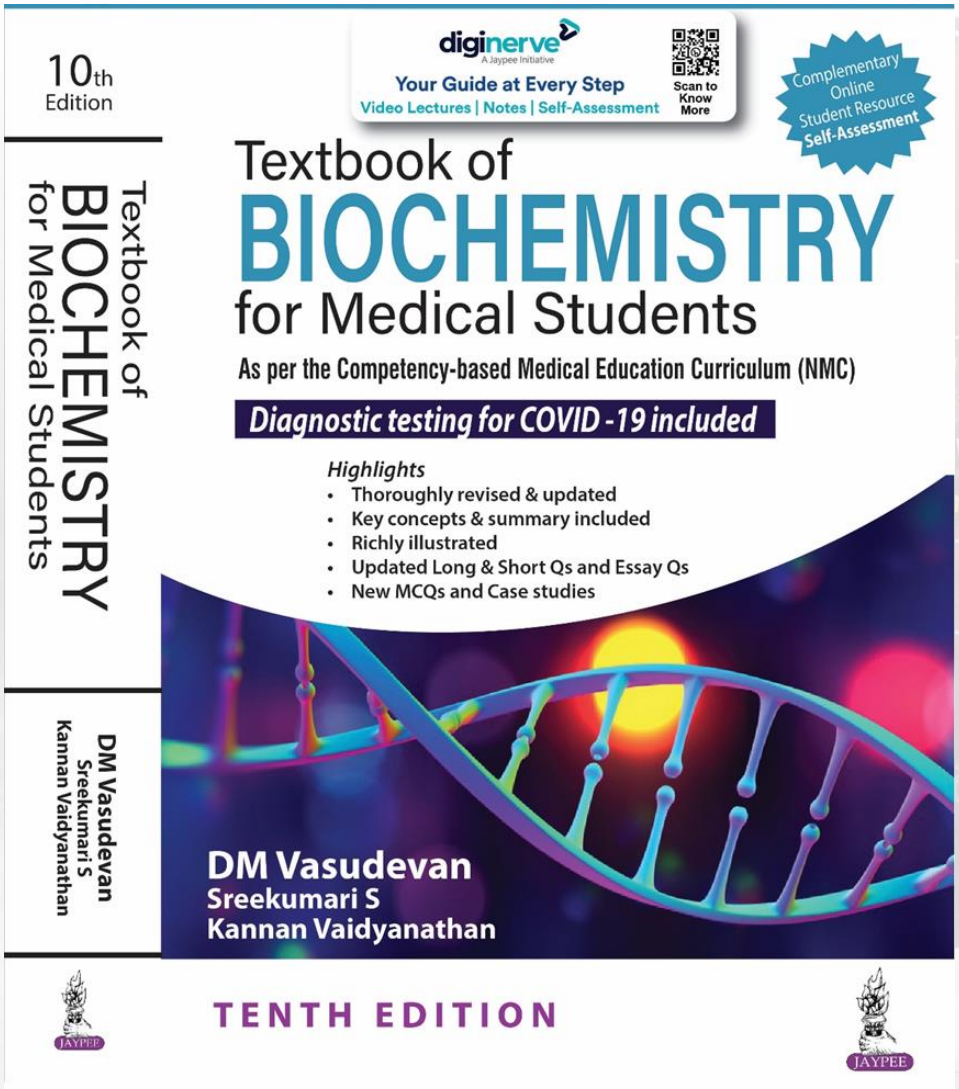
Chapter 29:

Water Soluble Vitamins (Thiamine, Riboflavin, Niacin, Pyridoxine, Pantothenic acid, Biotin, Folic Acid, Vitamin B12 and Ascorbic Acid)



Textbook of
BIOCHEMISTRY
for Medical Students
By DM Vasudevan, *et al.*

TENTH EDITION

Role of B complex coenzymes in TCA cycle

Enzyme	Reaction	Coenzymes used
Pyruvate dehydrogenase	Pyruvate → acetyl-CoA	TPP, FAD, NAD^+, Coenzyme A, Lipoamide
Isocitrate dehydrogenase	Isocitrate → alpha-ketoglutarate	NAD^+
Alpha-ketoglutarate dehydrogenase	Alpha-ketoglutarate → succinyl CoA	TPP, FAD, NAD^+, Coenzyme A, Lipoamide
Succinate dehydrogenase	Succinate → fumarate	FAD
Malate dehydrogenase	Malate → oxaloacetate	NAD^+

Role of B complex coenzymes in TCA cycle

Enzyme	Reaction	Coenzymes used
Alanine amino-transferase	Alanine → pyruvate	PLP
Aspartate amino-transferase	Aspartate → oxaloacetate	PLP
Pyruvate carboxylase	Pyruvate → oxaloacetate	Biotin
Propionyl-CoA carboxylase	Propionyl-CoA —> D-methyl malonyl-CoA	Biotin
Methyl malonyl-CoA mutase	L-methyl malonyl-CoA —> Succinyl-CoA	Vitamin B_{12}

THIAMINE (Vitamin B1)

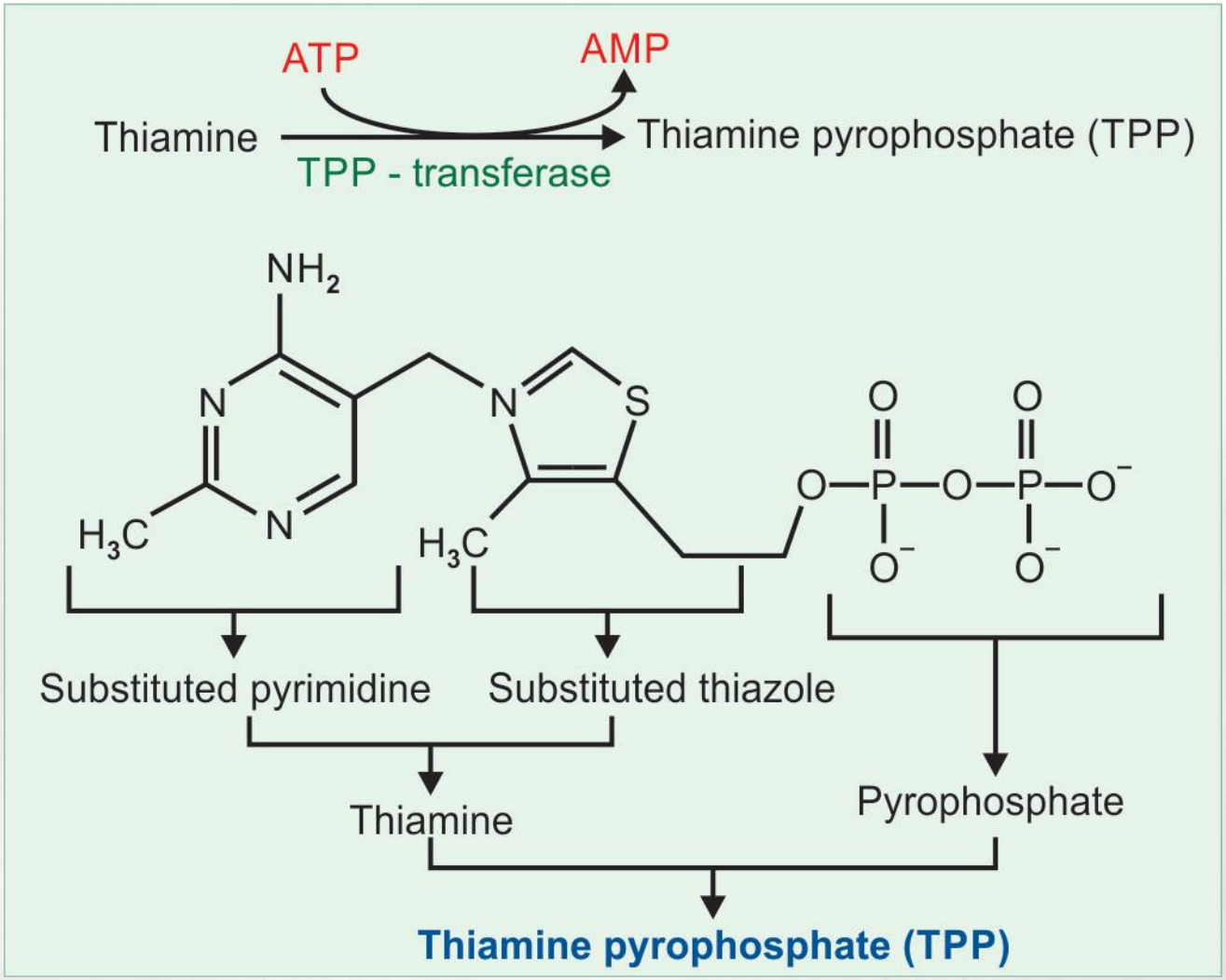
Sources

Aleurone layer of cereals (food grains) is a rich source of thiamine. Therefore, whole wheat flour and unpolished handpound rice have better nutritive value than completely polished refined foods.

Thiamine is partially destroyed by heat.

Structure of Thiamine

Thiamine contains a **pyrimidine** ring and a thiazole ring. The vitamin is then converted to its active coenzyme form by addition of two phosphate groups, by thiamine pyrophosphate transferase.

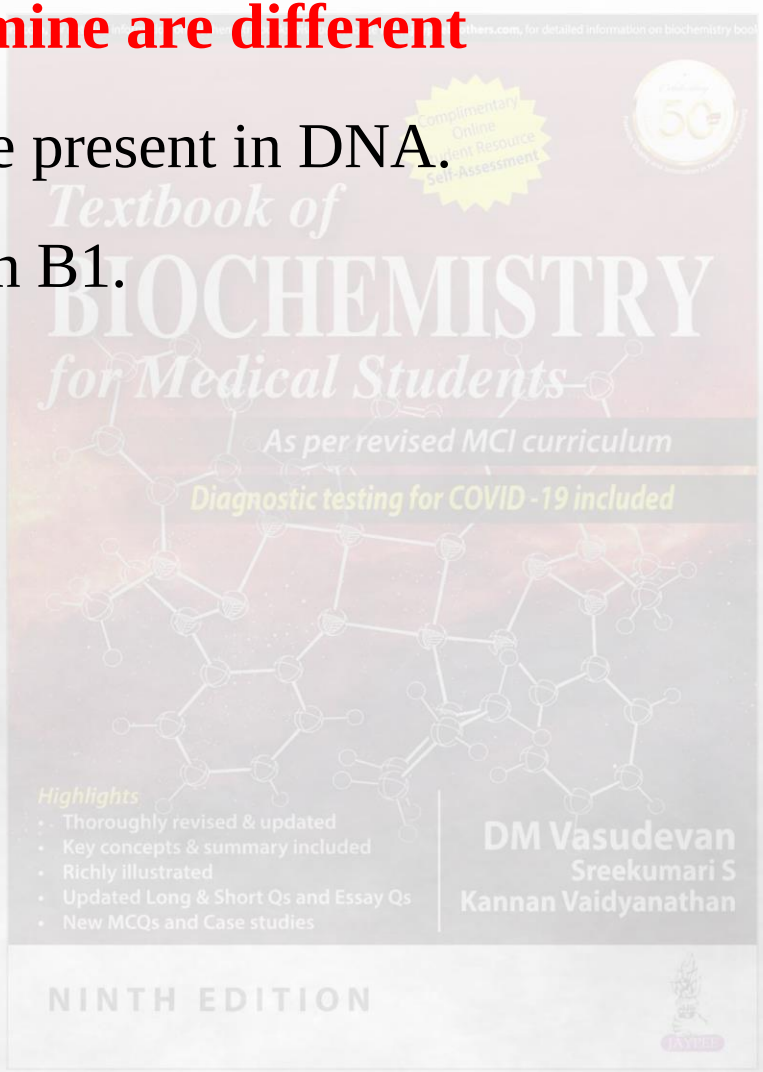


Structure of thiamine pyrophosphate.

Thiamine and thymine are different

Thymine is the base present in DNA.

Thiamine is vitamin B1.



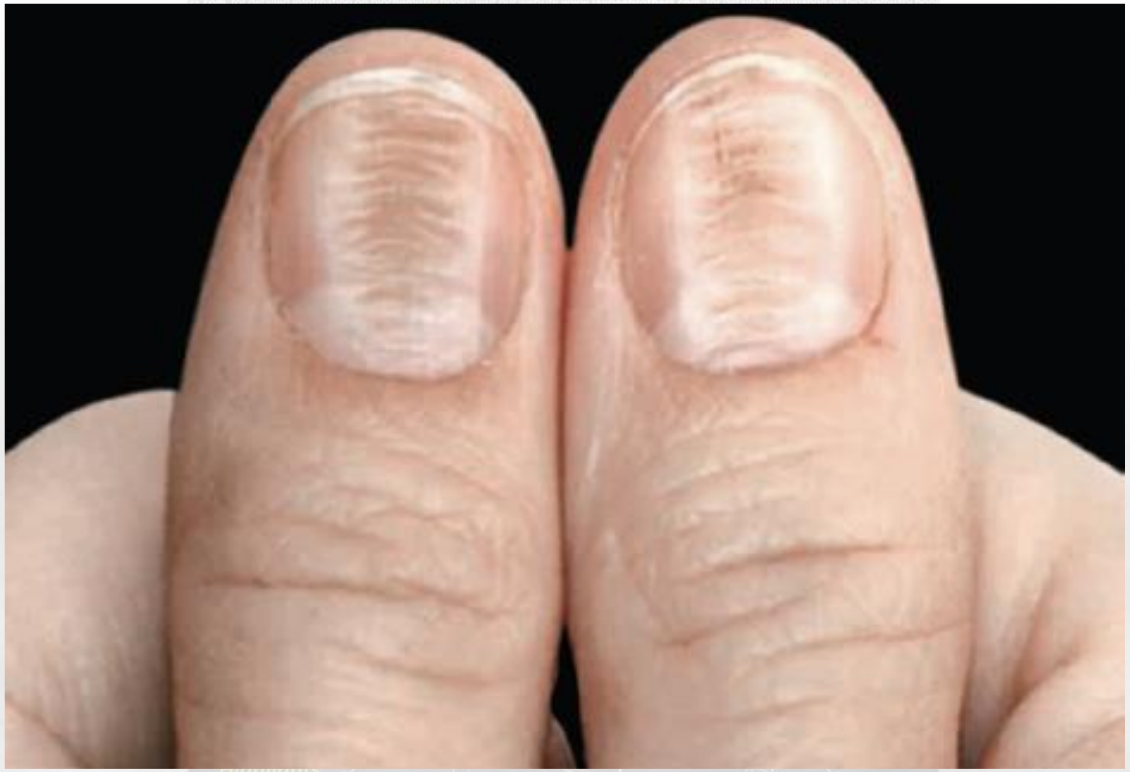
Physiological Role of Thiamine

1. **Pyruvate dehydrogenase:** The coenzyme form is **thiamine pyrophosphate** (TPP). It is used in oxidative decarboxylation of alpha-keto acids, e.g. pyruvate dehydrogenase catalyzes the breakdown of pyruvate, to acetyl-CoA, and carbon dioxide.
2. **Alpha-ketoglutarate dehydrogenase:** requires TPP is the oxidative decarboxylation of **alpha-ketoglutarate** to succinyl-CoA and CO2.
3. **Transketolase:** in the hexose monophosphate shunt pathway of glucose.
4. The main role of thiamine (TPP) is in **carbohydrate** metabolism. So, the requirement of thiamine is increased along with higher intake of carbohydrates.

Deficiency Manifestations of Thiamine

- **Beriberi:** The symptoms are anorexia, dyspepsia, heaviness and weakness. Subjects feel weak and get easily exhausted.
- **Wet beriberi:** Here cardiovascular manifestations are prominent. Edema of legs, face, trunk and serous cavities are the main features.
- **Dry beriberi: C**entral nervous system (CNS) manifestations are the major features. Peripheral neuritis with sensory disturbance leads to complete paralysis.
- **Infantile beriberi:** It occurs in infants born to mothers suffering from thiamine deficiency. Restlessness and sleeplessness are observed.

- **Wernicke-Korsakoff syndrome:** It is also called as **cerebral beriberi**. Clinical features are encephalopathy (ophthalmoplegia, nystagmus, cerebellar ataxia) plus psychosis.
- **Polyneuritis:** It is common in chronic alcoholics. Alcohol utilization needs large doses of thiamine. Alcohol inhibits intestinal absorption of thiamine, leading to thiamine deficiency.
- Polyneuritis may also be associated with pregnancy and old age.
- Thiamne deficiency may cause impairment of conversion of pyruvate to acetyl-CoA. The result is increased plasma concentration of pyruvate and lactate, leading to **lactic acidosis**.
- Thiamine deficiency is manifested as horizontal ridges in nails.



Horizontal ridges in nails in thiamine deficiency.

Biochemical Parameters

In thiamine deficiency, blood thiamine level is reduced, but pyruvate, alpha-ketoglutarate and lactate are increased. Erythrocyte **transketolase** activity is reduced; this is the earliest manifestation seen even before clinical disturbances.

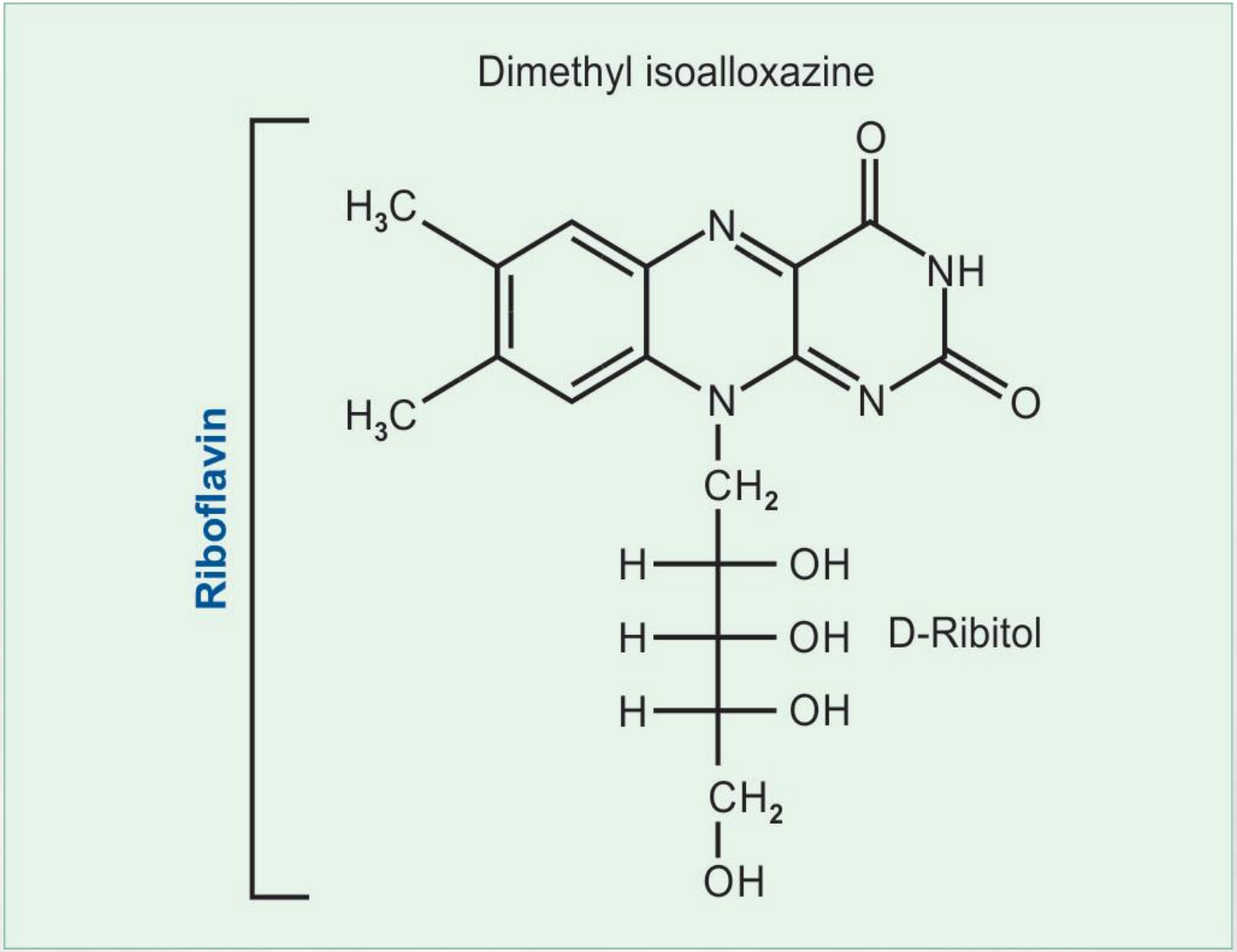
Recommended Daily Allowance of Thiamine

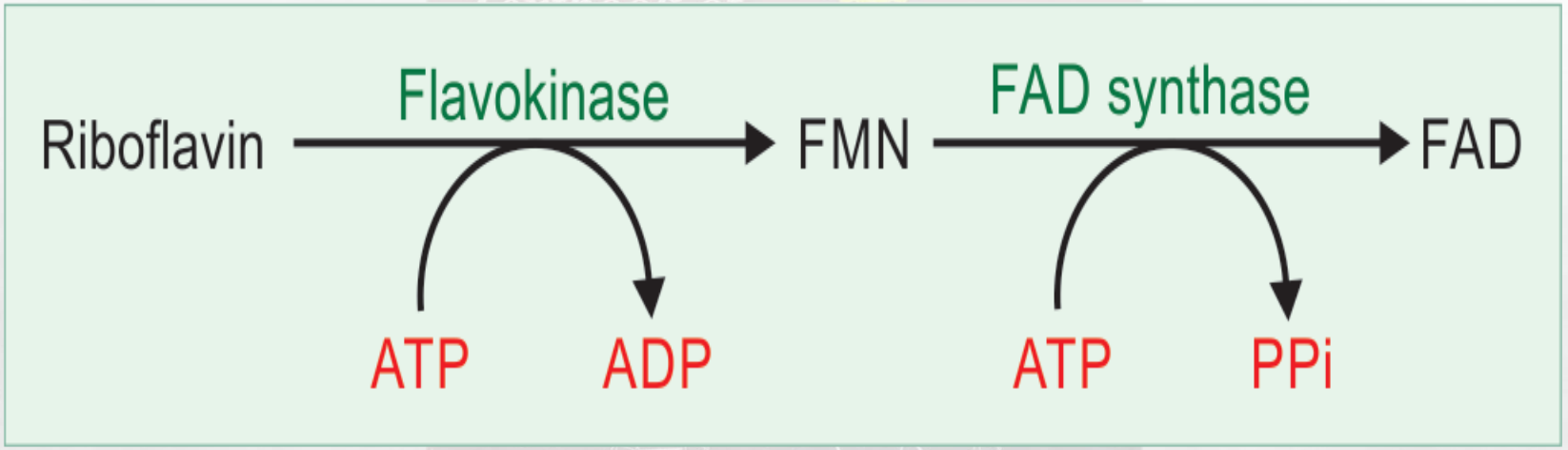
It depends on calorie intake (0.5 mg/1,000 calories).

Requirement is 1.5–2 mg/day.

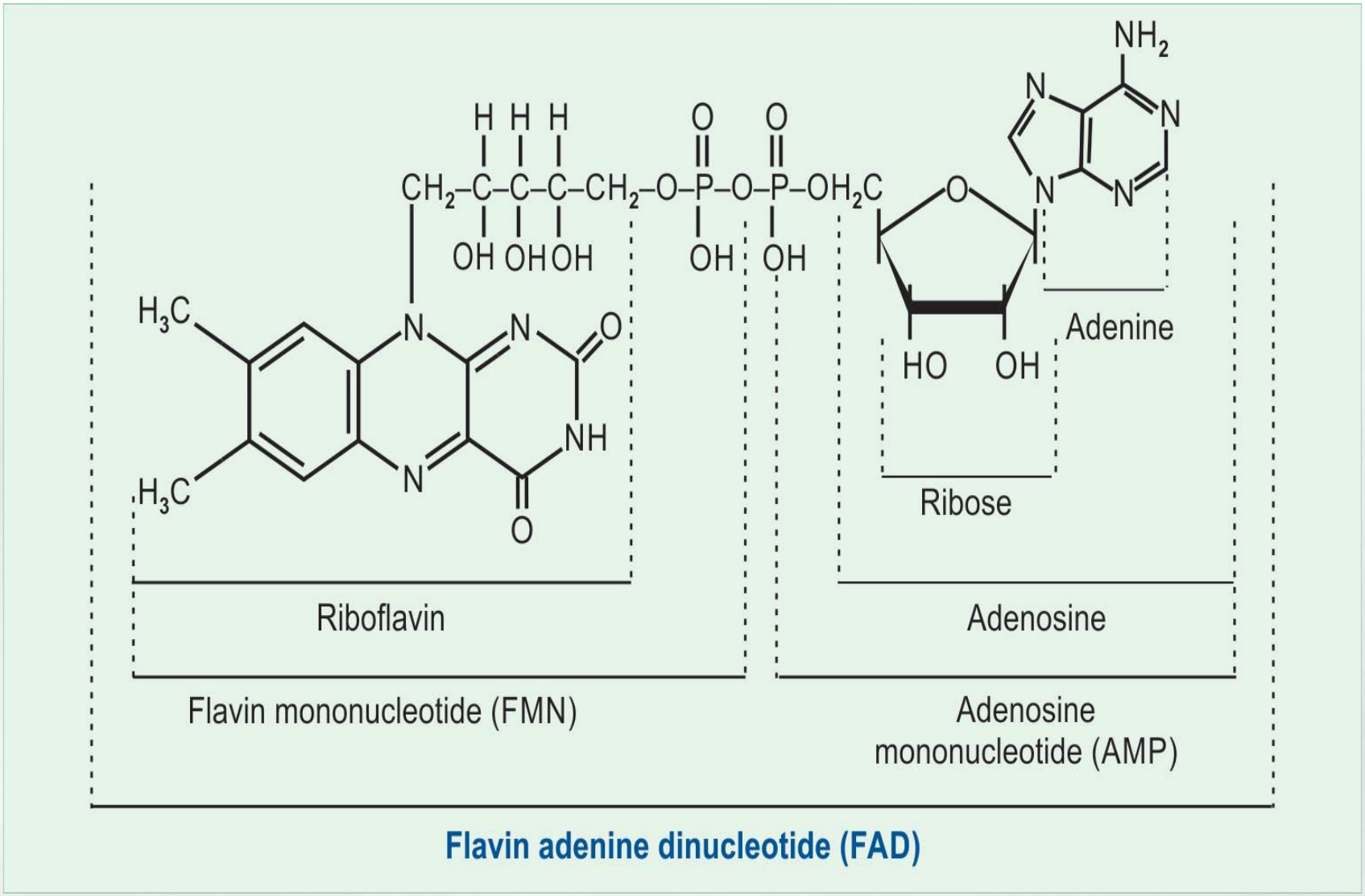
Thiamine is the drug used in the treatment of beriberi, alcoholic polyneuritis, neuritis of pregnancy and neuritis of old age.

Riboflavin (Vitamin B2)

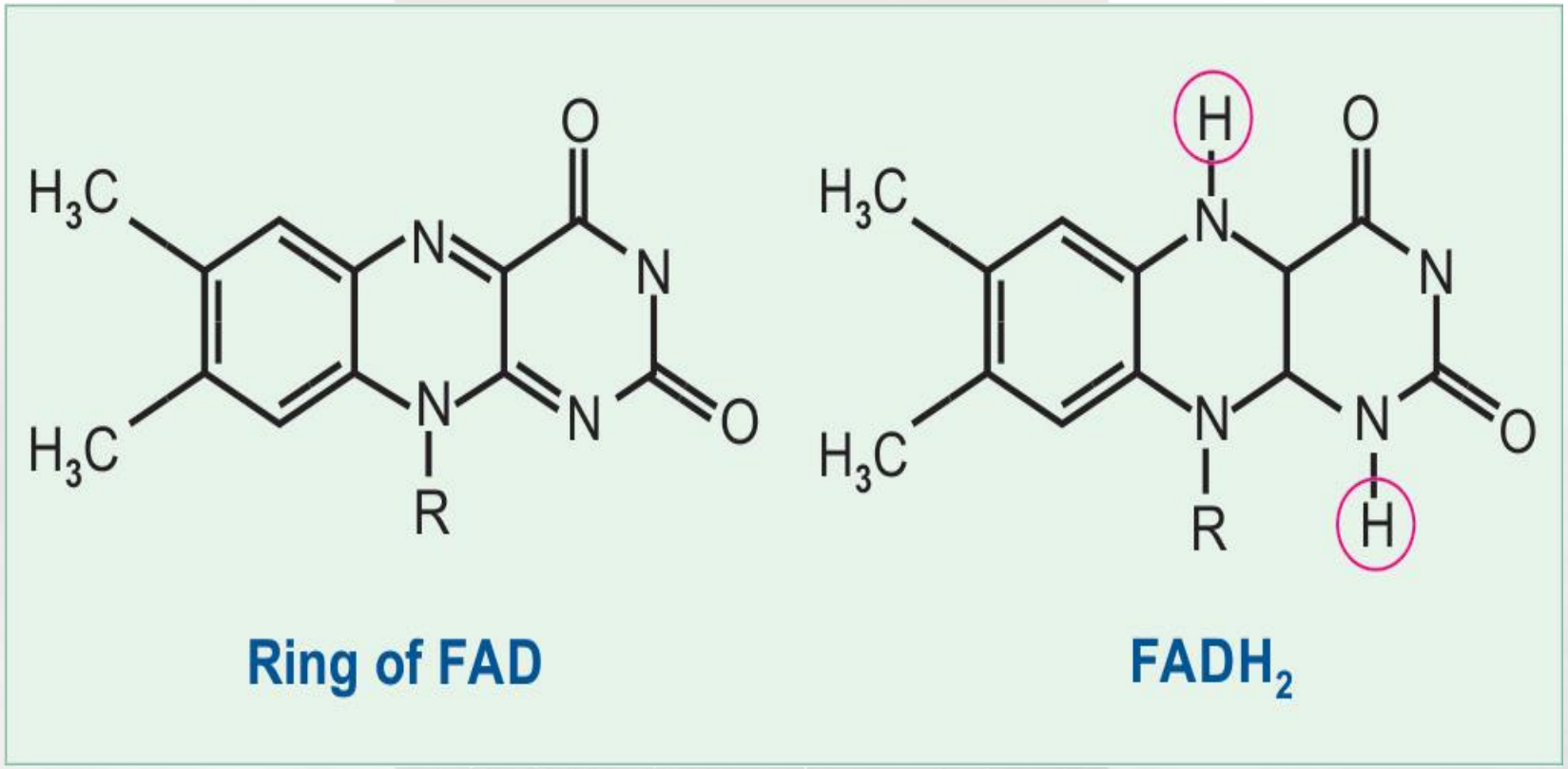




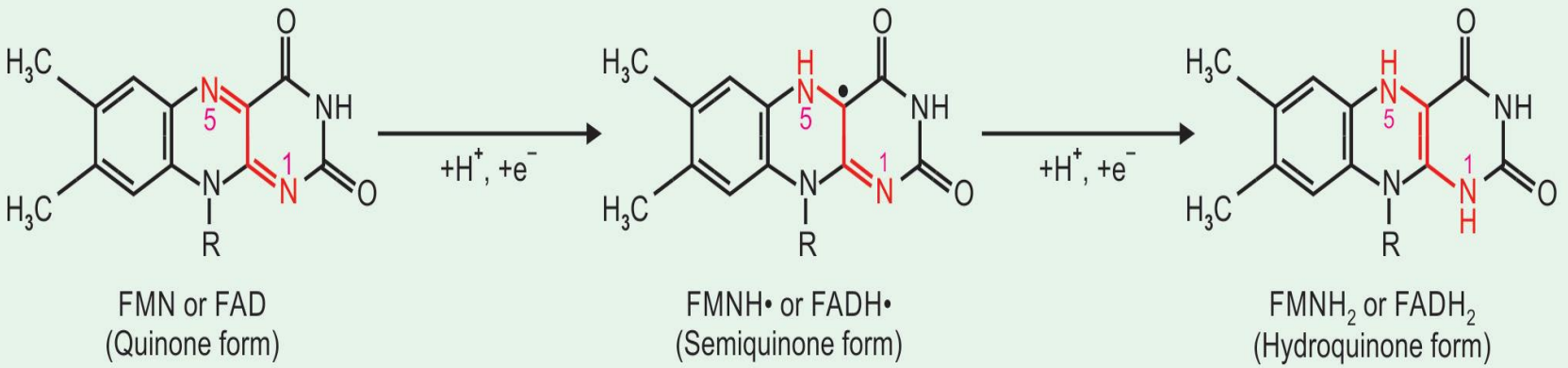
Generation of FMN and FAD with the help of ATP.



Flavin adenine dinucleotide (FAD)



Summary of acceptance of hydrogen by FAD.

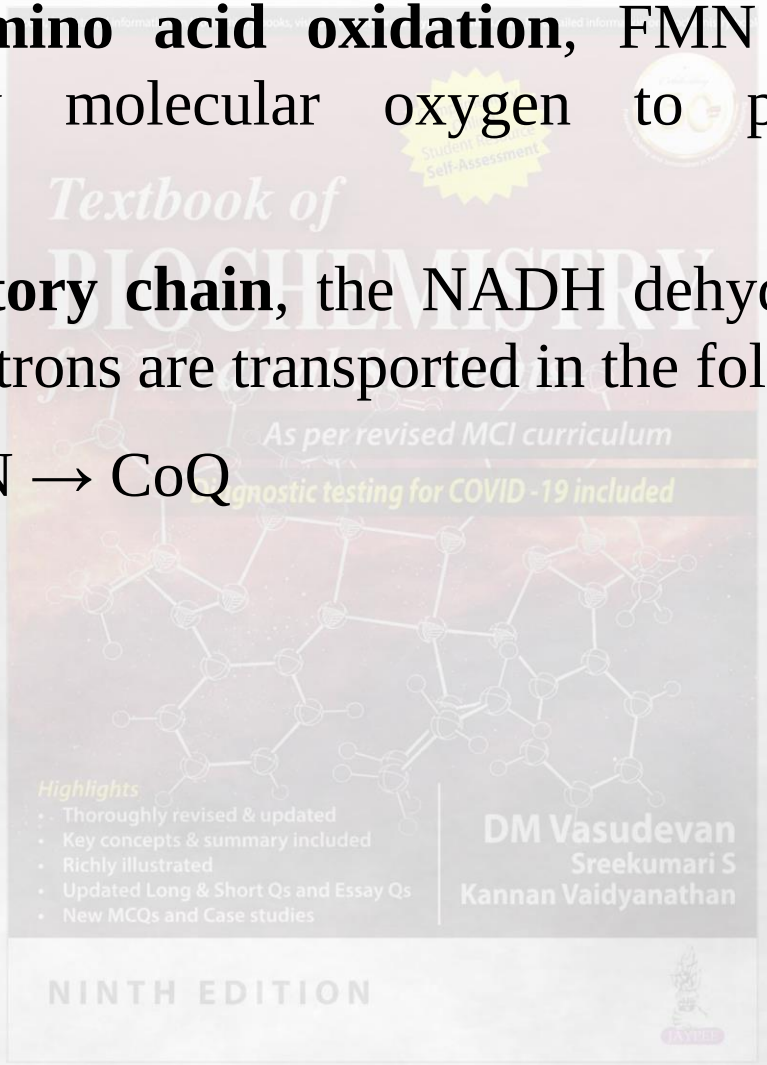


Details of acceptance of hydrogen by FAD.

FMN Dependent Enzymes

1. During the **amino acid oxidation**, FMN is reduced. It is reoxidized by molecular oxygen to produce hydrogen peroxide.
2. In the **respiratory chain**, the NADH dehydrogenase contains FMN. The electrons are transported in the following manner:

   NAD+ → FMN → CoQ



FAD-dependent Enzymes

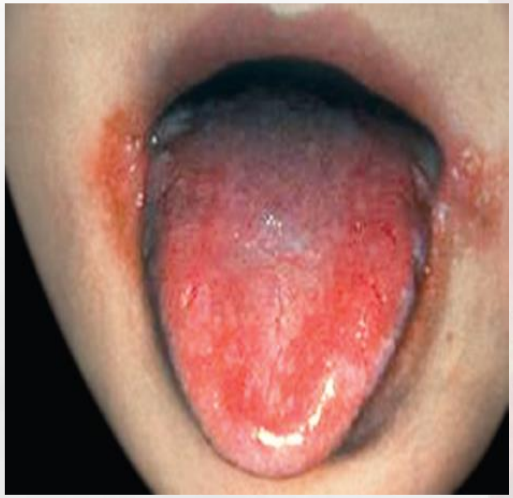
1. Succinate to fumarate by succinate dehydrogenase.
2. Acyl-CoA to alpha-beta unsaturated acyl-CoA by acyl-CoA dehydrogenase.
3. Xanthine to uric acid by xanthine oxidase.
4. Pyruvate to acetyl-CoA by pyruvate dehydrogenase.
5. Alpha-ketoglutarate to succinyl-CoA by alpha-ketoglutarate dehydrogenase.
6. Retinol (vitamin A) to retinoic acid by cytosolic retinal dehydrogenase.
7. Synthesis of 5-methyl tetrahydrofolate from 5,10-methylene tetrahydrofolate by methylene tetrahydrofolate reductase
8. Tryptophan to niacin.

Riboflavin Deficiency

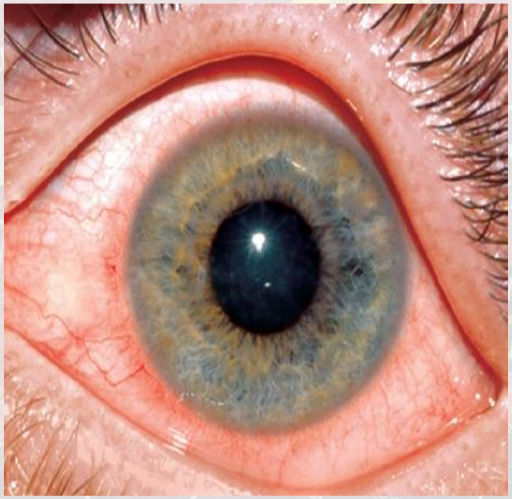
Deficiency of riboflavin in man is uncommon, because riboflavin is synthesized by the intestinal bacteria

Deficiency manifestations:

1. **Glossitis** (glossa = tongue)
2. Magenta colored tongue
3. **Cheilosis** (cheilos = lip) or fissured lip
4. **Angular stomatitis** (inflammation at the corners of mouth)
5. **Circumcorneal vascularization**
6. Proliferation of the bulbar conjunctival capillaries is the earliest sign
7. Normochromic normocytic anemia
8. Irregular ridges in the nails



Glossitis, magenta colored tongue and cheilosis.



Circumcorneal vascularization.

Irregular ridges in the nails in riboflavin deficiency.

Dietary Sources of Riboflavin

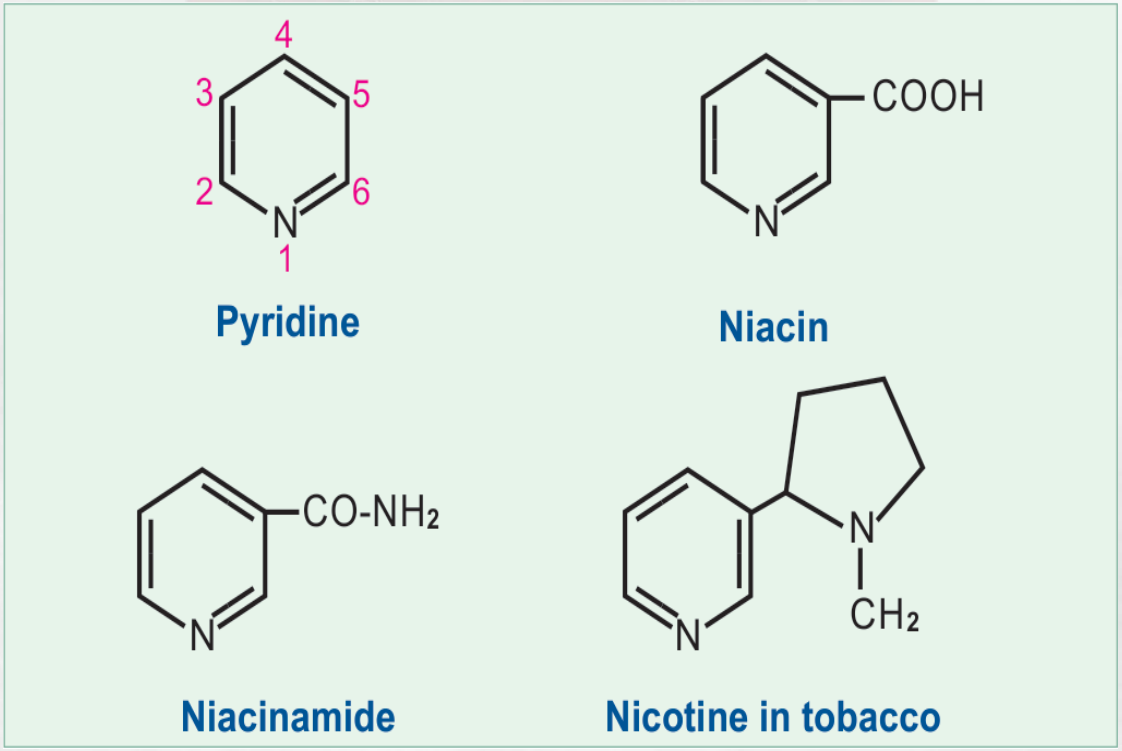
Rich sources are liver, dried yeast, egg and whole milk. Good sources are fish, whole cereals, legumes and green leafy vegetables.

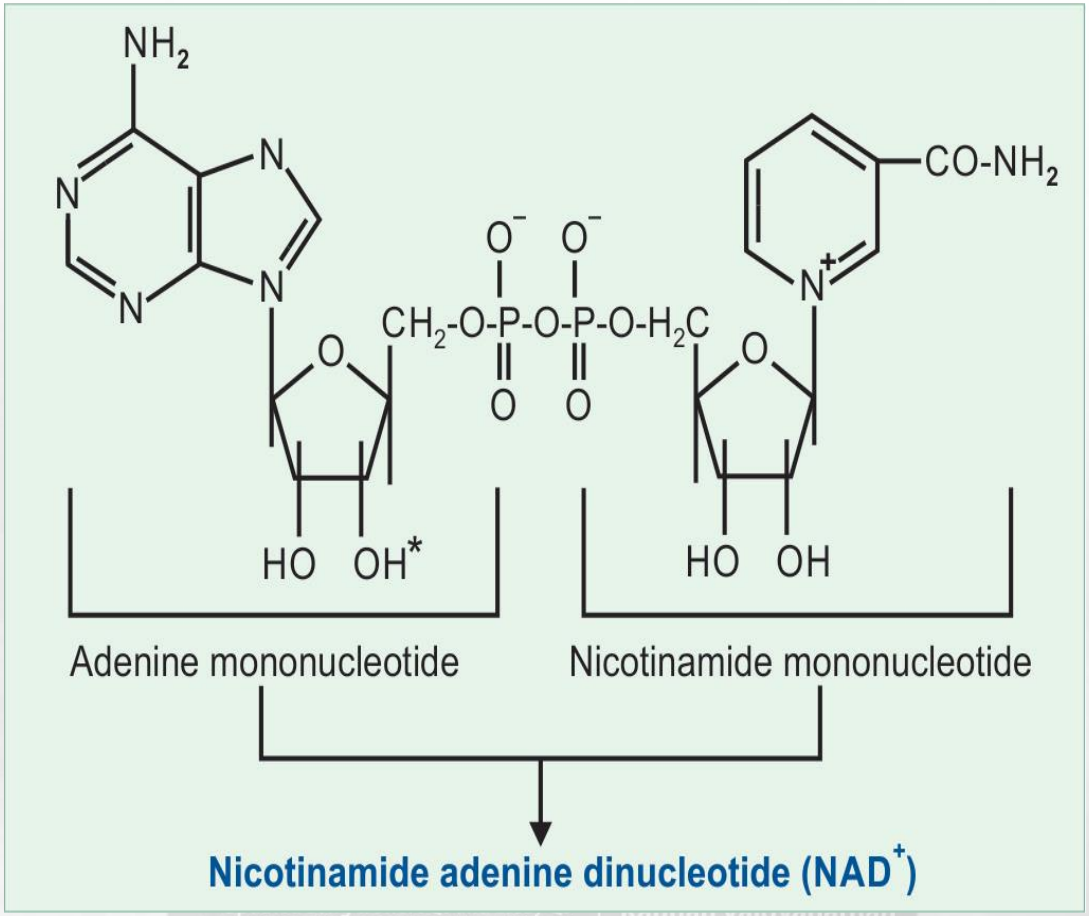
Daily Requirement

- Riboflavin is concerned mainly with the metabolism of **carbohydrates** and requirement is related to calorie intake.
- Adults on sedentary work require about 2 mg per day.
- During pregnancy, lactation and old age, requirement is 3 mg/day.

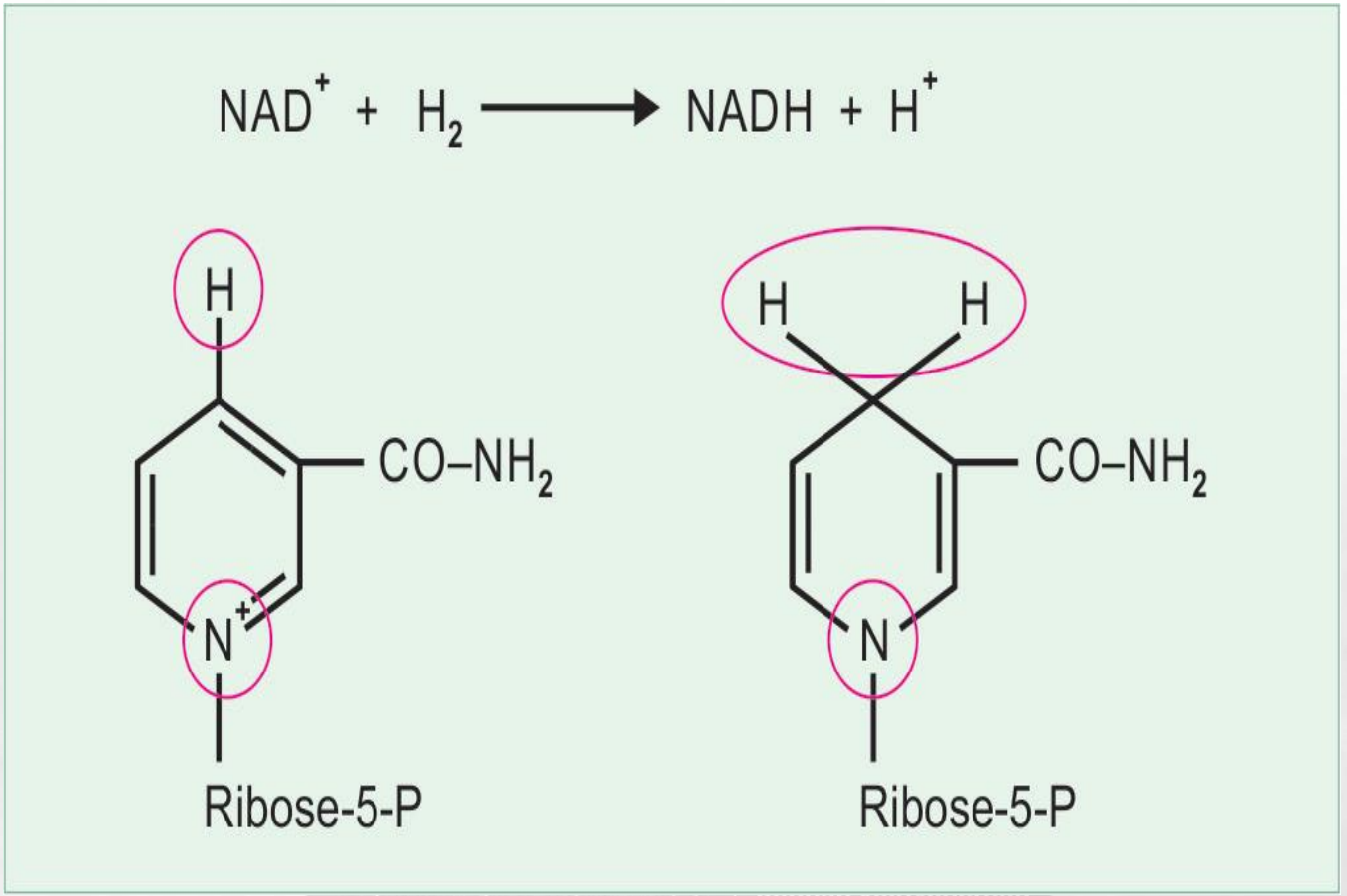
Niacin

Niacin and Nicotinic acid are synonyms. **Niacinamide** is the active form of the vitamin, present in tissues. The term nicotinic acid should not be confused with nicotine. Nicotinic acid is a vitamin; but, nicotine is the potent poison from tobacco.





Structure of NAD+ (In the case of NADP+, phosphoric acid residue is attached to the hydroxyl group marked with asterisk).



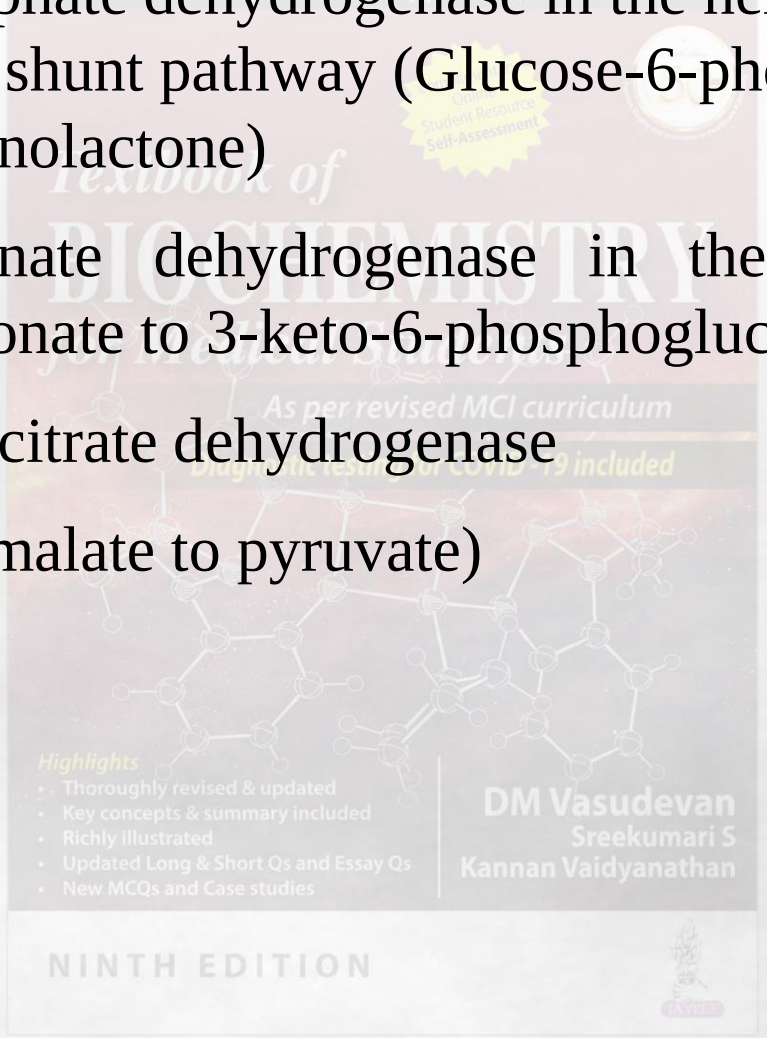
Acceptance of hydrogen by NAD+.

NAD^+ Dependent Enzymes

1. Lactate dehydrogenase (lactate to pyruvate)
2. Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate)
3. Pyruvate dehydrogenase (pyruvate to acetyl-CoA)
4. Alpha-ketoglutarate dehydrogenase (alpha-ketoglutarate to succinyl-CoA)
5. Beta hydroxyacyl CoA dehydrogenase (beta-hydroxyacyl-CoA to beta-ketoacyl-CoA
6. Glutamate dehydrogenase (Glutamate to alpha-ketoglutarate)

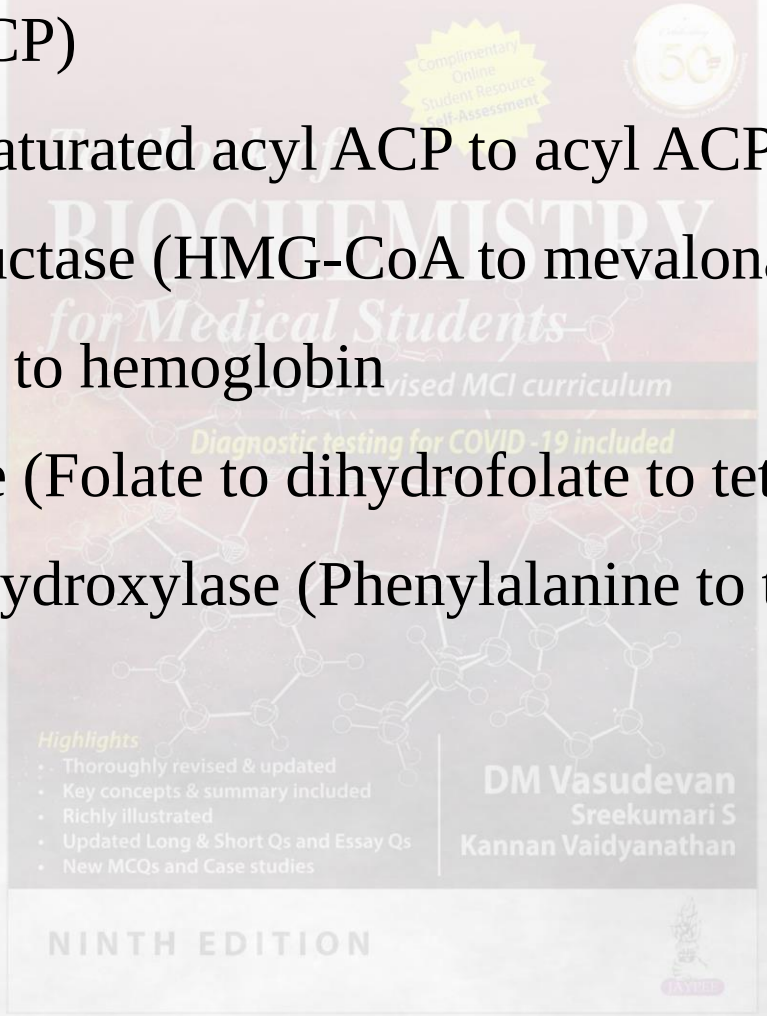
NADPH Generating Reactions

1. Glucose-6-phosphate dehydrogenase in the hexose monophosphate shunt pathway (Glucose-6-phosphate to 6-phosphogluconolactone)
2. 6-phosphogluconate dehydrogenase in the shunt pathway (6-phosphogluconate to 3-keto-6-phosphogluconate)
3. Cytoplasmic isocitrate dehydrogenase
4. Malic enzyme (malate to pyruvate)



NADPH Utilizing Reactions

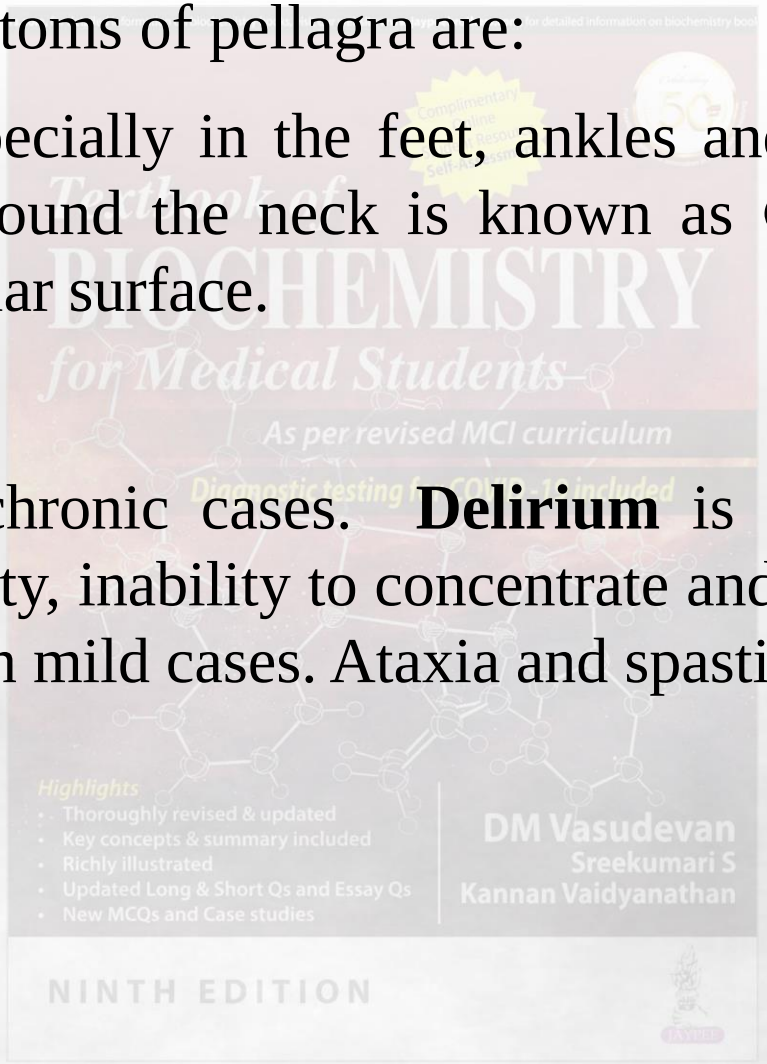
1. Ketoacyl ACP dehydrogenase (Beta ketoacyl ACP to beta hydroxyacyl-ACP)
2. Alpha, beta unsaturated acyl ACP to acyl ACP
3. HMG-CoA reductase (HMG-CoA to mevalonate)
4. Methemoglobin to hemoglobin
5. Folate reductase (Folate to dihydrofolate to tetrahydrofolate)
6. Phenylalanine hydroxylase (Phenylalanine to tyrosine)

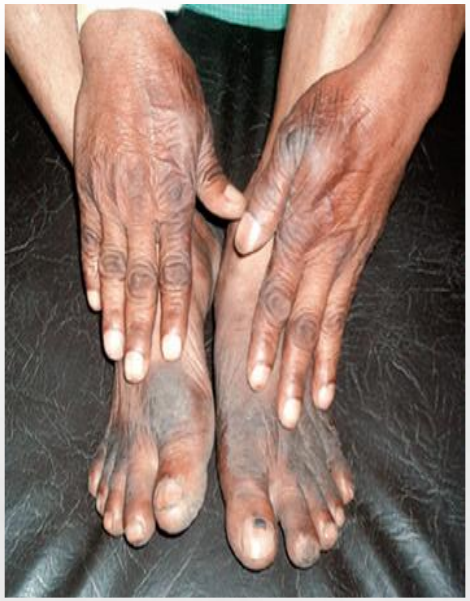


Niacin Deficiency

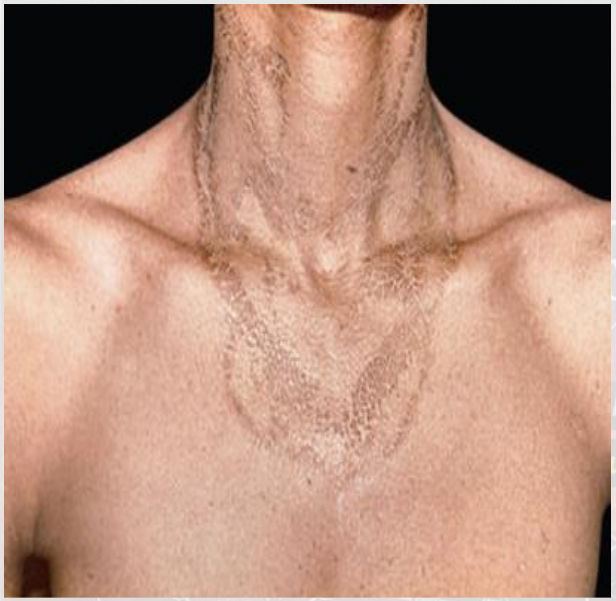
Pellagra: The symptoms of pellagra are:

1. **Dermatitis:** especially in the feet, ankles and face. Increased pigmentation around the neck is known as **Casal's necklace**. Nails has irregular surface.
2. **Diarrhea**.
3. **Dementia:** in chronic cases. **Delirium** is common in acute ellagra. Irritability, inability to concentrate and poor memory are more common in mild cases. Ataxia and spasticity are also seen.





Niacin deficiency causes pellagra with skin lesions.



Casal's necklace.

Niacin deficiency is manifested in nails as irregular or uneven surface.

Causes for Niacin Deficiency

Dietary deficiency of tryptophan: Pellagra is seen among people whose staple diet is maize and **sorghum** (jowar or guinea corn). Sorghum, contains leucine in high quantities. Leucine inhibits the quinolinate phosphoribosyl transferase (QPRT) enzyme, and so niacin cannot be converted to NAD+ (Leucine pellagra).

Deficient synthesis: Kynureninase, an important enzyme in the pathway of tryptophan, is pyridoxal phosphate dependent. So, conversion of tryptophan to niacin is not possible in pyridoxal deficiency.

Isoniazid (INH): It is an antituberculous drug, which inhibits pyridoxal phosphate formation.

Hartnup's disease: Tryptophan absorption from intestine is defective. Moreover, tryptophan is excreted in urine in large quantities.

Carcinoid syndrome: The tumor utilizes major portion of available tryptophan for synthesis of serotonin; so tryptophan is unavailable.

Dietary Sources of Niacin

The richest natural sources of niacin are dried yeast, rice polishing, liver, peanut, whole cereals, legumes, meat and fish.

About half of the requirement is met by the conversion of tryptophan to niacin. About 60 mg of tryptophan will yield 1 mg of niacin.

Recommended Daily Allowance (RDA)

Normal requirement is 20 mg/day. During lactation, additional 5 mg is required.

Therapeutic Use of Niacin

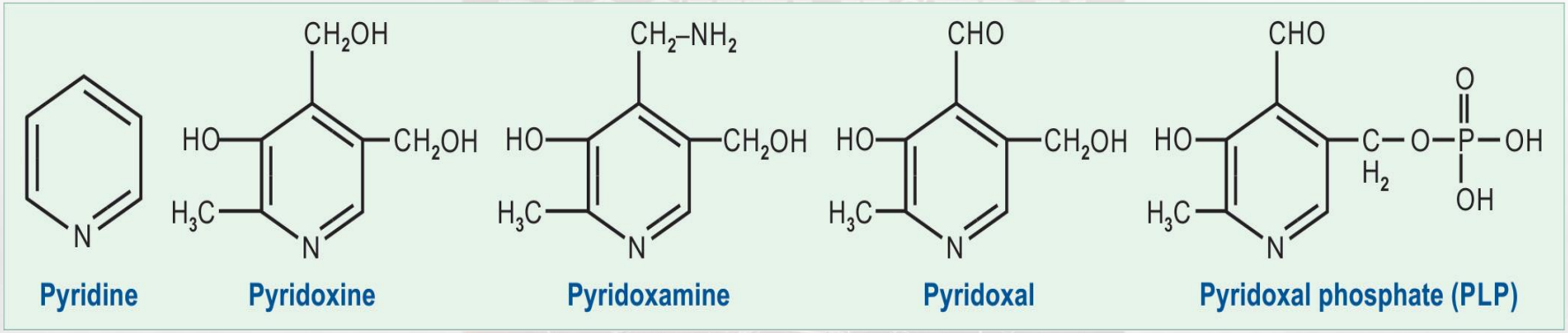
Nicotinic acid reduces serum **cholesterol level**. In high doses, niacin is useful to reduce lipoprotein(a) [Lp(a)] levels.

Toxicity of Niacin

Nicotinic acid when given orally or parenterally produces a transient vasodilatation of the cutaneous vessels and **histamine release**. The reaction is accompanied by itching, burning and tingling.

Vitamin B6

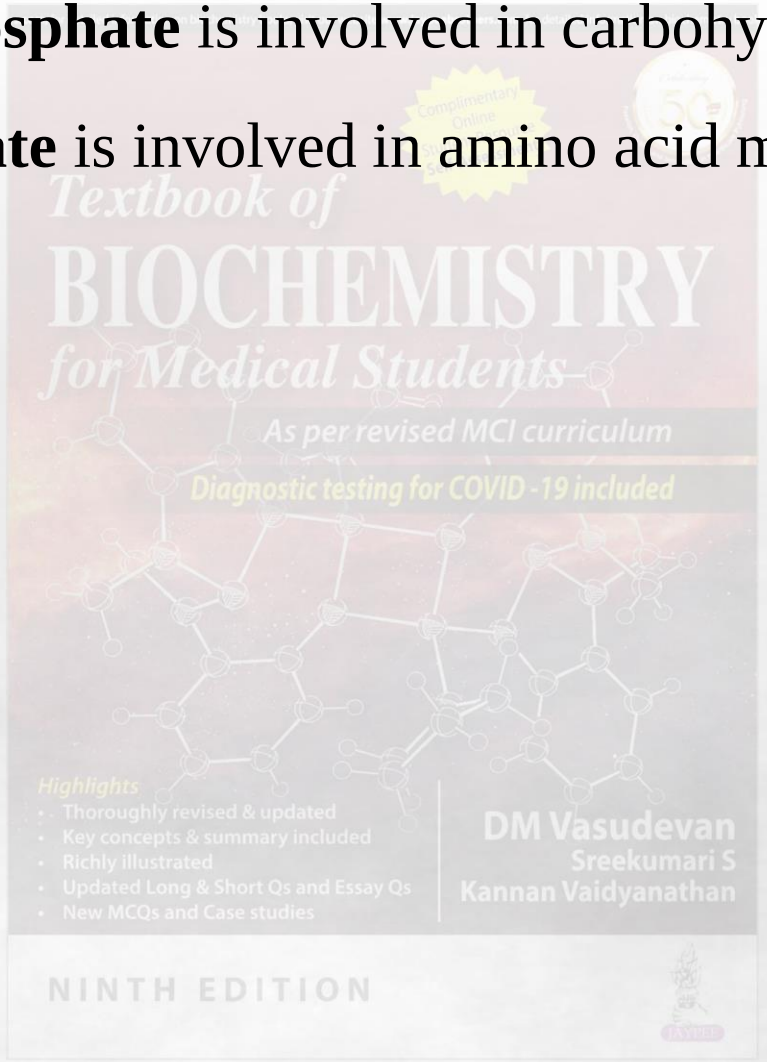
Vitamin B6 is the term applied to a family of 3 related compounds; **pyridoxine** (alcohol), **pyridoxal** (aldehyde) and **pyridoxamine**. Active form of pyridoxine is **pyridoxal phosphate (PLP).**



Functions of Thiamine and Pyridoxine

Thiamine pyrophosphate is involved in carbohydrate metabolism.

Pyridoxal phosphate is involved in amino acid metabolism.



Functions of Pyridoxal Phosphate

A. Transamination

1. These reactions are catalyzed by aminotransferases transaminases) which employ PLP as the coenzyme, For example:

Alanine + alphaketoglutarate → Pyruvate + Glutamic acid (Enzyme alanine transaminase)

B. Decarboxylation

1. Glutamate → gamma aminobutyric acid(GABA). GABA is an inhibitory neurotransmitter, and hence in B6 deficiency, especially in children, **convulsions** may occur.
2. Histidine → histamine, which is the mediator of **allergy**.
3. 5-hydroxy tryptophan → serotonin
4. Cysteine → taurine
5. Serine → ethanol amine

C. Metabolism of Sulfur-containing Amino acids

1. Homocysteine + Serine → Cystathionine (Enzyme Cystathionine synthase)
2. Cystathionine → Homoserine + Cysteine (Enzyme Cystathionase)

Both these reactions require PLP. Hence in vitamin B6 deficiency **homocysteine** in blood is increased. Therefore, pyridoxine is used in homocysteinemia.

D. Heme Synthesis

Aminolevulinic acid synthase is a PLP-dependent enzyme. So, in B6 deficiency, **anemia** may be seen.

E. Production of Niacin

Pyridoxal phosphate is required for the synthesis of niacin from tryptophan (**one vitamin is necessary for synthesis of another vitamin)**.

3-hydroxykynurenine → 3-hydroxyanthranilic acid (Enzyme ynureninase) Kynureninase is a PLP-dependent enzyme.

F. Glycogenolysis

Phosphorylase enzyme (glycogen to glucose-1-phosphate) requires PLP.

Deficiency Manifestations of Pyridoxine

A. Neurological Manifestations

In children, B6 deficiency leads to convulsions due to decreased formation of **GABA**. PLP is involved in the synthesis of sphingolipids; so B6 deficiency leads to demyelination of nerves and consequent **peripheral neuritis**.

B. Dermatological Manifestations

B6 deficiency in turn leads to niacin deficiency which is manifested as **pellagra**.

Another manifestation is the irregular indentations in nails.

C. Hematological Manifestations

In adults, hypochromic microcytic **anemia** may occur due to the inhibition of heme biosynthesis.

D. Other metabolic disorders

Xanthurenic aciduria and **homocystinuria** will respond to vitamin B6 therapy.

Effect of Drugs on Vitamin B6

1. **INH** or isonicotinic acid hydrazide (isoniazid) is an antituberculosis drug. It inhibits formation of PLP and causes vitamin B6 deficiency.
2. **Cycloserine** acts as B6 antagonist.
3. Mild vitamin B6 deficiency may be seen in women taking **oral contraceptive** pills.
4. **Ethanol** is converted to acetaldehyde, which inactivates PLP. Hence, B6 deficiency neuritis is quite common in alcoholics.

Deficiency of vitamin B6 is manifested as irregular indentations in nails.

Dietary Sources of Vitamin B6

Rich sources are yeast, rice polishing, wheat germs, cereals, legumes (pulses), oil seeds, egg, milk, meat, fish and green leafy vegetables.

Requirement of Vitamin B6

Adults need 2–3 mg/day.

During pregnancy and lactation, the requirement is

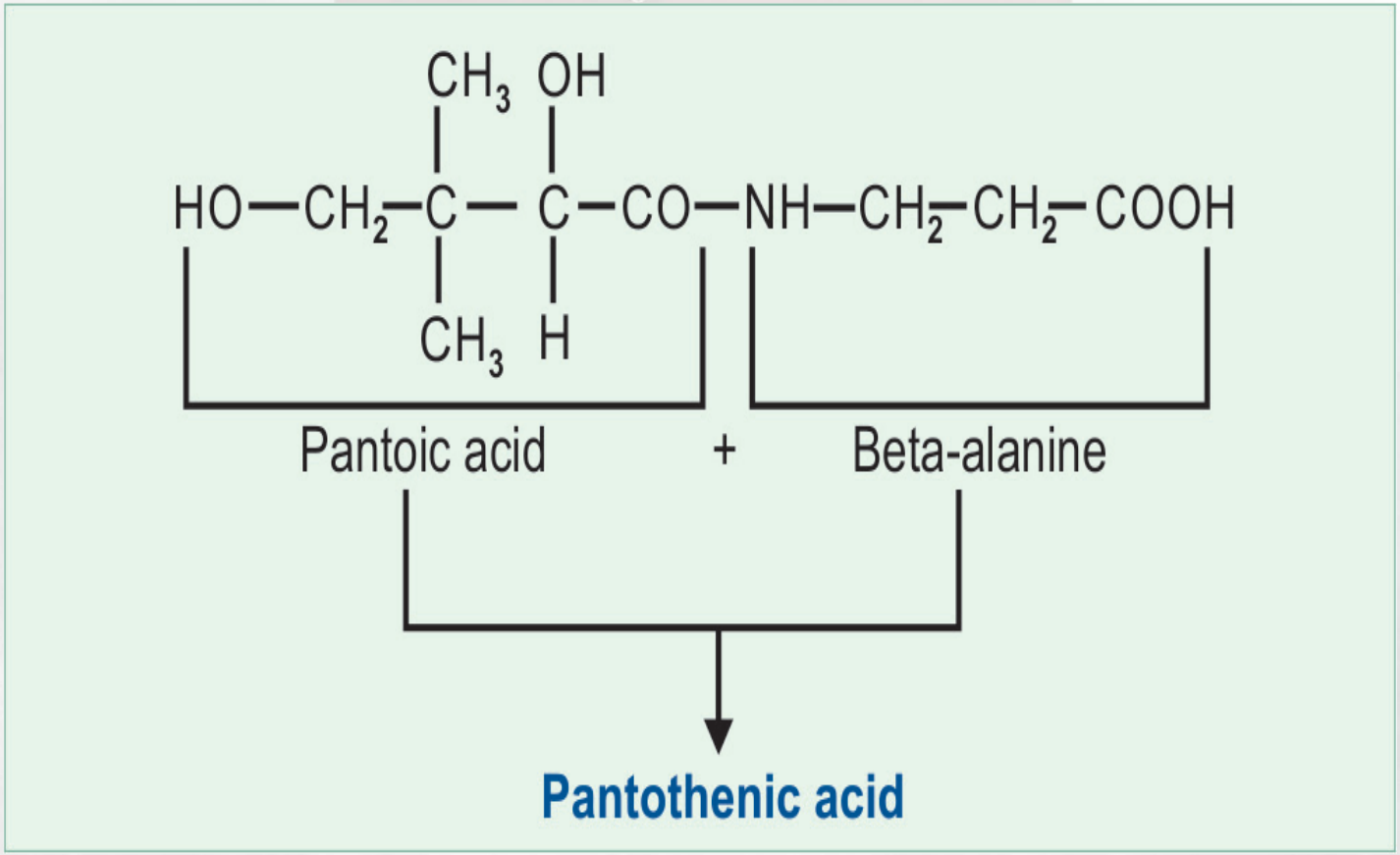
increased to 3 mg/day.

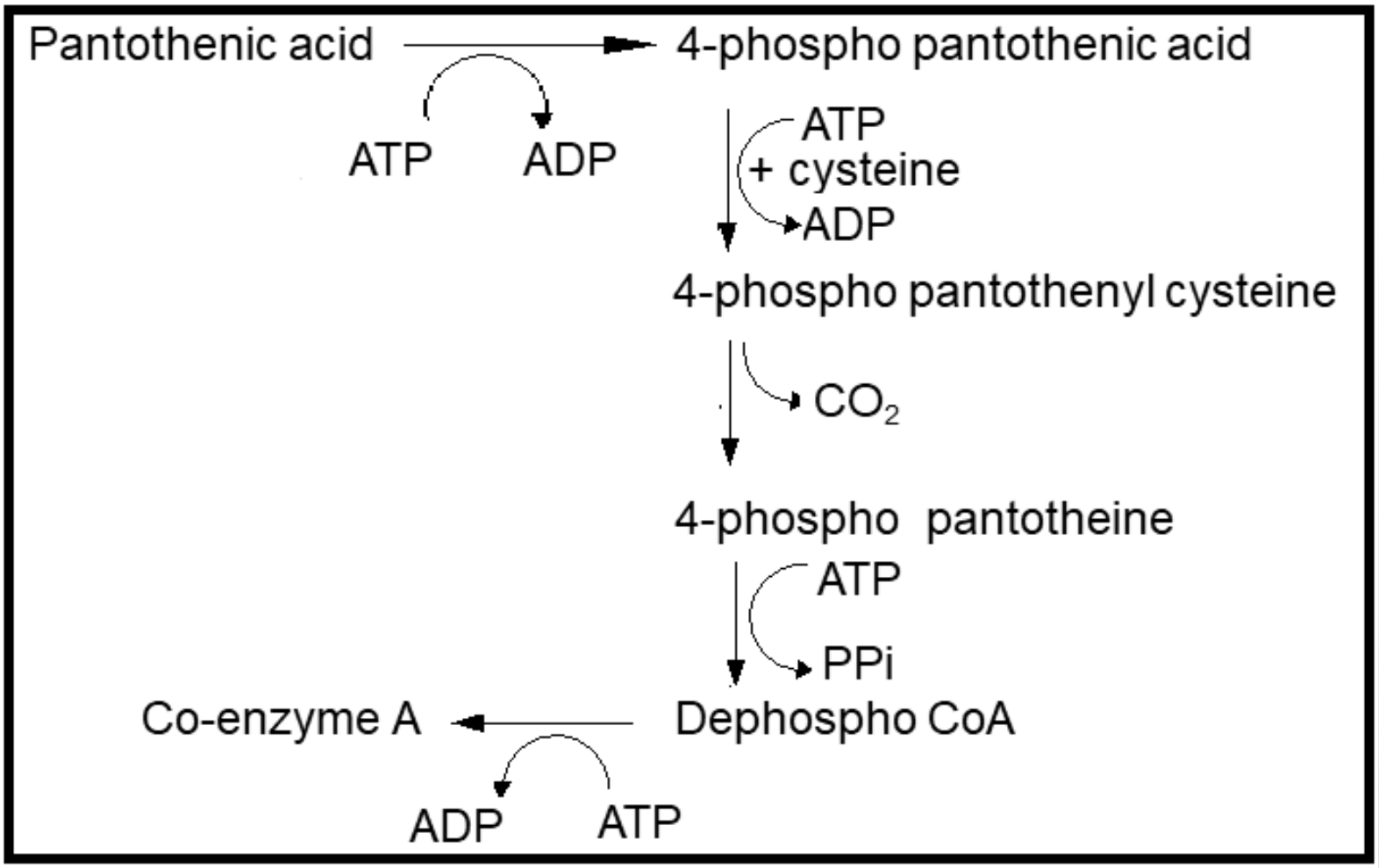
Toxicity of Vitamin B6

Doses over 100 mg may lead to **sensory neuropathy**.

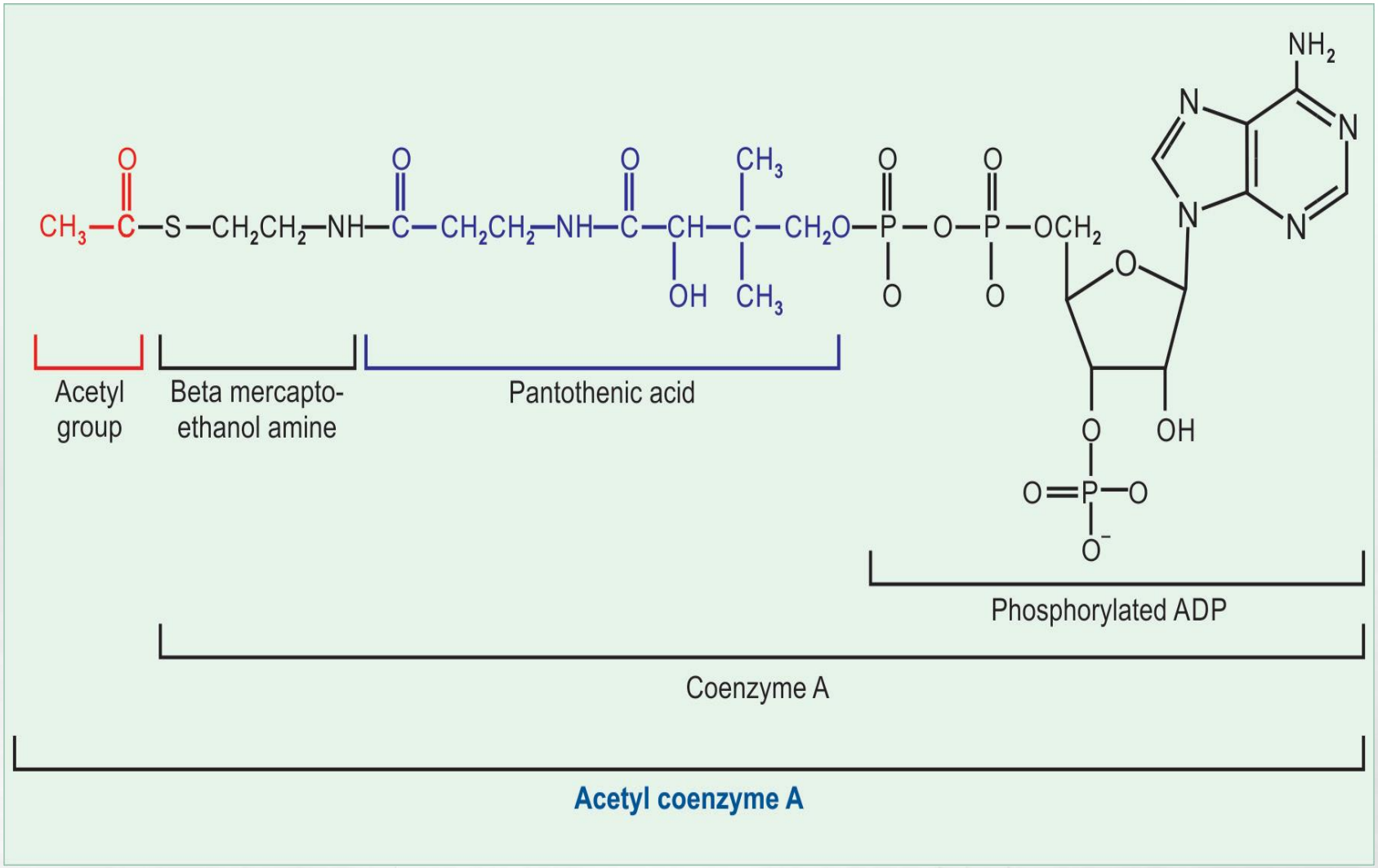
Pantothenic Acid

Pantothenic acid contains beta-alanine and D-pantoic acid. Pantothenic acid and beta-mercaptoethanolamine are parts of coenzyme A (CoA). The CoA contains a nucleotide.





Synthesis of CoA.



Structure of Coenzyme A (CoA).

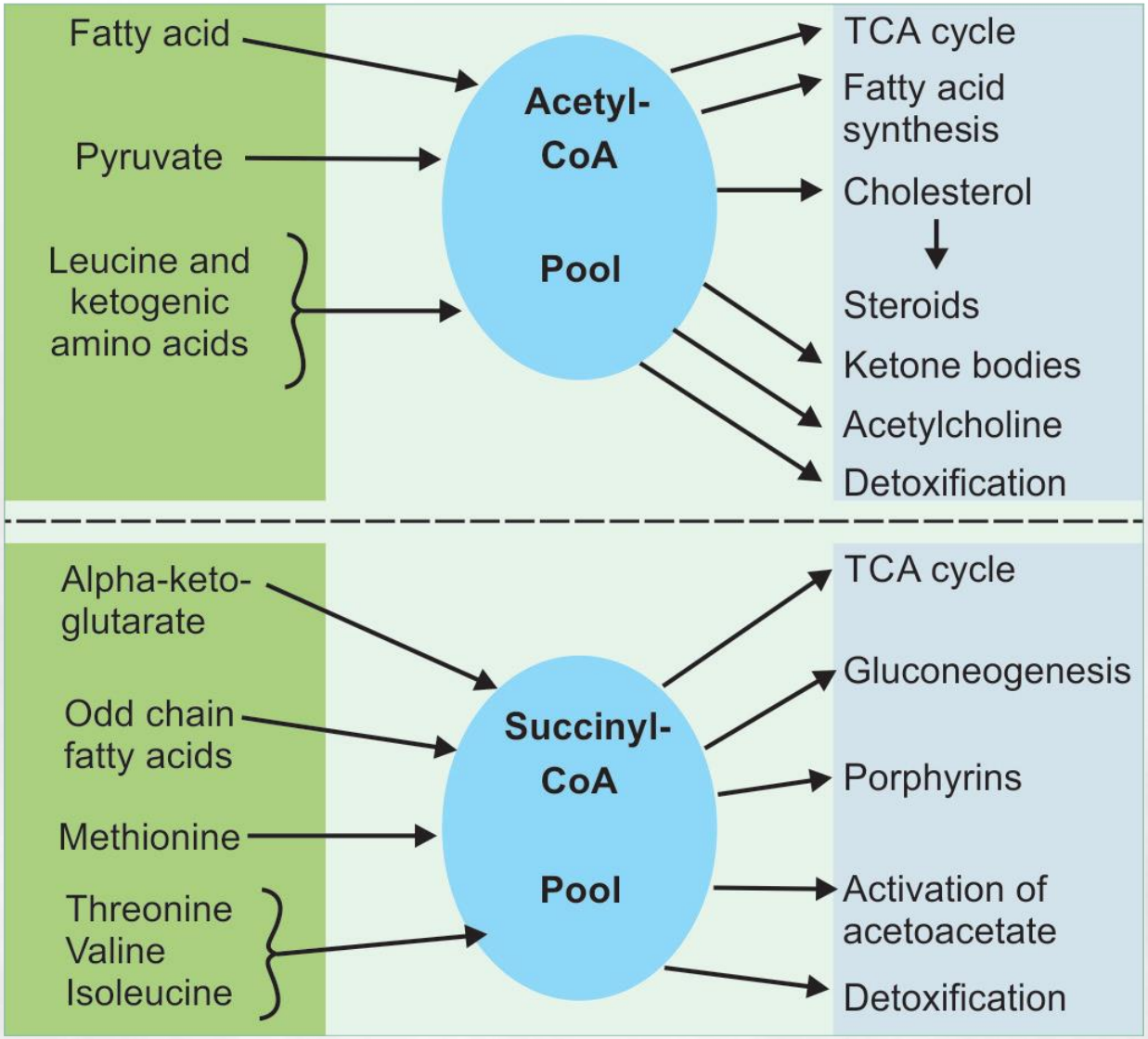
Coenzyme Activity of Pantothenic Acid

- Acetyl-CoA + Choline → Acetylcholine + CoA (enzyme acetylcholine synthase)
- Pyruvate + CoA + NAD+ → Acetyl-CoA + CO2 + NADH (Enzyme pyruvate dehydrogenase)

The important CoA derivatives are:

- Acetyl-CoA
- Succinyl-CoA
- HMG-CoA
- Acyl-CoA.

Coenzyme A is an important component of **fatty acid synthase complex**. The **acyl carrier protein (ACP)** also contains pantothenic acid.


Fatty acid
Pyruvate
Leucine and ketogenic amino acids
Acetyl-CoA Pool
TCA cycle
Fatty acid synthesis
Cholesterol
Steroids
Ketone bodies
Acetylcholine
Detoxification
Alpha-keto-glutarate
Odd chain fatty acids
Methionine
Threonine
Valine
Isoleucine
Succinyl-CoA Pool
TCA cycle
Gluconeogenesis
Porphyrins
Activation of acetoacetate
Detoxification

Deficiency of Pantothenic Acid

Gopalan's **Burning foot syndrome** is manifested as paresthesia (burning, lightning pain) in lower extremities, staggering gait. The syndrome is seen during famine, in prison camps, in chronic alcoholics and in renal dialysis patients.

Sources of Pantothenic Acid

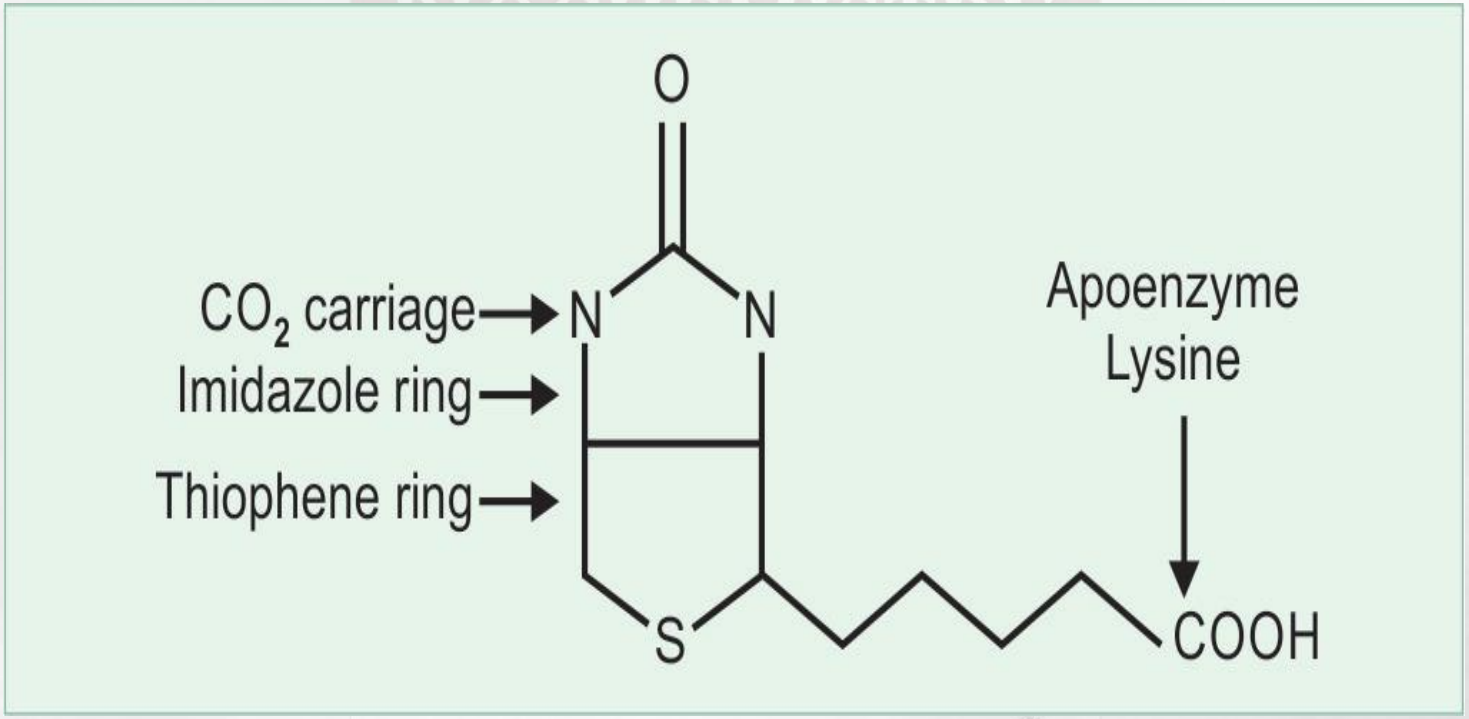
It is widely distributed in plants and animals. Moreover, it is synthesized by the normal bacterial flora in intestines. Therefore, deficiency is very rare. Yeast, liver and eggs are good sources.

Requirement of Pantothenic Acid

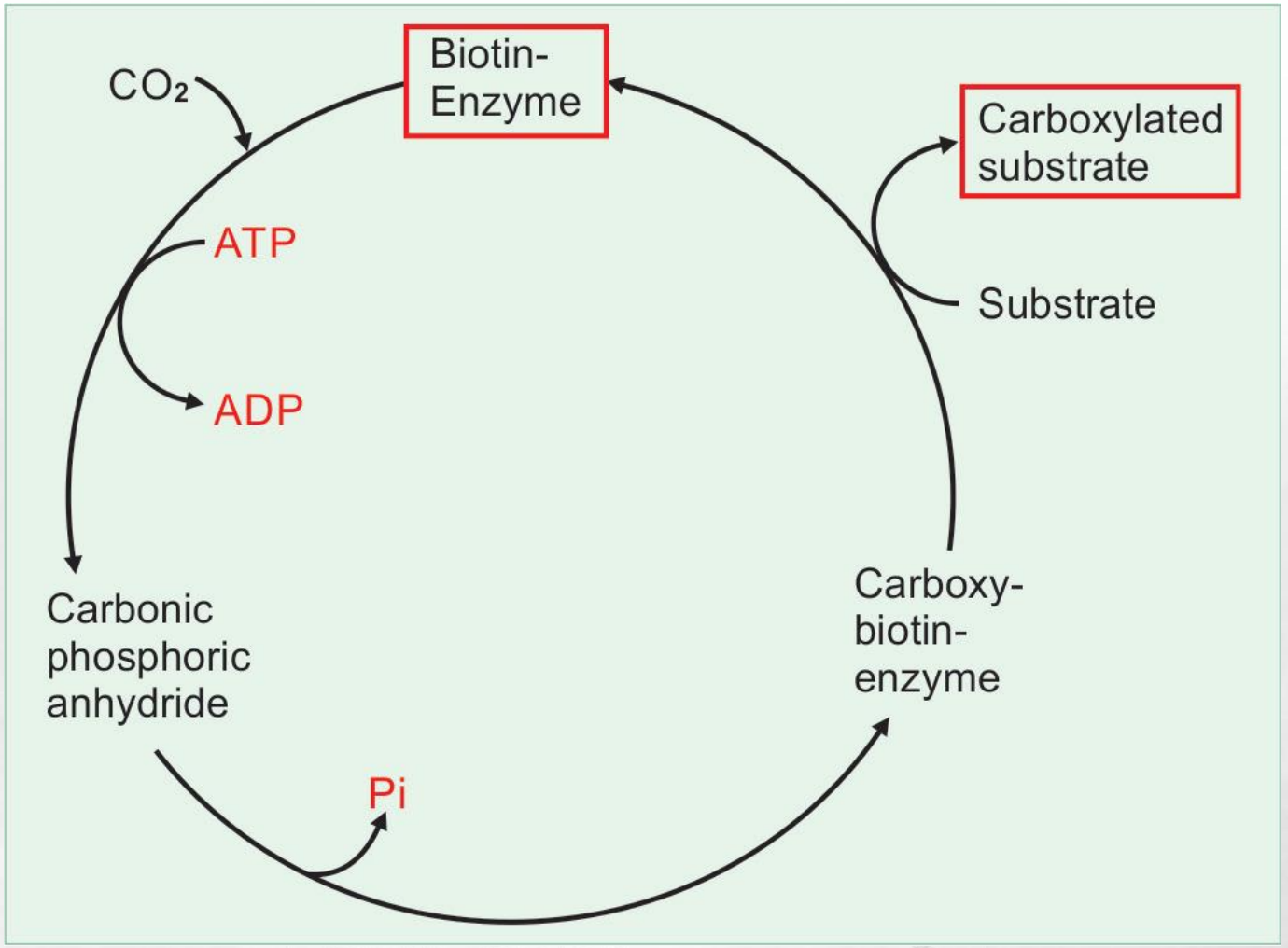
Recommended dietary allowance is assumed to be about 5-10 mg/day.

Biotin

It consists of an imidazole ring fused with a thiophene ring. The carboxyl group forms an amide linkage with the epsilon nitrogen of a lysine residue in the apoenzyme.



Structure of biotin.



Biotin helps in carboxylation reactions.

Coenzyme Activity of Biotin

Biotin acts as coenzyme for **carboxylation reactions**.

Biotin Requiring CO_2 Fixation Reactions

1. Acetyl-CoA Carboxylase

This is the rate limiting reaction in the biosynthesis of fatty acids

$$\text{Acetyl-CoA} + CO_2 + \text{ATP} \rightarrow \text{Malonyl-CoA} + \text{ADP} + \text{Pi}$$

2. Propionyl-CoA Carboxylase

$$\text{Propionyl-CoA} + CO_2 + \text{ATP} \rightarrow \text{Methyl malonyl-CoA} + \text{ADP} + \text{Pi}$$

3. Pyruvate Carboxylase

$$\text{Pyruvate} + CO_2 + \text{ATP} \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

Biotin-Independent Carboxylation Reactions

- Carbamoyl phosphate synthetase
- Addition of CO_2 to form C6 in purine ring
- Malic enzyme, converting pyruvate to malate.

Biotin Antagonists

Avidin, a protein present in **egg white** has a great affinity to biotin. Hence intake of raw (unboiled) egg may cause biotin deficiency.

Avidin is heat labile and boiling of egg will neutralize the inhibitory activity.

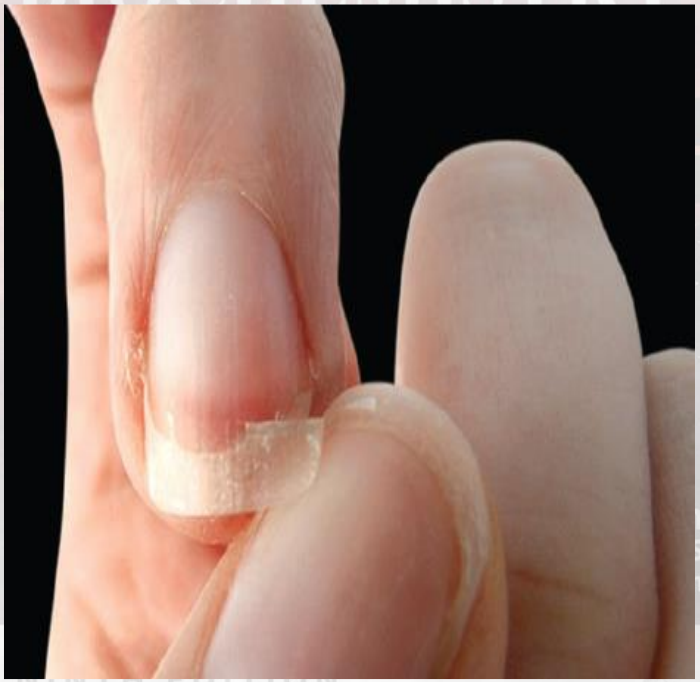
Egg white contains avidin and egg yolk contains biotin.

Avidin-biotin system is commonly utilized for detection of pathogens in the enzyme-linked immunosorbent assay (**ELISA) test**.

Deficiency of Biotin

Deficiency is seen during prolonged use of antibacterial drugs.

Symptoms include dermatitis, atrophic glossitis, hyperesthesia, muscle pain, anorexia and hallucinations. Deficiency of biotin may be manifested as split nails.



Split nail in biotin deficiency.

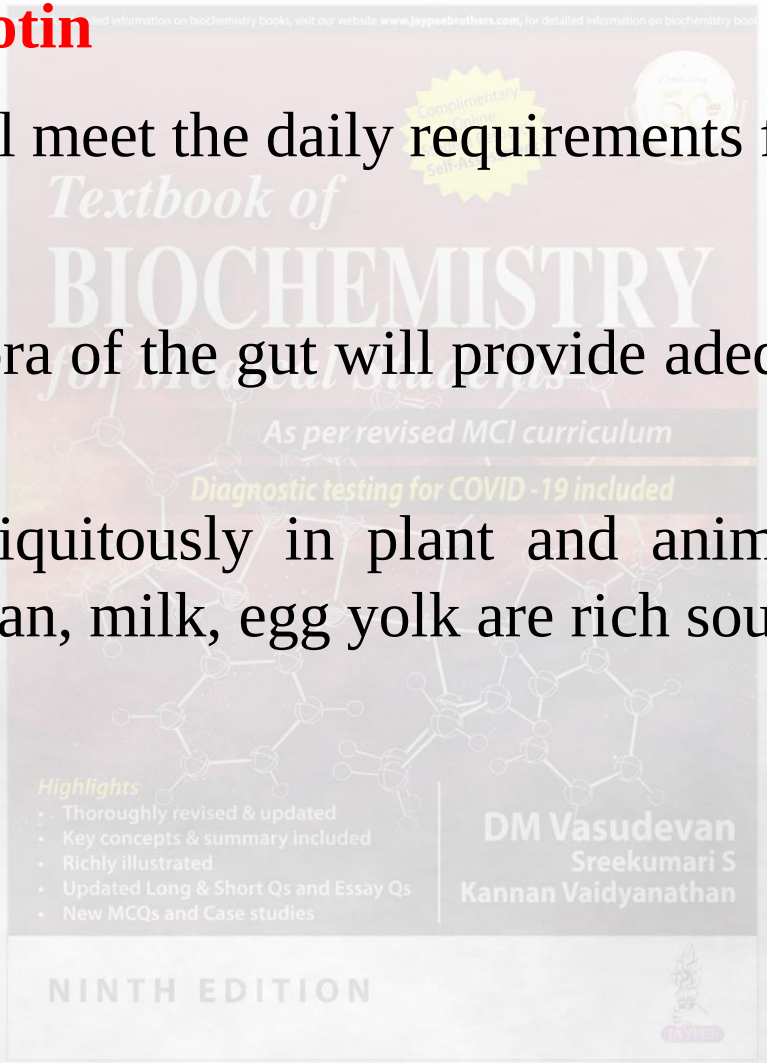
Requirement of Biotin

About 30–40 μg will meet the daily requirements for adults.

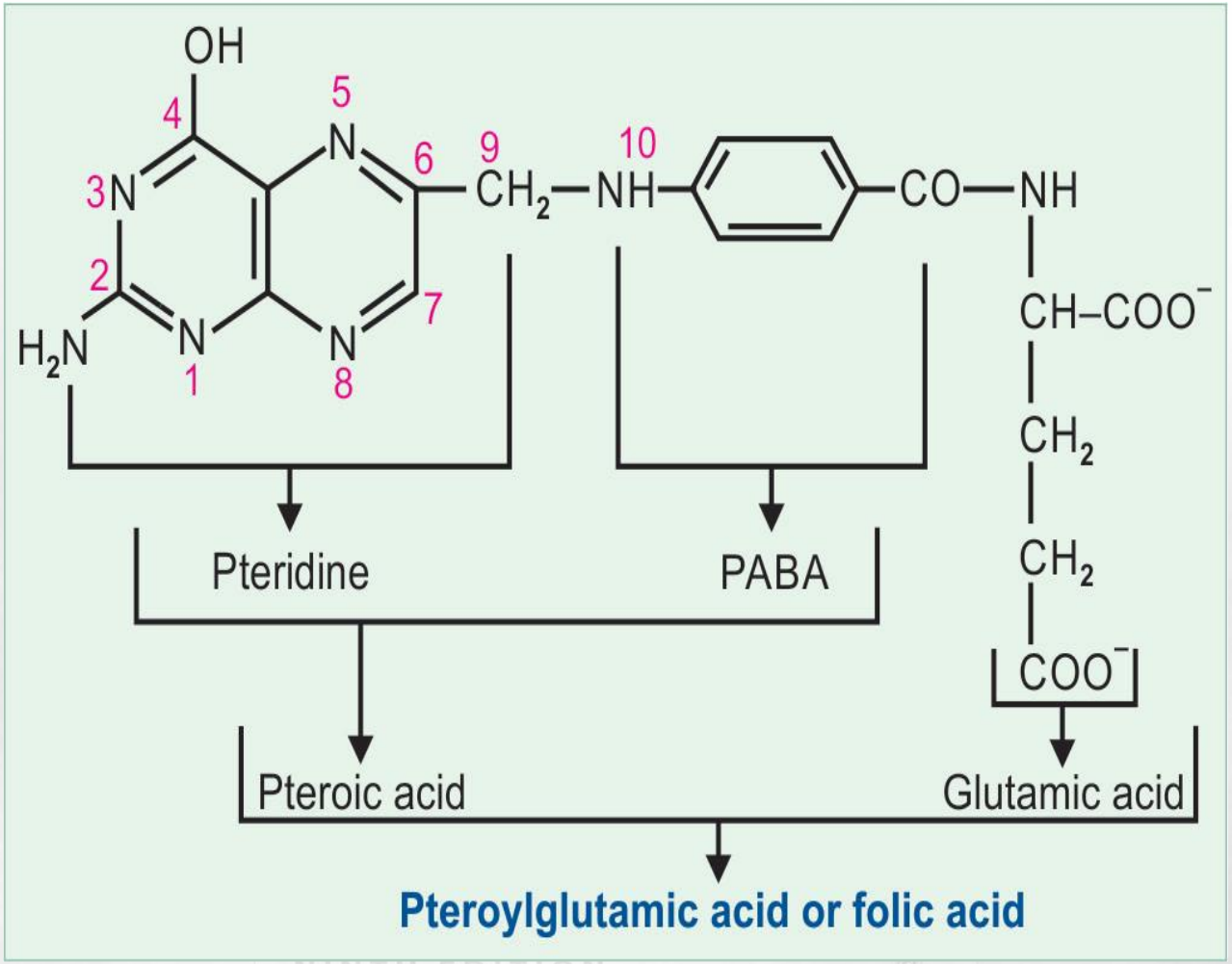
Sources of Biotin

Normal bacterial flora of the gut will provide adequate quantities of biotin.

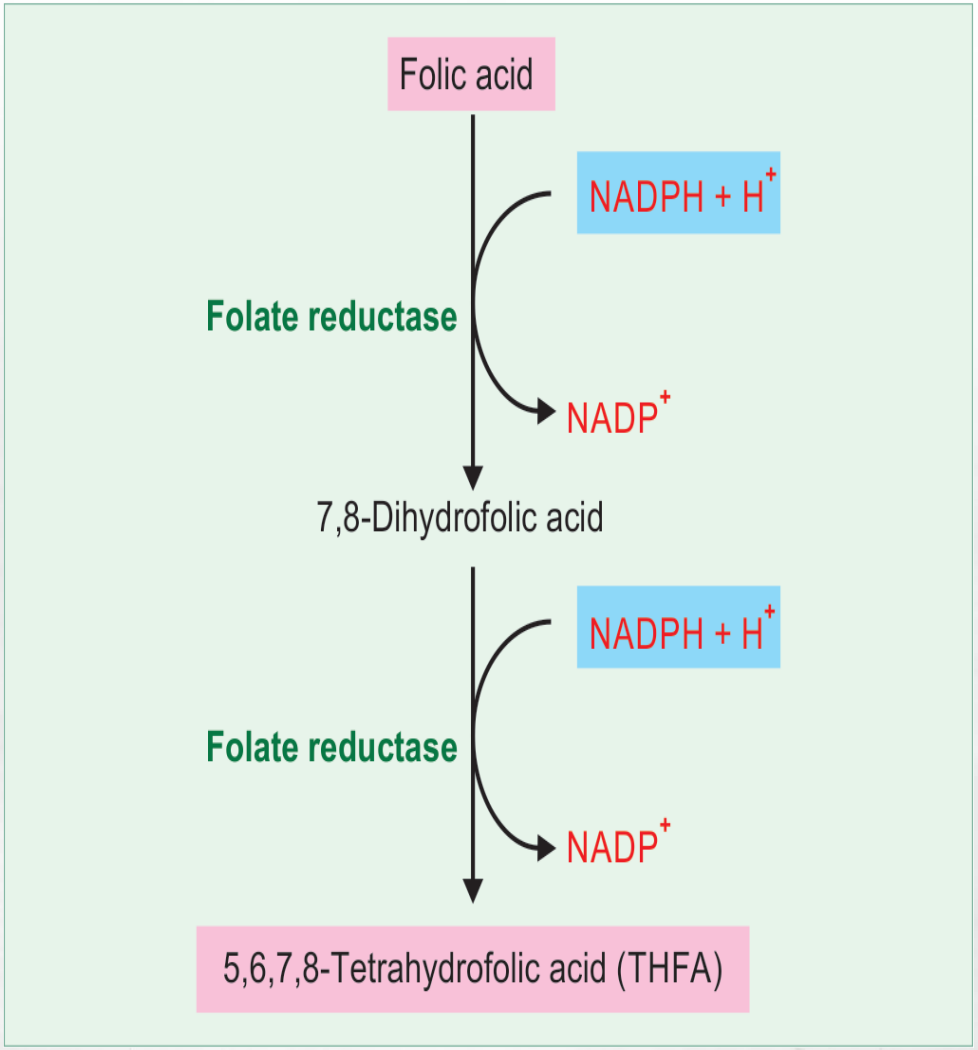
It is distributed ubiquitously in plant and animal tissues. Liver, yeast, peanut, soybean, milk, egg yolk are rich sources.



Folic Acid



Structure of folic acid.



Production of tetrahydro folic acid

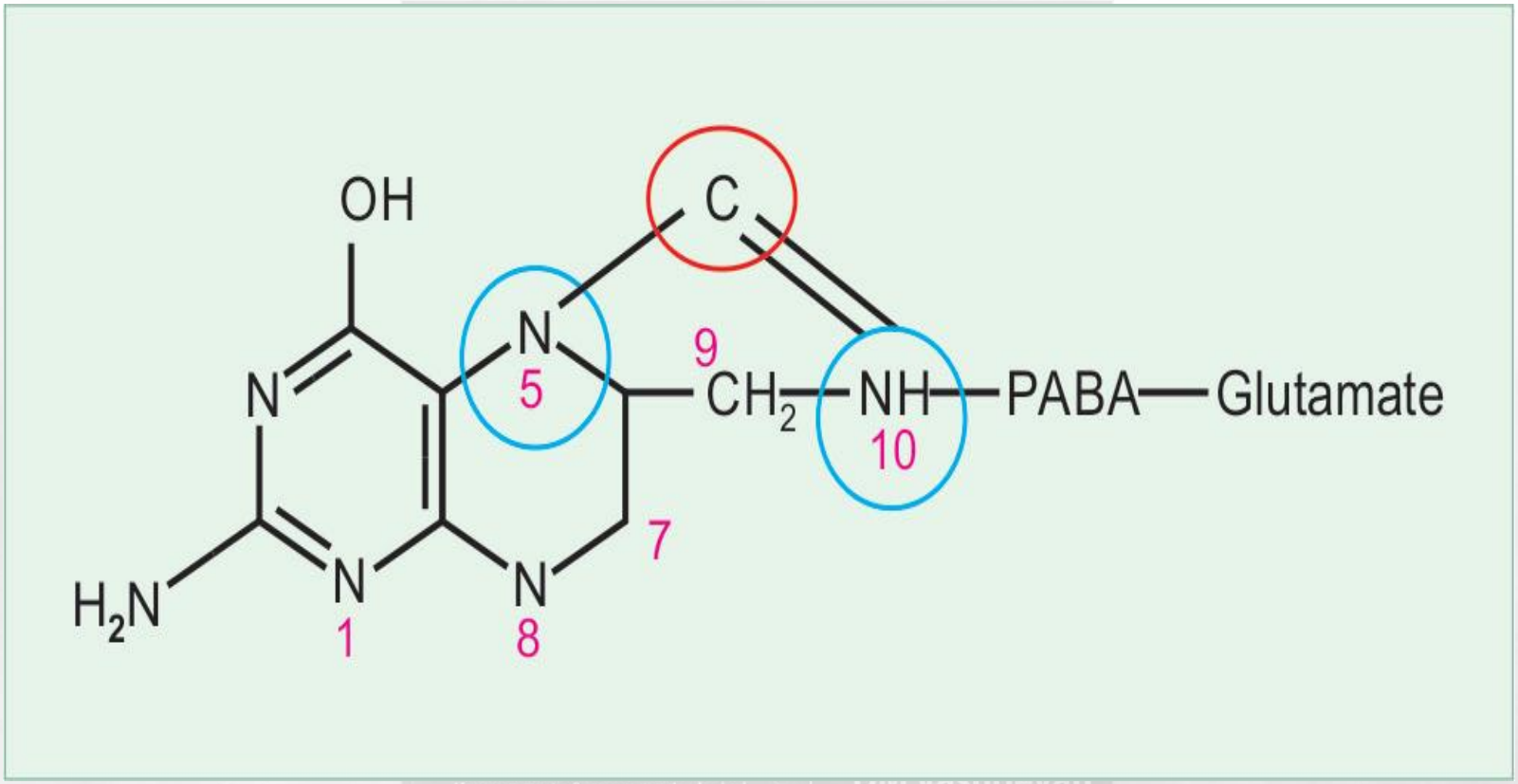
Coenzyme Functions of Folic Acid

The THFA is the **carrier of one-carbon** groups. The following groups are one Carbon compounds:

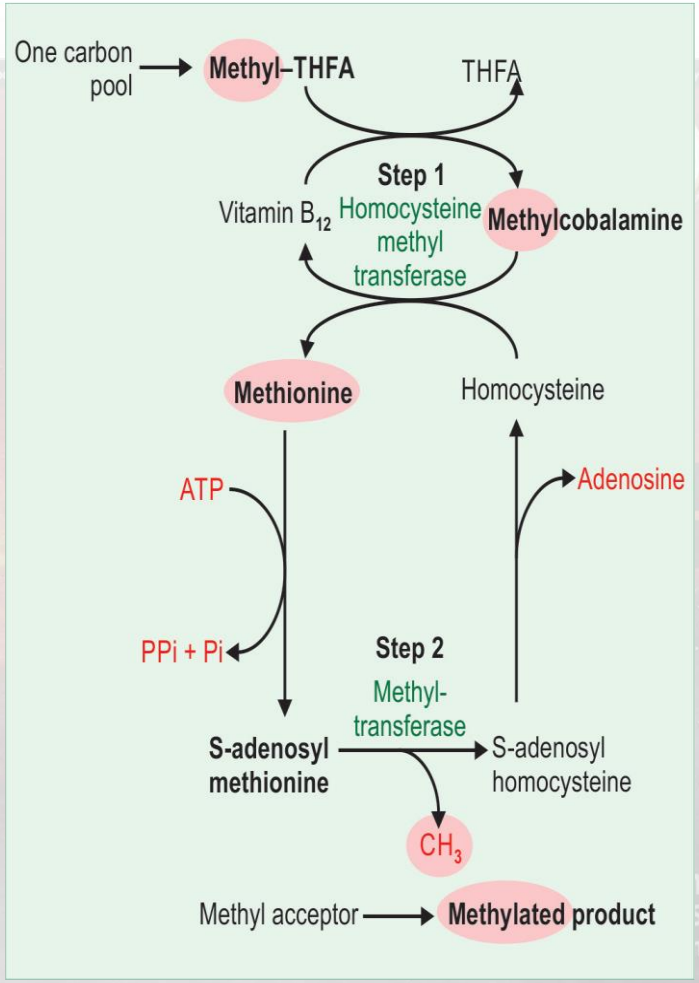
1. Formyl (-CHO)
2. Formimino (-CH=NH)
3. Methenyl (-CH=)
4. Methylene (-CH2–)
5. Hydroxymethyl (-CH2OH)
6. Methyl (-CH3).

These one carbon compounds are attached either to the 5th or to the 10th or to both 5 and 10 nitrogen atoms of THFA.

The methyl group in N5-methyl THFA is used for synthesis of active methionine, which takes part in transmethylation reactions. Such **transmethylation** reactions are required for synthesis of choline, epinephrine, creatine, etc.



N5, N10-methenyl THFA. The one carbon unit (red ring) is attached to the N5 and N10 groups (blue rings) of tetrahydrofolic acid.



Step 1 explains the folate trap. Step 2 is important for methyl transfer reactions

Causes of Folate Deficiency

Pregnancy: where requirement is increased.

Defective absorption: in sprue, celiac disease, gluten induced enteropathy, resection of jejunum and short-circuiting of jejunum in gastroileostomy.

Drugs: Anticonvulsant drugs (hydantoin, dilantin, phenobarbitone) will inhibit the intestinal enzyme, so that folate absorption is reduced.

Hemolytic anemias: **R**equirement of folic acid becomes more.

Dietary deficiency: Absence of vegetables in food for prolonged periods.

Folate trap: The only way for regeneration of free THFA is the homocysteine methyl transferase, with the help of vitamin B12. When B12 is deficient, this reaction cannot take place, leading to folate deficiency.

Deficiency Manifestations

1. Reduced DNA synthesis

In folate deficiency, THFA is reduced and thymidylate synthase is inhibited. Hence dUMP is not converted to dTMP. So dTTP is not available for DNA synthesis. Thus, cell division is arrested.

2. Macrocytic Anemia

The asynchrony or dissociation between the maturity of nucleus and cytoplasm is manifested as **immature looking nucleus** and mature eosinophilic cytoplasm in the bone marrow cells. **Reticulocytosis** is often seen. These abnormal RBCs are rapidly destroyed in spleen. This **hemolysis** leads to the reduction of lifespan of RBC. Reduced generation and increased destruction of RBCs result in anemia.

3. Homocysteinemia

Folic acid deficiency may cause increased homocysteine levels in blood. Plasma homocysteine levels above 15 mmol/L will increase the risk of **cardiac diseases**.

Deficiency Manifestations, Continued

4. Histidine load test or FIGLU excretion test

Histidine is normally metabolized to formiminoglutamic acid (FIGLU) from which formimino group is removed by THFA. Therefore, in folate deficiency, FIGLU is excreted in urine. This test is rarely done nowadays.

5. AICAR excretion

Addition of C2 with the help of N10-formyl THFA is the last step in purine synthesis. When this reaction is blocked, the precursor, amino imidazole carboxamide ribose-5-phosphate (AICAR) accumulates and is excreted.

6. Birth Defects

Folic acid deficiency during pregnancy may lead to **neural tube defects** such as spina bifida in the fetus. Administration of folic acid to mother prevents birth defects and fetal malformations.

Sources of Folic Acid

Rich sources of folate are yeast, green leafy vegetables. Moderate sources are cereals, pulses, oil seeds and egg. Milk is a poor source for folic acid.

Recommended Daily Allowance

The requirement of free folate is 200 μg/day. In pregnancy the requirement is increased to 600 μg/day and during lactation to 350 μg/day.

Folic Acid Therapy

In macrocytic anemia, the therapeutic dose is 1 mg of folic acid per day orally. Folic acid alone should not be given in macrocytic anemia; because it may aggravate the neurological manifestation of B12 deficiency. So, folic acid and vitamin B12 are given in combination to patients.

Folate Antagonists

1.Sulfonamides

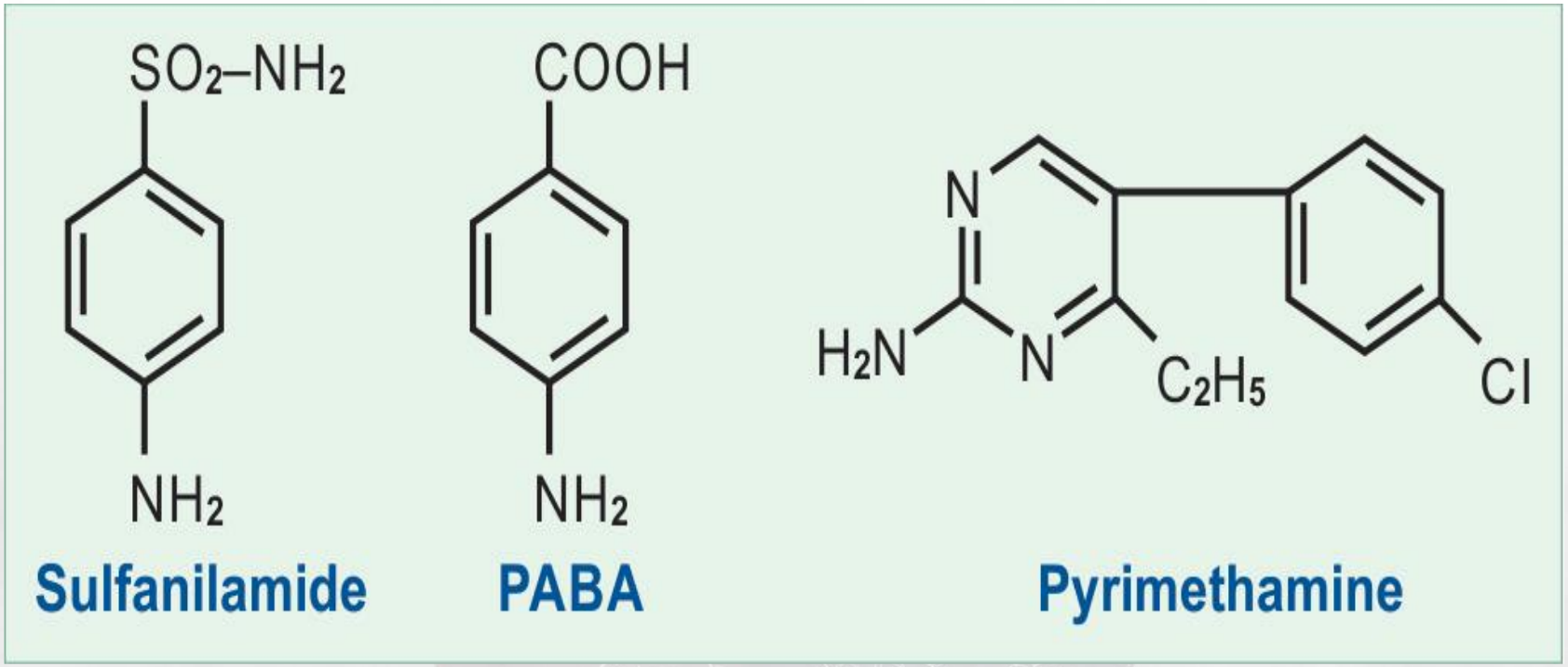
They have structural similarity with PABA. Therefore, they competitively inhibit the enzyme responsible for the incorporation of PABA into folic acid. When sulfonamides are given, such microorganisms cannot synthesize folic acid and hence their growth is inhibited. Sulfonamides are very good **antibacterial** agents, which do not affect the human cells.

2. Pyrimethamine

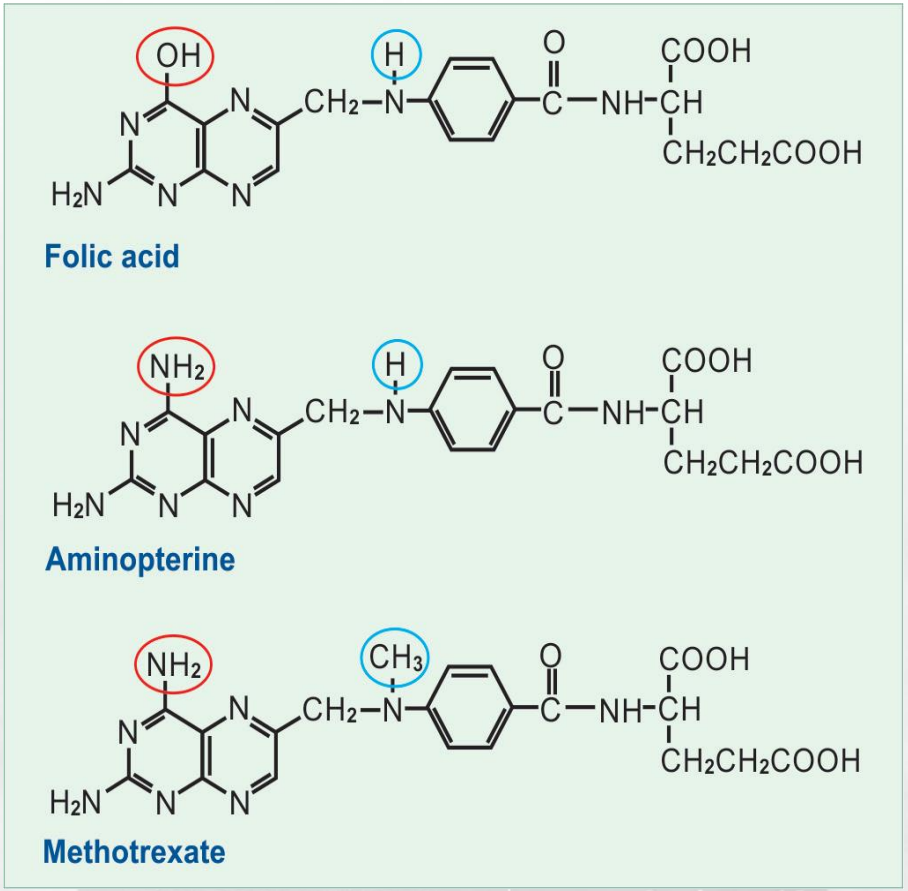
This antifolate agent is used as **antimalarial drug**.

3. Aminopterin and Amethopterin

Aminopterin (4-amino folic acid) and Amethopterin (methotrexate) are structurally similar to folic acid (Fig. 29.38). Therefore, they are powerful inhibitors of folate reductase and THFA generation. Thus, these drugs decrease the DNA formation and cell division. They are widely used as **anticancer drugs or antimetabolites**, especially for leukemias and choriocarcinomas.

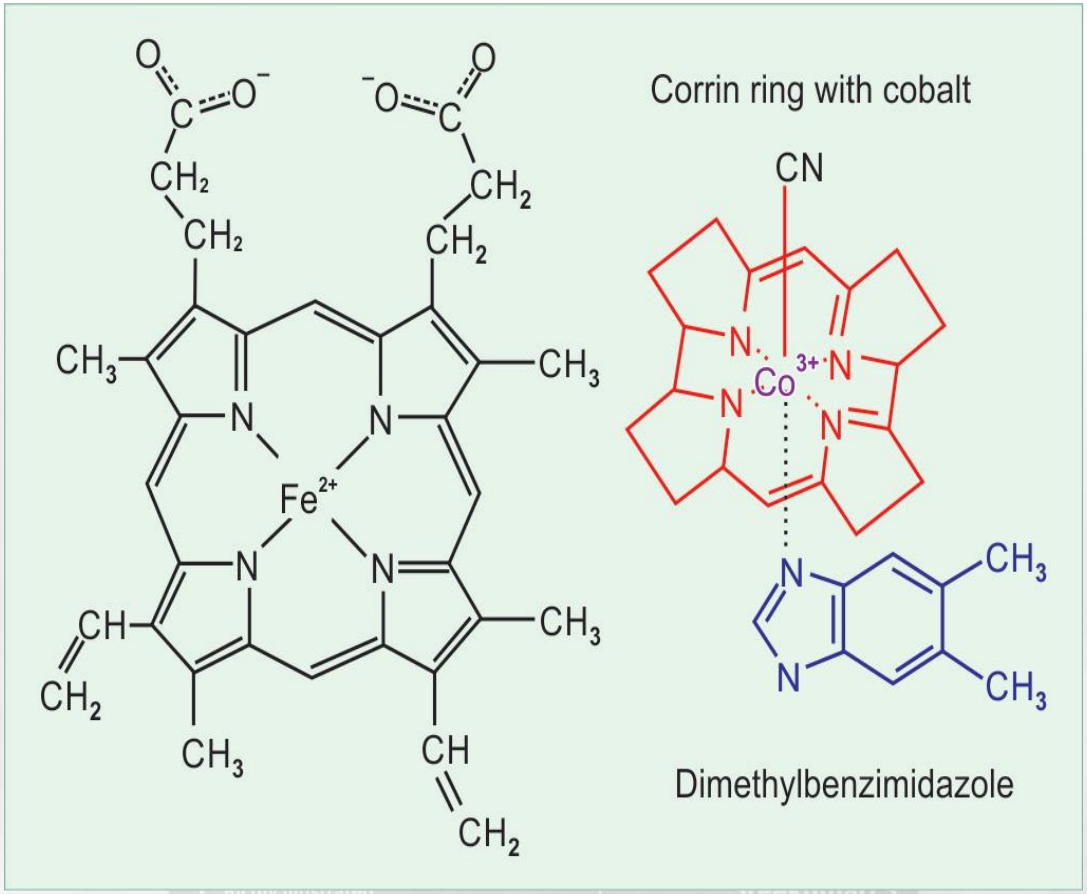


Folate antagonists. Sulfa drug has structural similarity with para-aminobenzoic acid (PABA) which is a constituent of folic acid. So sulfa drugs will competitively inhibit the synthesis of folic acid in bacteria. Pyrimethamine is an antimalarial drug.



Comparison of structures of folic acid, aminopterin and methotrexate. The structural differences are marked with red and blue circles.

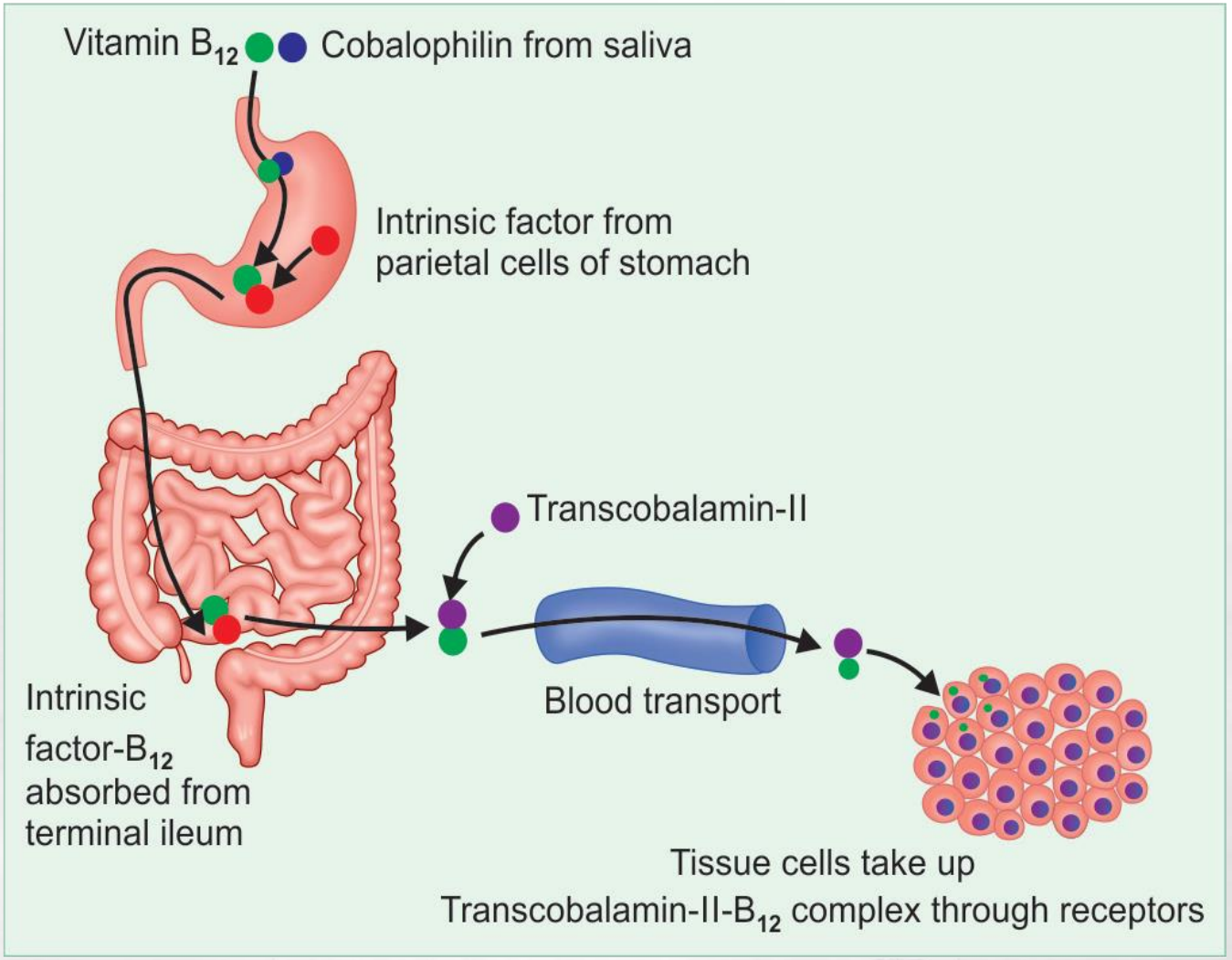
Vitamin B12



Structure of vitamin B12. The CN group is present in cyanocobalamin. The CN may be substituted with –OH, –adenosyl or –methyl groups. (A) Heme group is shown for comparison; (B) Simplified structure of Vitamin B12.

Absorption of Vitamin B12

The binding protein is the **intrinsic factor** (IF) of Castle. The B12 is otherwise known as extrinsic factor (EF). Intrinsic factor is secreted by the gastric parietal cells. The second factor is **cobalophilin**, secreted in the saliva. Gastric **pepsin** release the vitamin from proteins of the food, and then B12 binds with cobalophilin. In duodenum, cobalophilin is hydrolyzed by **trypsin** of pancreatic juice; vitamin is released, and then vitamin binds to intrinsic factor. This IF-B12 complex is attached with specific receptors on mucosal cells. The whole IF-B12 complex is internalized. The B12 is absorbed from **ileum**, while folic acid is from jejunum.



Absorption and storage of vitamin B12.

Functional Role of B12

1. Methylmalonyl-CoA Isomerase

D-methylmalonyl-CoA is formed in the body from propionyl-CoA. It is then converted to L form by a racemase and then isomerized by methylmalonyl-CoA mutase (containing Ado-B12) to succinyl-CoA, which enters into citric acid cycle. In B12 deficiency, methylmalonyl-CoA is excreted in urine as **methylmalonic aciduria**. The metabolism of odd chain fatty acids, valine, isoleucine, methionine and threonine leads to the production of methylmalonyl-CoA.

2. Homocysteine Methyltransferase and Methyl Folate Trap

The production of methyl THFA is an irreversible step. Therefore, the only way for generation of free THFA is this reaction. When B12 is deficient, this reaction cannot take place. This is called the methyl **folate trap**. This leads to the associated **folic acid scarcity in B12 deficiency**.

Causes of B12 Deficiency

1. Nutritional

The only source for B12 in vegetarian diet is curd/ milk, and lower income group may not be able to afford it.

2. Decreased Absorption

Gastrectomy, resection of ileum and malabsorption syndromes.

3. Addisonian Pernicious Anemia

It is very rare in India, but common in European countries. It is manifested usually in persons over 40 years. Antibodies are generated against IF. So, IF becomes deficient, leading to defective absorption of B12.

4. Gastric Atrophy

In chronic **iron deficiency** anemia, there is generalized mucosal atrophy.

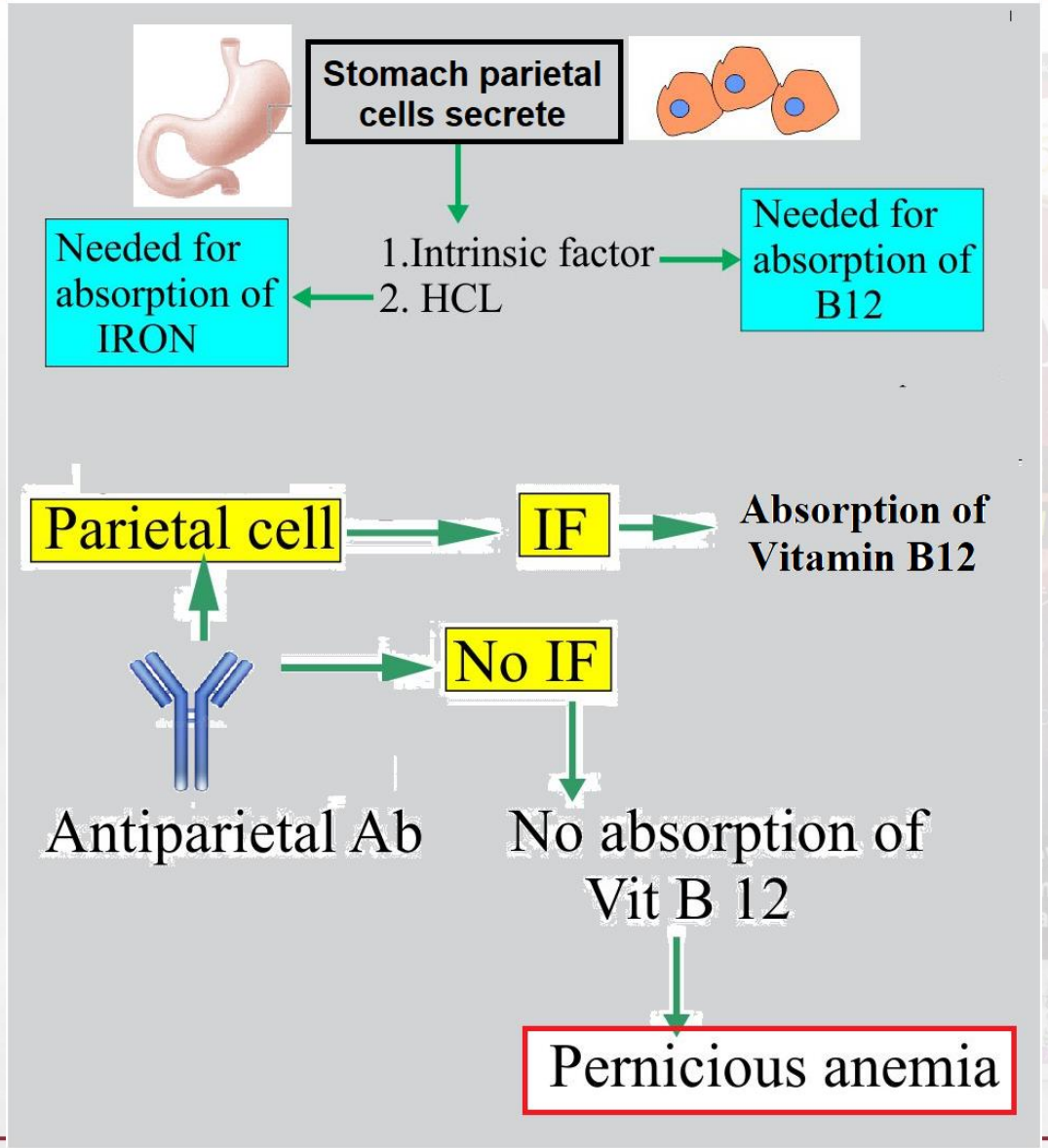
5. Pregnancy

Increased requirement of vitamin in pregnancy.

6. Fish Tapeworm

Seen in Scandinavian countries. This tapeworm has a special affinity to B12 causing reduction in available vitamin.

Pernicious anemia



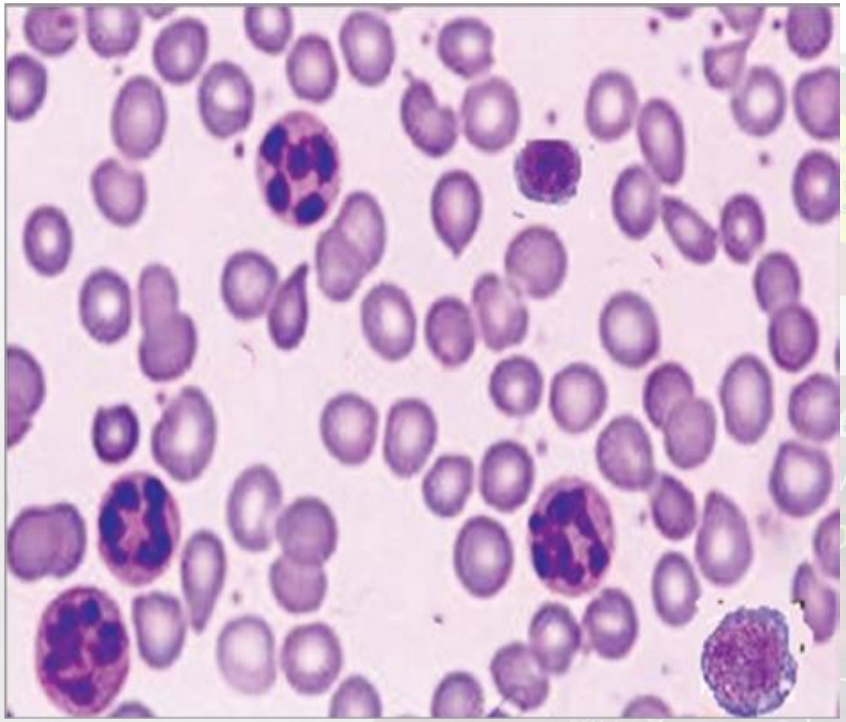
Antibodies against IF (intrinsic factor) will inhibit the absorption of vitamin B12.

Deficiency Manifestations

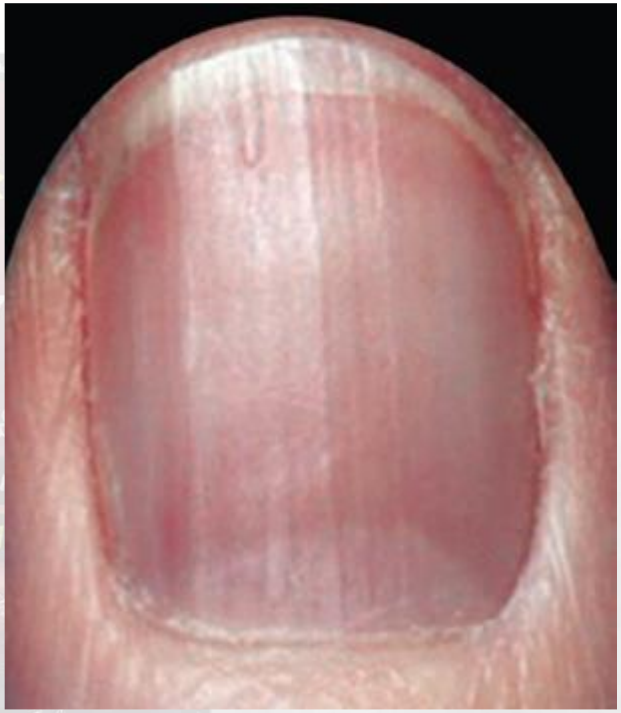
1. **Folate trap:** Vitamin B12 deficiency causes simultaneous folate deficiency due to the folate trap.
2. **Megaloblastic anemia:** In the peripheral blood, megaloblasts, immature nucleated RBCs and hyperpigmented neutrophils are observed.
3. **Abnormal homocysteine level:** Due to folate trap, homocysteine is accumulated, leading to **homoystinuria**. Homocysteine level in blood has a positive correlation with myocardial infarction.
4. **Demyelination:** As active methionine is not available, methylation of phosphatidylethanolamine to phosphatidylcholine is not adequate. This leads to deficient formation of myelin sheaths of nerves, demyelination and neurological lesions.

Deficiency Manifestations, Continued

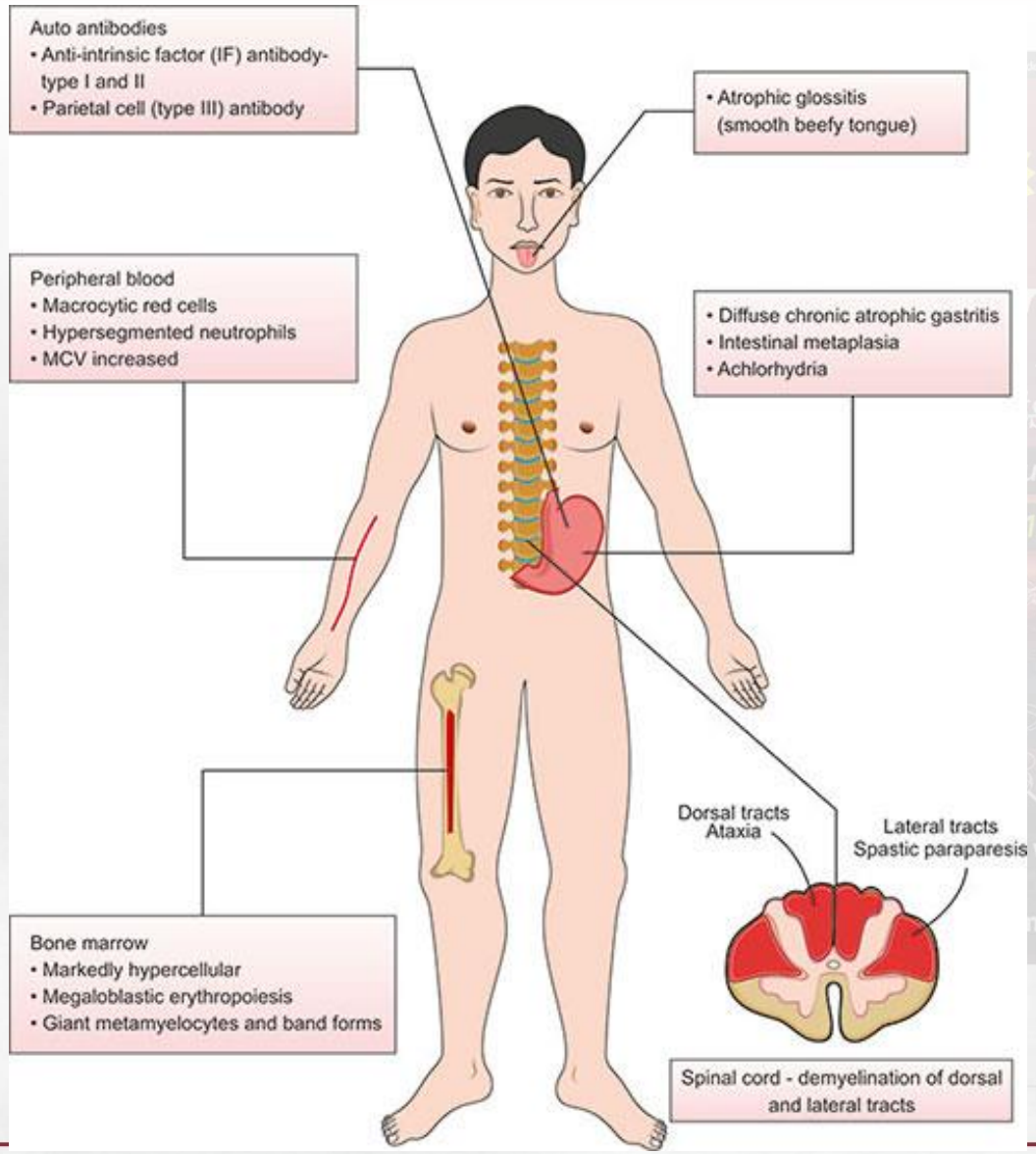
5. **Subacute combined degeneration:** Damage to nervous system is seen in B12 deficiency (but not in folate deficiency). There is **demyelination** affecting dorsal column and pyramidal tract of spinal cord. Since sensory and motor tracts are affected, it is named as combined degeneration. Symmetrical paresthesia of extremities, alterations of tendon and deep senses and reflexes, loss of position sense, unsteadiness in gait, positive **Romberg's** sign (falling when eyes are closed) and positive **Babinski's** sign (extensor plantar reflex) are seen.
6. **Achlorhydria:** Absence of acid in gastric juice is associated with vitamin B12 deficiency.
7. **Nail:** Vitamin B12 deficiency is manifested in the nail as parallel ridges.



Peripheral blood picture of megaloblastic anemia in vitamin B12 deficiency. Normoblasts (immature nucleated RBCs) and hypersegmented neutrophils are seen.



Vitamin B12 deficiency is manifested in the nail as parallel ridges



Clinical manifestations of vitamin B_{12} deficiency.

Assessment of B12 Deficiency

1. **Serum B12 level:** by radioimmunoassay (RIA) or by ELISA.
2. **Peripheral smear:** Peripheral blood and bone marrow shows typical picture of megaloblastic anemia.
3. **Schilling test:** Radioactive labelled (Cobalt-60) vitamin B12, 1 μg is given orally. Simultaneously, an intramuscular injection of unlabeled vitamin B12 is given, in order to saturate tissues with normal vitamin B12. So, radioactive vitamin B12 will not bind to body tissues. Therefore, in normal persons, the entire absorbed radioactivity will pass into the urine. In patients with pernicious anemia, less than 5% of the radioactivity is detected in urine.
4. **Methylmalonic acid** is seen in urine.
5. **Formiminoglutamic acid (FIGLU) excretion test** will be positive.
6. **Homocystinuria** is also observed

Treatment

All macrocytic anemias are generally treated with folate and vitamin B12. Therapeutic dose of B12 is 500 to 1,000 μg by intramuscular injections.

Requirement of Vitamin B12

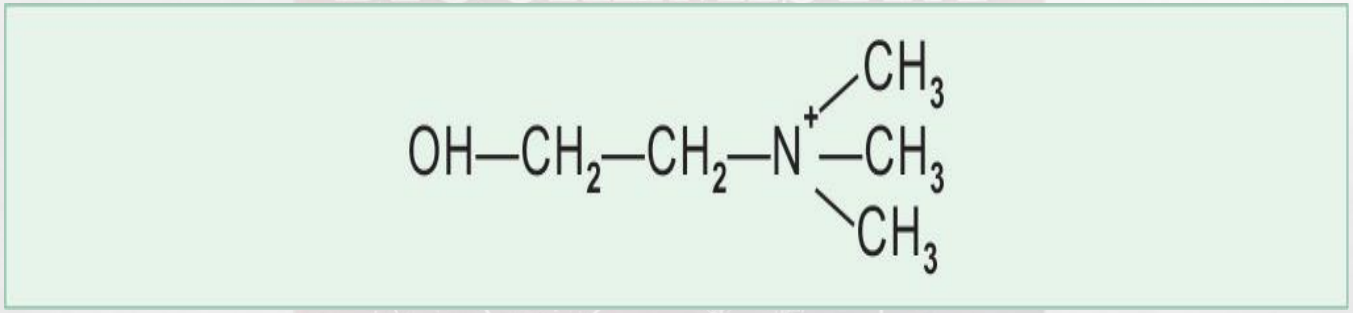
Normal daily requirement is 2 μg/day. During pregnancy and lactation, this is increased to 2.5 μg/day. Those who take folic acid, should also take vitamin B12. Elderly people are advised to take vitamin B12 supplementation.

Dietary Sources

Vitamin B12 is not present in vegetables. Liver is the richest source. Curd is a good source, because lactobacillus can synthesize B12.

Choline

Choline is synthesized in the body. It is synthesized from serine. Rice polishing, vegetables, milk, egg and liver are good sources.



Structure of choline is hydroxyethyl trimethylamine, or trimethyl ethanolamine

Biochemical Functions of Choline

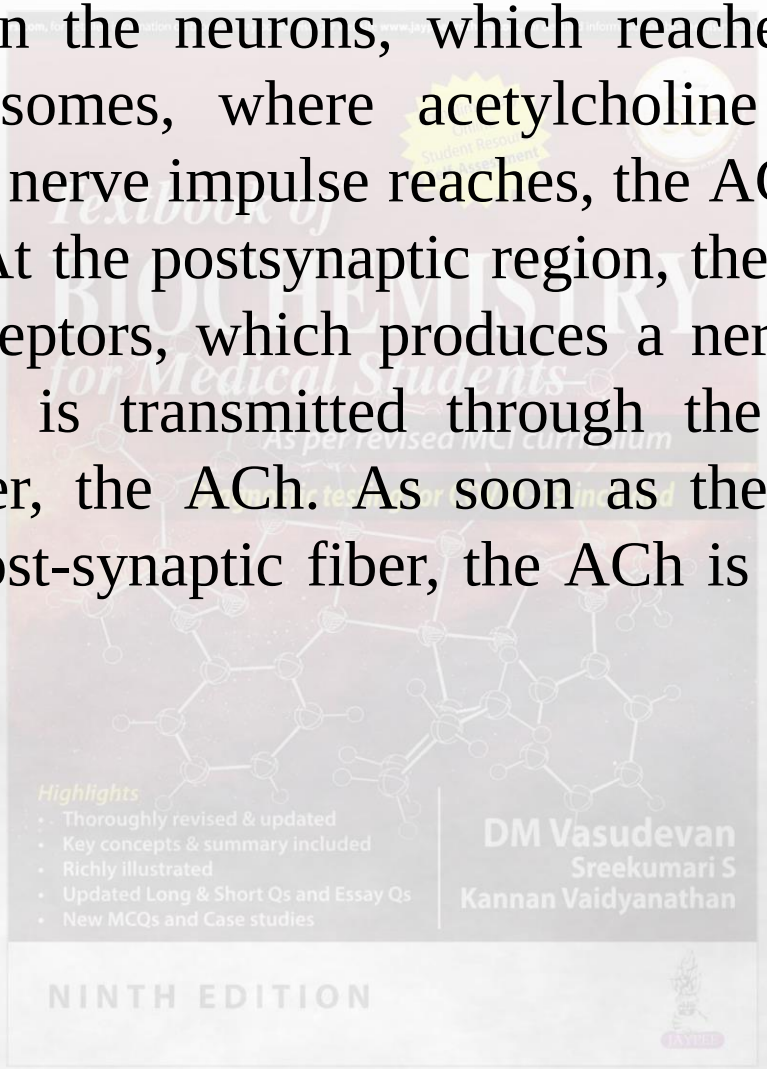
1. **Phospholipid synthesis :** Choline is a part of **phospholipids** which are seen in membranes of all cells.
2. **Myelin sheath:** is made by phospholipids.
3. **Fatty liver:** Choline is able to prevent fatty liver and cirrhosis.
4. **Transmethylation reactions:** Choline can donate three methyl groups to the one-carbon pool. Finally, these methyl groups are transferred to homocysteine to produce methionine, which is used for transmethylation reactions.
5. **Acetylcholine (ACh) synthesis:**

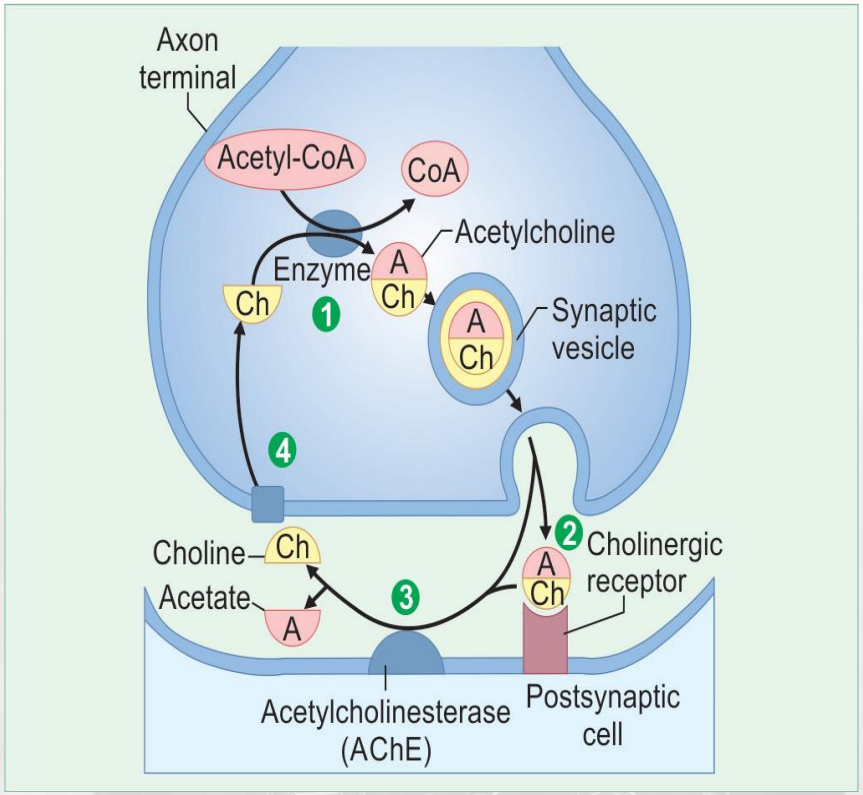
Choline + Acetyl-CoA → Acetylcholine + CoA (enzyme, choline acetylase)

Acetylcholine → Choline + Acetate (enzyme, Acetylcholine esterase)

Acetylcholine

It is synthesized in the neurons, which reaches the presynaptic region of synaptosomes, where acetylcholine (ACh) is stored in packets. When a nerve impulse reaches, the ACh is liberated into the synaptosome. At the postsynaptic region, the ACh is picked up by the specific receptors, which produces a nerve impulse. Thus, the nerve impulse is transmitted through the synapses by the chemical messenger, the ACh. As soon as the nerve impulse is generated in the post-synaptic fiber, the ACh is hydrolyzed by the ACh-esterase.





Acetylcholine transmits the nerve impulse from presynaptic region to postsynaptic region of the nerve. (1) ACh is synthesized in the presynaptic region; (2) ACh attached to receptor on postsynaptic cell; (3) ACh is hydrolyzed by ACh-esterase in the synaptic cleft; (4) Choline is transported back to axon and is reused.

Clinical Applications of Acetylcholine

1. The postsynaptic receptors for ACh may be competitively blocked by **succinylcholine**, a structural analogue of ACh. Then ACh cannot act, producing muscle relaxation. It was used widely as a **muscle relaxant** during surgery.
2. Cholinesterase inhibitors (e.g. **neostigmine**) will allow prolonged action of ACh and therefore nerve impulse is sustained. These drugs are used in **myasthenia gravis**. Myasthenia is an autoimmune condition, where antibodies are produced against neuromuscular end plate proteins destroying the end plates.
3. Pseudocholinesterase is seen in RBCs, more in immature cells.

Role of B complex vitamins in brain function

Vita-min	Co-enzyme	Role in metabolism	Role in brain
Thia-mine	TPP	Oxidative decarboxylation of alpha-keto acids. Transketolase of HMP pathway	Modulatory role in neurotransmitter function of acetylcholine
Ribo-flavin	FAD and FMN	Oxidation-reduction reactions and electron transport chain	FMN and FAD are required for recycling of Niacin, B6, folate, and hemoproteins like Hb, NOS, P450 enzymes

Role of B complex vitamins in brain function

Vita-min	Co-enzyme	Role in metabolism	Role in brain
Niacin	NAD and NADP	NAD for Oxidation-reduction reactions and ETC.	A high affinity NIACR1 is responsible for the peripheral flushing whereas a low affinity NIACR2 is significant in immune cells and adipose tissue lipolysis

Role of B complex vitamins in brain function

Vita-min	Co-enzyme	Role in metabolism	Role in brain
Pyri-doxine	PLP	Amino acid metabolism. Transamination, gluconeogenesis, alpha decarboxylation of amino acids	Synthesis of neurotransmitters like dopamine, serotonin and melatonin. Even mild deficiency can cause down regulation of GABA and serotonin synthesis.
Panto-thenic acid	Co-enzyme A	Active form of fattyacyl groups in fatty acid and cholesterol metabolism	Essential for the synthesis of cholesterol, steroid, phospholipids and neurotransmitters in the brain cells.

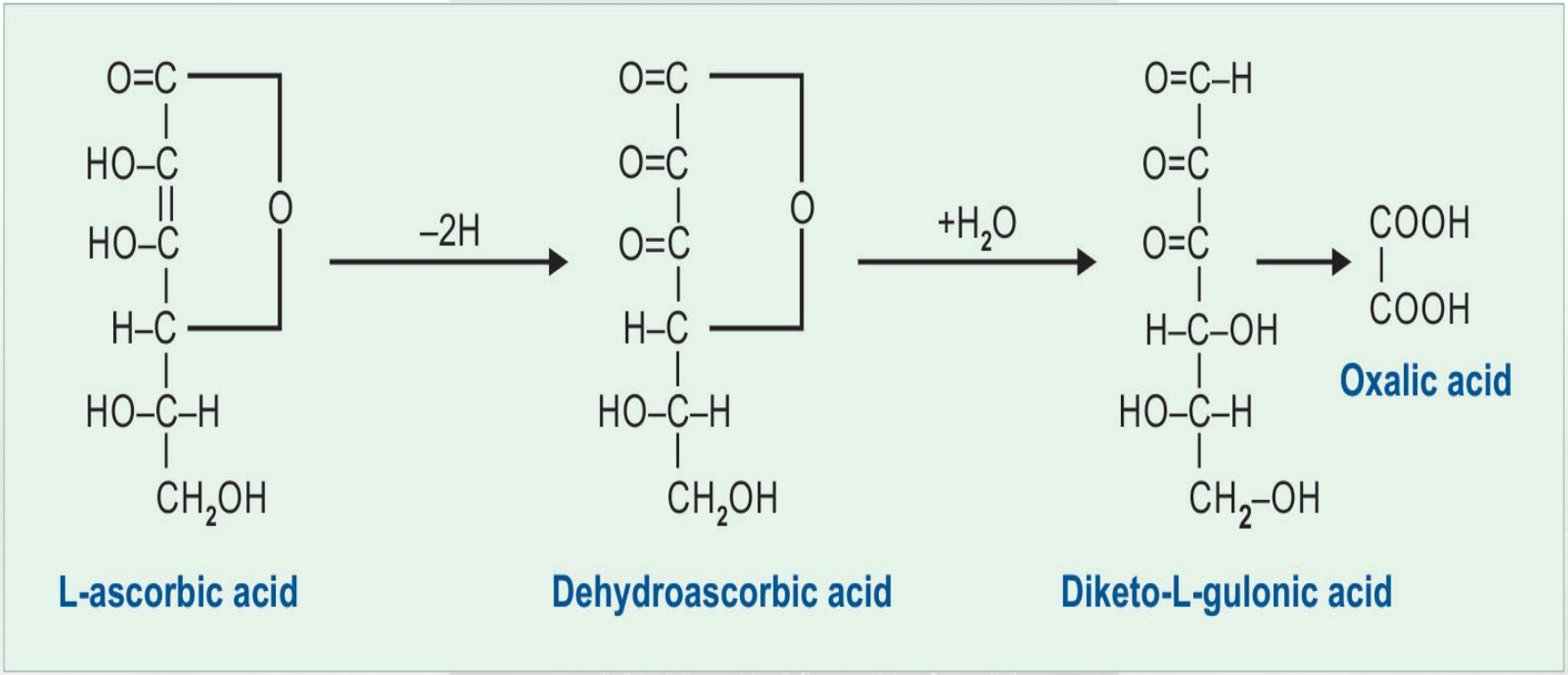
Role of B complex vitamins in brain function

Vita-min	Co-enzyme	Role in metabolism	Role in brain
Biotin	Biotin	Carboxylation reactions in gluconeogenesis and lipogenesis.	Deficiency affects uptake of glucose by hepatocytes and brain cells.
Folic acid	THFA	One-carbon group transfer for synthesis of nucleotides, amino acid metabolism, methyl group transfer.	A decrease in purine and pyrimidine nucleotide synthesis and non-genomic methylation of DNA. The effects of folate and B_{12} deficiencies are linked by the folate trap.

Role of B complex vitamins in brain function

Vita-min	Co-enzyme	Role in metabolism	Role in brain
B_{12}	Ado-B_{12}	Recycling of THFA and carrier of methyl groups.	In B_{12} deficiency, neurological signs and symptoms manifest before hematological effects

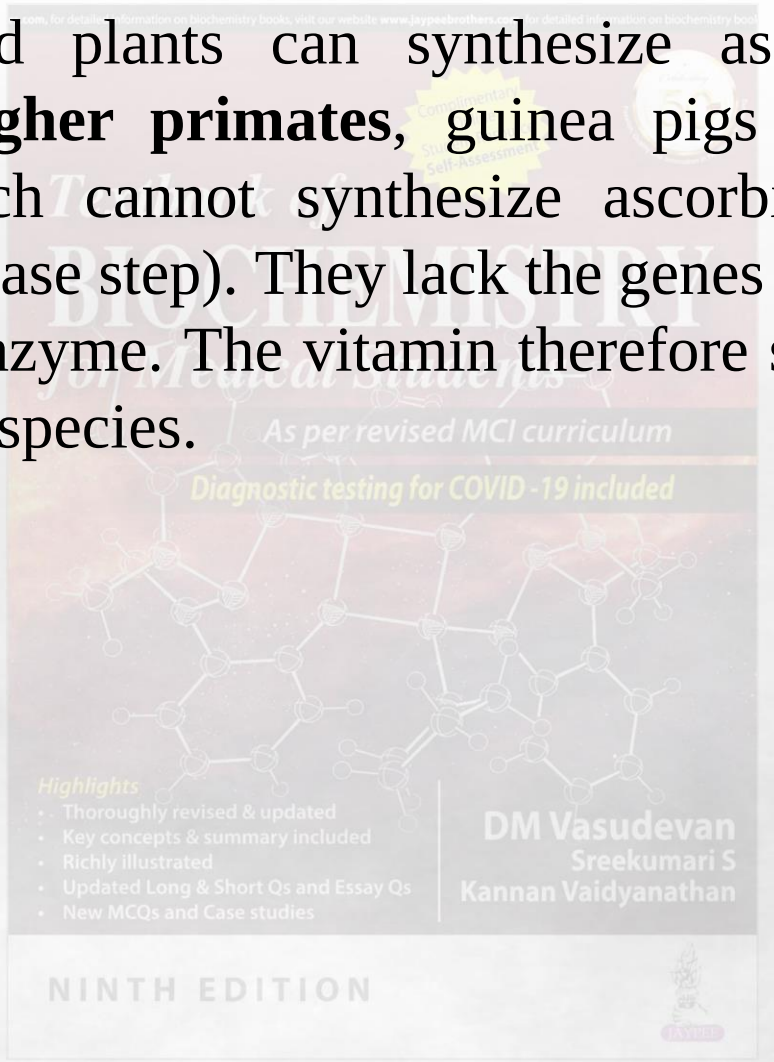
Ascorbic Acid (Vitamin C)



Vitamin C; structure and catabolism.

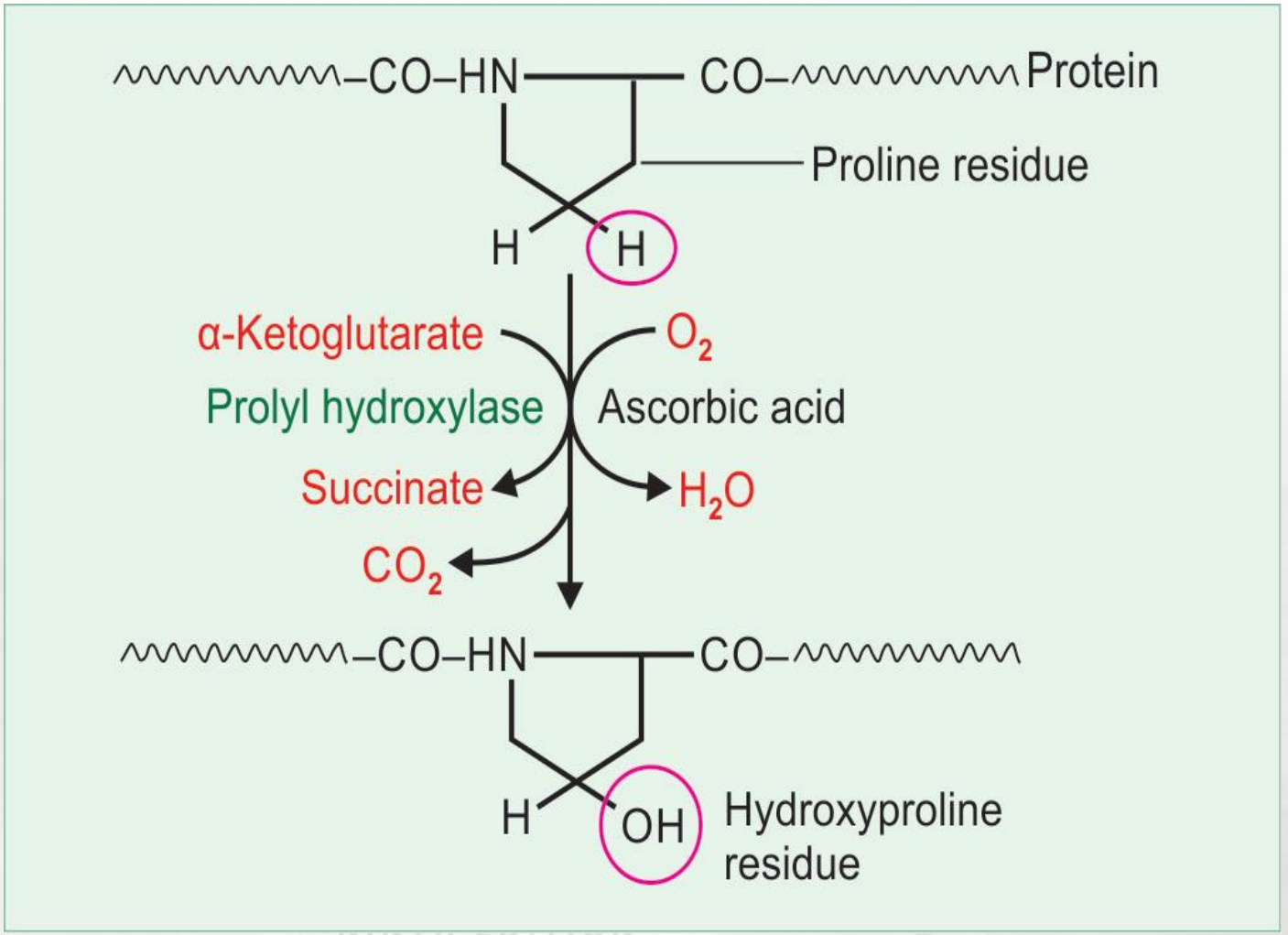
Biosynthesis of Ascorbic Acid in Animals

Most animals and plants can synthesize ascorbic acid from glucose. **Man, higher primates**, guinea pigs and bats are the only species which cannot synthesize ascorbic acid (block in gulonolactone oxidase step). They lack the genes responsible for the synthesis of this enzyme. The vitamin therefore should be supplied in the diet of these species.



Biochemical Functions of Vitamin C

1. **Hydroxylation of proline and lysine:** Hydroxyproline and hydroxylysine are essential for the formation of cross links in the **collagen**, which give the tensile strength to the fibers.
2. **Tryptophan metabolism:** hydroxylation of tryptophan to 5-hydroxy tryptophan, for synthesis of serotonin.
3. **Tyrosine metabolism:** para-hydroxy phenylpyruvate to homogentisic acid.
4. **Iron metabolism:** Ascorbic acid reduces ferric iron to ferrous state.
5. **Hemoglobin metabolism:** Reconversion of met-hemoglobin to hemoglobin.
6. **Folic acid metabolism:** Helps the enzyme folate reductase.
7. **Steroid synthesis:** Vitamin C helps in the synthesis of bile acids from cholesterol. The initial 7 alpha-hydroxylase step is stimulated by the vitamin.
8. **Phagocytosis:** Ascorbic acid stimulates phagocytic action of leukocytes.
9. **Antioxidant property:** Aniline dyes are known to induce bladder cancer in factory workers. Daily intake of vitamin C reduces this risk for cancer.



Hydroxylation of proline to hydroxyproline needs ascorbic acid.

Deficiency Manifestations of Vitamin C

Scurvy

Gross deficiency of vitamin C results in scurvy. Deficiency in children is named as infantile scurvy or **Barlow's disease**.

Hemorrhagic Tendency

In ascorbic acid deficiency, **collagen is abnormal** and the intercellular cement substance is brittle. So, capillaries are fragile, leading to the tendency to bleed even under minor pressure. Subcutaneous hemorrhage may be manifested as **petechia** in mild deficiency and as **ecchymosis** or even hematoma in severe conditions.

Internal Hemorrhage

In severe cases, hemorrhage may occur in the conjunctiva and retina. Internal bleeding may be seen as epistaxis, hematuria or melena.

Deficiency Manifestations of Vitamin C, Continued

Oral Cavity

In severe cases of scurvy, the gum becomes painful, swollen, and spongy. The pulp is separated from the dentine and finally teeth are lost. Wound healing may be delayed.

Bones

In the bones, the deficiency results in the failure of the osteoblasts to form the intercellular substance, **osteoid**. The deposition of bone is arrested. The resulting scorbutic **bone is weak** and fractures easily. There may be hemorrhage into joint cavities. Painful swelling of joints may prevent locomotion of the patient.

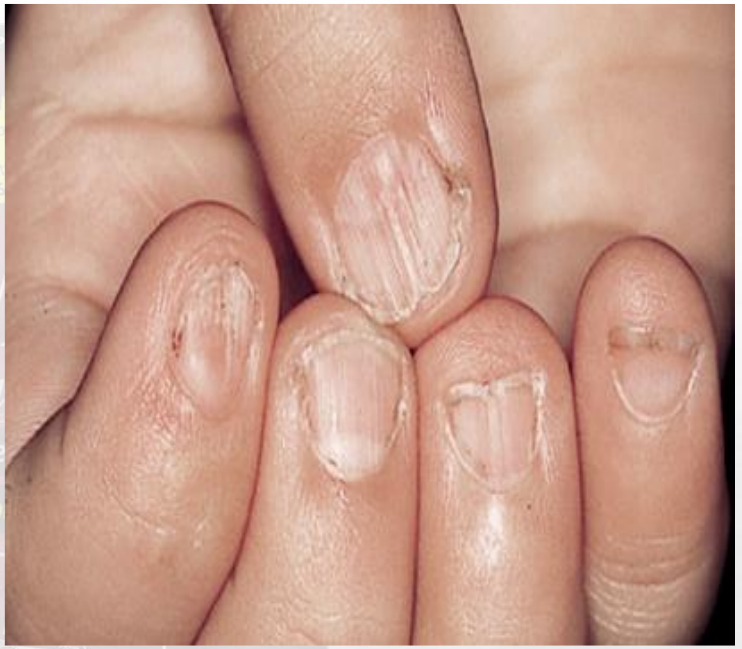
Nails

Spoon-shaped nails are characterized by their concave shape.

Anemia

In vitamin C deficiency, microcytic, hypochromic **anemia** is seen. Poikilocytosis and anisocytosis are also common in anemia due to deficiency of vitamin C.

Gingivitis and bleeding gum in Vitamin C deficiency.



Vitamin C deficiency anifestation in nails. Spoon-shaped nails with red spots in the nail bed, known as splinter hemorrhage.

Dietary Sources of Vitamin C

Rich sources are amla (Indian gooseberry), guava, lime, lemon and green leafy vegetables.

Requirement of Vitamin C

Recommended daily allowance is 75 mg/day (equal to 50 mL orange juice). During pregnancy, lactation, and in aged people requirement may be 100 mg/day.

Therapeutic Use of Vitamin C

Vitamin C is used as an adjuvant in infections. Beneficial effect of ascorbic acid is reported in the treatment of tuberculosis, when plasma level is kept near to saturation point. Clinical dose is 500 mg/day.

Because of its power to **heal wounds**, vitamin C has been recommended for treatment of ulcer, trauma, and burns.

Name	Coenzyme form	RDA	Main reaction using the coenzyme	Deficiency disease
Thiamine	Thiamine pyrophosphate (TPP)	2–3 mg	Oxidative decarboxy-lation of alpha keto acids	Beriberi
Riboflavin	Flavin adenine dinucleotide (FAD)	2-3 mg	Dehydrogenation, oxidised in ETC (1.5 ATP)	Glossitis, angular stomatitis
Niacin	NAD and NADP	20 mg	Dehydrogenation, oxidised in ETC (2.5 ATP)	Pellagra
Pyri-doxine	Pyridoxal phosphate (PLP)	2–3 mg	Transamination, decarboxylation of amino acids	Seizures, homocysti-nuria

Name	Coenzyme form	RDA	Main reaction using the coenzyme	Deficiency disease
Pantothe-nic acid	Coenzyme A, ACP	5-10 mg	CoA derivatives, acyl carrier proteins	Burning foot syndrome
Biotin	Biotin	40 microg	Carboxylation	No specific disease
Folic acid	Tetrahydrofolic acid (THFA)	300 microg	One-carbon group carrier	Macrocytic anemia
Vitamin B12	Adenosyl B12, methylcobalamin	2 microg	Isomerisation of methylmalonyl-CoA, remethylation of homocysteine to met	Megaloblastic anemia, Sub-acute combined degeneration
Ascorbic acid	No specific form	75 mg	Antioxidant property	Scurvy