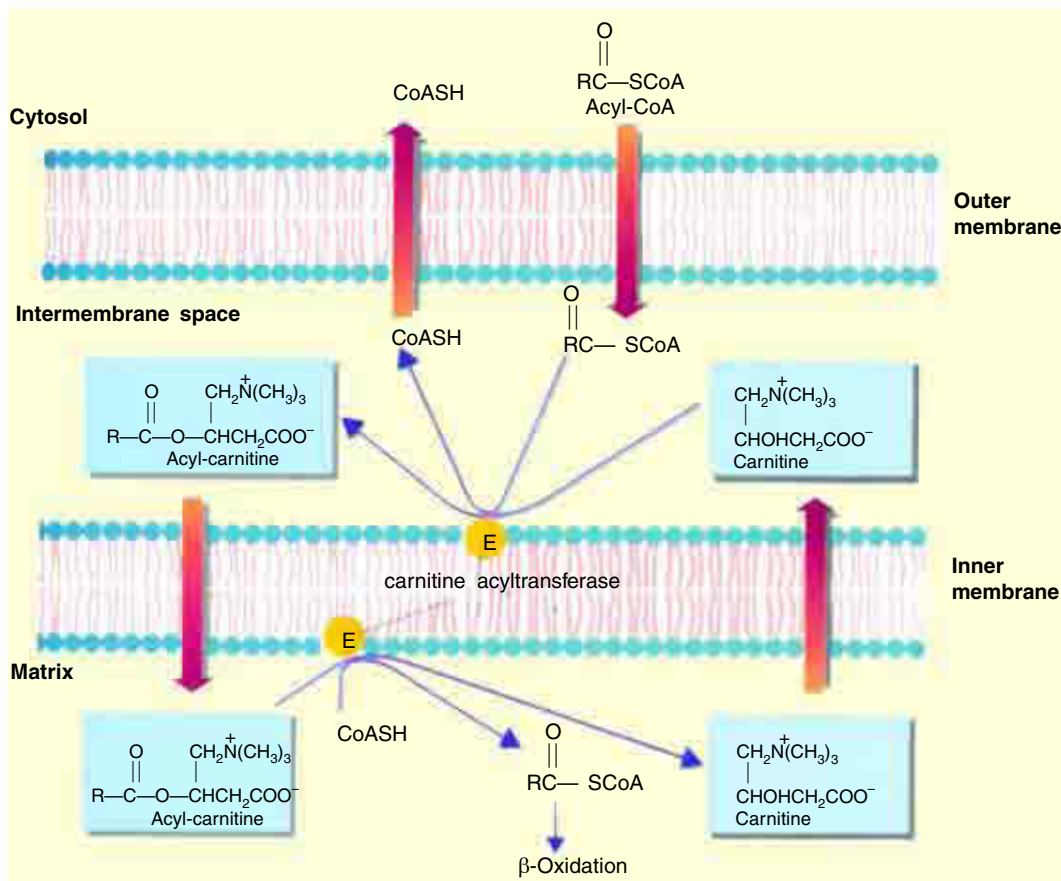


Fig. 34–26. The role of carnitine in the transfer of acyl groups to the mitochondrial matrix

[The acyl groups of acyl-CoA from intramitochondrial compartment are shuttled off to extramitochondrial compartment.]

BIOFLAVONOIDS

A. History. Albert Szent-Györgyi and his associates, in 1936, reported the presence, in lemon peel, of a material which they named **citrin**. It consisted of a mixture of flavonoids and was shown to be associated with the maintenance of normal capillary permeability and fragility. The active principle in citrin was found to be **hesperidin**. It shows physiologic roles similar to those exhibited by other structurally-related compounds such as flavanones, flavones and flavonols. The term **vitamin P** (for permeability) was at first assigned to this group of compounds. They are now more commonly referred to as **bioflavonoids**.

B. Occurrence. The bioflavonoids are widely distributed in nature. *They are always of plant origin.* They are present in the juice, peel and pulp of citrus fruits, in tobacco leaves, in buckwheat (*Fagopyrum esculentum*), in grapes and in many other fruits and vegetables.

C. Structure. Hesperidin is 5,3'-dihydroxy-4'-methoxy-7-rhamnoglucosidoflavanone. Its aglycone hesperitin, rutin (5,7,3',4'- tetrahydroxy-3-glucorhamnosidoflavone) and its aglycone, quercetin all have comparable physiologic roles.

D. Properties. The bioflavonoids are water-soluble.

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E. Metabolism. The bioflavonoids act as antioxidants and thus protect ascorbic acid from oxidative destruction (Clemetson and Andersen, 1966). The effect is indirect due to the chelation of heavy metal ions (Cu^{2+} etc.,) that catalyze oxidative degradation of ascorbic acid. Bioflavonoids, thus, decrease oxidative losses of ascorbic acid from foods during storage or in intestinal tract.

F. Deficiency. Bioflavonoid deficiency in animals results in a syndrome characterized by increased capillary permeability and fragility. In man, however, the deficiency symptoms have not been observed.

G. Human requirements. The dietary allowances for man are not known.

VITAMERS (= ISOTELS)

The various forms of any vitamin are referred to as **vitamers**. Williams, however, prefers to call these as isotels or isotelic vitamins, since the name vitamer is misleading. Although the isotels, in general, are not the isomers but a few of them may be isomers. *All the fat-soluble vitamins and a few water-soluble vitamins (vitamins B_5 and B_6) have isotels.* The various isotelic forms of a vitamin may differ with respect to either the β -ionone ring (vitamin A), the side chain attached at carbon 17 of the steroid nucleus (Vitamin D), the substituents present at carbon atoms 6, 7 and 8 in the chroman ring (vitamin E) or the side chain attached at carbon 3 of naphthoquinone radical (vitamin K). The study of isotels helps in a better understanding of the various physiologic functions which the vitamins perform.

Table 34-4. Water-soluble vitamins

Vitamin	Common name(s)	Chemical name	Sources	Metabolic functions	Deficiency diseases(s)*	Daily requirement of man
B ₁	Antiberberi factor or Antineuritic factor	Thiamine or Aneurin C ₁₂ H ₁₇ ON ₄ S	All plant and animal foods; Cereals, heart, liver, kidney and milk	Thiamine pyrophosphate (TPP) as coenzyme in many decarboxylation reactions	Beriberi (in man); Polyneuritis (in birds)	1.4–1.7 mg
B ₂	'Yellow enzyme'	Riboflavin or Lactoflavin C ₁₇ H ₂₀ O ₆ N ₄	Milk, liver, kidney, heart and green vegetables; Occur almost exclusively as a constituent of either FMN or FAD	Phototropic curvature of plant organs; FAD as a cofactor in respiration; Also in bioluminescence	Glossitis; Cheilosis; Corneal vascularization; Dermatitis	1.4–1.7 mg
B ₃	Filtrate factor or Yeast factor	Pantothenic acid C ₉ H ₁₇ O ₅ N	Yeast, liver and eggs; Also fruits, vegetables and skimmed milk; In most animal tissues, it occurs as its coenzyme	Participates in the formation of coenzyme A	Adrenal cortical insufficiency in man; Depigmentation of hair in rats, pigs and dogs; Depigmentation of feathers in chicks	5.0–10.0 mg
B ₅	Pellagra-preventive factor or Antiblacktongue factor	Niacin, C ₆ H ₅ O ₂ N Niacinamide, C ₆ H ₆ ON ₂	Yeast, liver, Meat, poultry, vegetables and fruits; Milk and eggs devoid of niacin	As a constituent in two pyrimidine nucleotide coenzymes, NAD and NADP	Pellagra (in man); Blacktongue (in dogs)	15–20 mg
B ₆	Antidermatitis factor	Pyridoxine C ₈ H ₁₁ O ₃ N Pyridoxal, C ₈ H ₉ O ₃ N Pyridoxamine, C ₈ H ₁₂ O ₂ N ₂	Cereal grams, yeast, egg yolk and meat ; Pyridoxal and pyridoxamine also occur as their phosphates	Serve as growth factor to a number of bacteria; Also act as a carrier in active transport of amino acids across cell membranes	Convulsions and anemia in human infants; Acrodyndia in rats; Anemia and nervous lesions in dogs and chicks	2.0 mg

Table 34-4. (Cont'd)

Vitamin	Common name(s)	Chemical name	Sources	Metabolic functions	Deficiency diseases(s)*	Daily requirement of man
B ₇	Antiegg white injury factor or Coenzyme R	Biotin C ₁₀ H ₁₆ O ₃ N ₂ S	Yeast, liver, peanuts and eggs	As a prosthetic group for many enzymes which bring about carboxylation and synthesis of fatty acids	Very rare; Sterilization of intestine, however, may lead to dermatitis, loss of hair and edema	25 mg
B ₉	Liver Lactobacillus casei factor	Folic acid or folacin C ₁₉ H ₁₉ O ₆ N ₇	Liver, kidney, yeast and wheat	Enzymatic synthesis of purines, pyrimidines and amino acids	Megaloblastic anemia (in man); Macrocytic anemia (in monkey)	1.0–2.0 mg
B ₁₂	Anti-pernicious anemia factor (APA factor)	Cyanocobalamin C ₆₃ H ₈₈ O ₁₄ N ₁₄ PCo	Animals and microorganisms only; Plants devoid of it	Nucleic acid metabolism	Pernicious anemia	1.0 mg
C	Antiscorbatic factor or Cevitamin	Ascorbic acid or Cevitamic acid C ₆ H ₈ O ₆	Fresh fruits and vegetables- Acerola fruit, citrus fruit, tomatoes, green pepper and new potatoes	Reducing agent; Biosynthesis of adrenal steroid hormones; Synthesis of collagen	Scurvy	75–100 mg
P	Bioflavonoids	Hesperidin	Of plant Origin-juice, peel and pulp of citrus fruits, in grapes etc.	As antioxidant; Maintenance of capillary permeability and fragility	Hemorrhage (in animals)	Not known
Choline	–	Trimethyl-hydroxyethyl-ammonium hydroxide	Widely distributed; Richest source is egg yolk; liver, kidney, meat and cereals are other good sources.	Part of phospholipids; A lipotropic agent; Acts as a methyl group donor.	Anorexia (in puppies); Fatty liver (in rats)	Not known

*Deficiency diseases have been mentioned in relation to man, if not stated otherwise.

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PROBLEMS

- 1.** Individuals with thiamine deficiency display a number of characteristic neurological signs: loss of reflexes, anxiety, and mental confusion. Suggest a reason why thiamine deficiency is manifested by changes in brain function.
- 2.** The neurological disorders seen in vitamin B₁₂ deficiency are caused by progressive demyelination of nervous tissue. How does lack of B₁₂ interfere with formation of the myelin sheath ?
- 3.** What should a person eat to get all the vitamin C his body can use ?
- 4.** Scurvy is to vitamin C as fatty liver is to :
 - (a) vitamin K
 - (b) pantothenic acid
 - (c) riboflavin
 - (d) choline
- 5.** If maize is the staple diet, the deficiency to develop is likely to be that of :
 - (a) cholecalciferol
 - (b) nicotinic acid
 - (c) pyridoxine
 - (d) thiamine
- 6.** Macrocytic anemia can result from the deficiency of :
 - (a) folic acid
 - (b) vitamin B₂
 - (c) inositol
 - (d) vitamin B₁₂
- 7.** If you are a complete vegetarian, do you miss any vitamins ?
- 8.** Flour is often enriched with :
 - (a) vitamin A
 - (b) vitamin B₁
 - (c) vitamin B₁₂
 - (d) vitamin E
- 9.** Can B vitamins cut the risk of developing Alzheimer's disease ?
- 10.** Rain drops contain which of the following vitamins :
 - (a) vitamin A
 - (b) vitamin B₂
 - (c) vitamin B₁₂
 - (d) vitamin D
- 11.** Can vitamins help in the treatment of depression ?
- 12.** Why do doctors prescribe vitamin tablets along with antibiotics ?