Al-Mustaqbal University

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

1. Prof. Dr. Fadhil Jawad Al-Tu'ma, Ph.D., Professor of Clinical Biochemistry.
2. Dr. Dalya Shakir Obaida, Ph.D. Lecturer of Clinical Biochemistry.

Lecture No. 5

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Nutrition, Vitamins and Coenzymes – Lecture 2

Riboflavin or Vitamin B2

Chemistry

1. It is an orange-yellow compound containing a D-ribose alcohol (D-Ribitol) and a heterocyclic parent ring structure **isoalloxazine (*Flavin* nucleus).**
2. ***Stability:*** It is stable to heat in neutral acid solution but not in alkaline solutions.

Biological Active Forms: The biological active forms, in which riboflavin serves as a prosthetic group (coenzyme) of a number of enzymes, are the phosphorylated derivatives, Fig. 1.

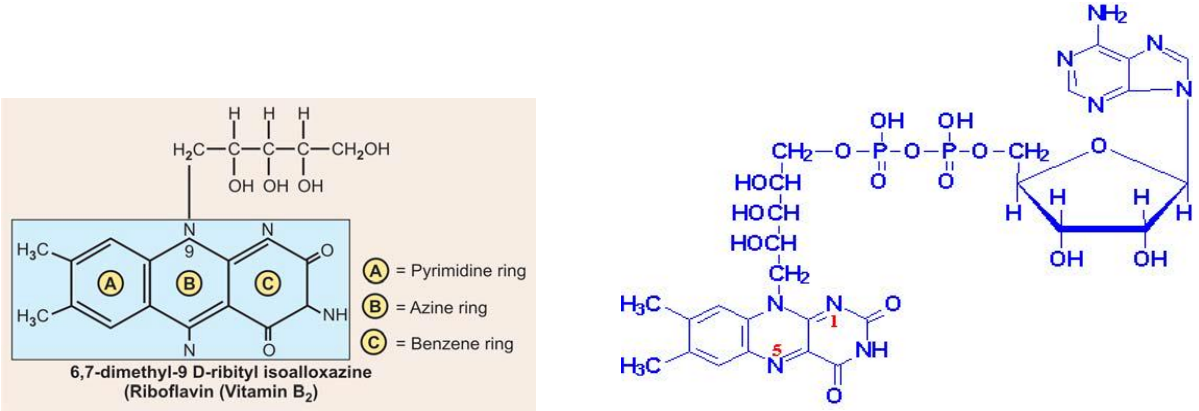

Fig. 1: Riboflavine Coenzymes

Two main derivatives are:

1. **FMN (Flavin mononucleotide):** In this the phosphoric acid is attached to ribityl alcoholic group in position 5.

   Flavin-Ribityl-PO_4

2. **FAD (Flavin adenine dinucleotide):** It may be linked to an adenine nucleotide through a pyrophosphate linkage to form FAD.

   Flavin-ribityl-P-P-ribose-Adenine

Thus, **FMN and FAD are two coenzymes of this vitamin.**

Biosynthesis

All higher plants can synthesize riboflavin. In nature, it occurs both as "free form" and also as "nucleotide" form or as flavoproteins.

Human beings and animals cannot synthesize and hence solely dependent on dietary supply. ***In man, considerable amounts can be synthesized by intestinal bacteria,*** but the quantity absorbed is not adequate to maintain normal nutrition.

FMN	FAD
1. Warburg's yellow enzyme	Xanthine oxidase (Xanthine → Uric acid)
2. Cytochrome-C-reductase	D-amino acid oxidase
3. L-amino acid oxidase	Aldehyde oxidase
	Fumarate dehydrogenase (Succinate → Fumarate)
	Glycine oxidase
	Acyl-CoA dehydrogenase
	Diaphorase

Metabolism

Absorption: Flavin nucleotides are readily absorbed in small intestine. **Free riboflavin undergoes phosphorylation, a prerequisite for absorption.**

Blood or Plasma level: Human blood plasma contains 2.5 to 4.0 µg%, two-third as FAD and bulk of remainder as FMN. Concentration in red bloods cells 15 to 30 µg/100 gm. Leucocytes and platelets is 250 µg/100 gm. ***These values remain quite constant even in severe riboflavin deficiency; hence determination of riboflavin in blood is not useful.*** Riboflavin present in all tissues as nucleotides bound to proteins (FP), highest concentration in liver and kidney.

Excretion: Daily urinary excretion ranged between 0.1 to 0.4 mg (10 to 20% of intake).

a. **Milk:** Riboflavin is secreted in milk, 40 to 80 per cent in 'free' form.
b. **Feces:** Free and nucleotides tend to remain quite constant, 500 to 750 µg daily, largely from the unabsorbed bacterial synthesis.

Occurrences and Food Sources

Widely distributed in nature, in all plant and animal cells.

a. ***Plant sources:*** High concentration occurs in yeasts. Appreciable amount present in whole grains, dry beans and peas, nuts, green vegetable.
b. ***Animal source:*** Liver (2–3 mg/100 gm), kidney, milk, eggs.

Metabolic Roles:

FMN and FAD act as coenzymes in various Hydrogen-transfer reactions in metabolism. The hydrogen is transported by reversible reduction of the coenzyme by two hydrogen atoms added to the 'N' at positions 1 and 10, thus forming dihydro or leucoriboflavin.

Clinical Aspects:

Deficiency Manifestations

There is **no definite disease entity**. Deficiency is usually associated with deficiencies in other B-vitamins.

In protein synthesis there is impaired in severe riboflavin deficiency; since protein malnutrition interferes with utilization and retention of riboflavin.

Daily Requirement: Exact human requirement is not known and r***elated to degree of protein utilization.***

Recommended Daily Intake

1. Adults: 1.5 to 1.8 mg
2. Women in later half of pregnancy: 2.0 mg
3. During lactation: 2.5 mg
4. Infants: 0.6 mg
5. Children: 1.0 to 1.8 mg

Requirement increases after severe injury / burns, during increased protein utilization, in pregnancy and lactation, during oral broad spectrum antibiotic therapy.

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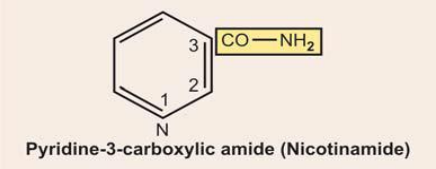
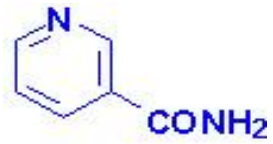
Niacin or Vitamin B3:

Chemistry: Nicotinic acid (niacin) is chemically **pyridine-3-carboxylic acid.**

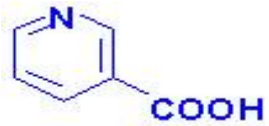
In tissues: Occurs principally as the **amide** (nicotinamide, niacinamide).



Pyridine-3-carboxylic amide (Nicotinamide)



Nicotinamide



Nicotinic Acid

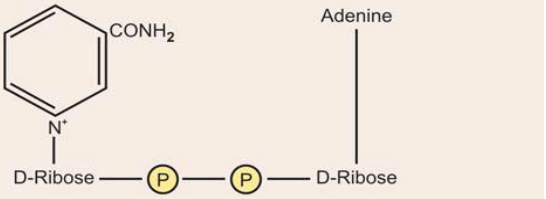
Biological "Active" Forms

In tissues, nicotinamide is present largely as a "dinucleotide", the pyridine 'N' being linked to a D-ribose residue.

Two such nucleotide active forms are known, Figure 2:

1. **Nicotinamide adenine dinucleotide (NAD$^+$)**. The compound contains:

- One molecule of nicotinamide,
- Two molecules of D-ribose,
- Two molecules of phosphoric acid, and
- One molecule of adenine. Structure may be shown schematically as follows:



- ***Nicothinamide adenine dinucleotide phosphate (NADP$^+$)*** Other names are TPN$^+$, *co-enzyme II.*

This compound differs from NAD$^+$ in that **it contains an additional molecule of phosphoric acid attached to 2-position of D-ribose attached to N-9 of Adenine.** The reduced form of either coenzymes is designated by the prefix "dihydro-nicotinamide adenine dinucleotide (NADH).

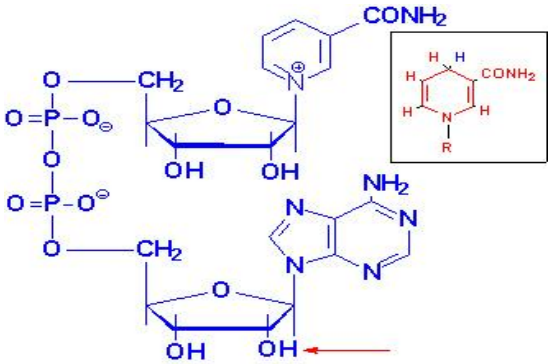


Fig. 2: Coenzyme forms of nicotiamide

Biosynthesis

1. ***Amino acid tryptophan is a precursor of nicotinic acid*** in many plants, and animal species including human beings. ***60 mg of tryptophan can give rise 1 mg of Niacin.*** Pyridoxal-P is required as a coenzyme in this synthesis.
2. **It can be synthesized also by intestinal bacteria**.
3. **In human beings:** In addition to dietary source, it is synthesized in tissues from amino acid tryptophan, and to a limited extent supplemented by bacterial synthesis in intestine.

Metabolism

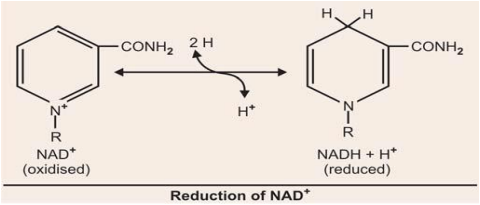
Absorption: Nicotinic acid and its amide are absorbed from the small intestine.

Blood/plasma level:

- **a.** Whole blood: 0.2 to 0.9 mg/100 ml (average 0.6 mg%)
- **b.** RBCs: 1.3 mg%. Most of the nicotinic acid and its amide in the blood are in RB cells, presumably as coenzyme.
- **c.** Plasma-total activity: 0.025 to 0.15 mg% (average 0.075 mg%)

Excretion: ***In urine,*** it is excreted as follows:

- ***a. As nicotinic acid and nicotinamide:*** Normal adults on normal diet excrete both nicotinic acid and its amide in urine. **Nicotinic acid:** 0.25 to 1.25 mg daily. **Nicotinamide:** 0.5 to 4 mg daily.
- ***b. As N'-methyl nicotinamide***.
- **c.** Glycine conjugates of methyl derivatives are also excreted. Methylation, oxidation and conjugation take place in liver.



Occurrence and Food Sources

1. Both nicotinamide and coenzyme forms are distributed widely in plants and animals.
2. Important food sources are:
   - **a.** ***Animal source:*** Liver, kidney, meat, fish
   - **b.** ***Vegetable source:*** Legumes (peas, beans, lentils), nuts, certain green vegetables, coffee and tea. Poor sources are: Fruits, milk and eggs.

Metabolic Roles

The coenzymes NAD^+ and $NADP^+$ operate as hydrogen and electron transfer agents by virtue of reversible oxidation and reduction, see **Box-1.**

Clinical Aspects:

Deficiency Manifestations: Nicotinic acid deficiency produces a disease called **pellagra.** Cardinal features described as **"3 D's"** are

a. **Dermatitis**
b. **Diarrhea**
c. **Dementia**

Clinical Features

a. **Skin lesions:**
b. **GI manifestations:** Include vomiting, abdominal pain, with alternating constipation / diarrhea.
c. **Cerebral manifestations:** These include headache and other mental symptoms ranging from mild psychoneuroses to severe psychosis.
d. **General effects:** These include inadequate growth, loss of weight and strength, anemia which may be due to associated deficiency of other vitamins, dehydration and its consequences resulting from diarrhea.
e. **In Hartnup disease**: Pellagra may be associated. Tryptophan deficiency occurs due to genetic defect in membrane transport mechanism for tryptophan resulting to great **loss of the amino acid.**

Daily Requirement

In adult: 17 to 21 mg daily and Infants: 6 mg

Requirement Increases in:

Increased calorie intake, acute illness or early convalescence, after severe injury, infection and burns, pregnancy and lactation.

Effect on plasma Lipids: Nicotinic acid and NOT nicotinamide have been found to reduce the plasma lipid concentration in certain cases of hyperlipidaemia. Large doses of nicotinic acid from 3 to 6 grams per day have been found to reduce the levels of cholesterol and TG.

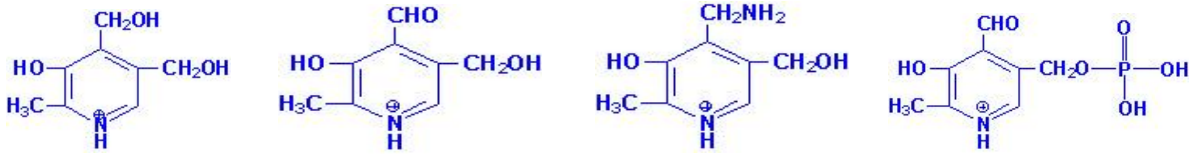
Box-1

NAD^+	$NADP^+$
• *Alcohol dehydrogenase* (Ethanol → Acetaldehyde) • *Lactate dehydrogenase (LDH)* (PA ↔LA) • *Malate dehydrogenase* (Malate → OAA) • *Glyceraldehyde-3-P-dehydrogenase* (Gly-3-P → 1, 3-di-phosphoglycerate) • α-Glycero-P-dehydrogenase • *Pyruvate dehydrogenase complex (PDH)* (PA → Acetyl-CoA) • α-Ketoglutarate dehydrogenase complex (α-ketoglutarate → succinyl-CoA)	• *Glucose-6-P-dehydrogenase (G-6-PD)* (G-6-P → 6-Phosphogluconate) • *Glutathione reductase* **Either NAD^+ or $NADP^+$** • *Glutamate dehydrogenase* (Glutamate → α-ketoglutarate + NH_3) • *Isocitrate-dehydrogenase* (I C D) (Isocitrate → Oxalosuccinate)

Pyridoxine or Vitamin B6:

Chemistry

1. **Pyridoxal (Pyridoxine) or** chemically 2-methyl-3-OH-4,5-di (hydroxy methyl) pyridine.
2. It occurs in association with an aldehyde-**pyridoxal** and an amine **pyridoxamine** form. **All three forms exhibit vitamin B6 activity.**



Pyridoxine **Pyridoxal** **Pyridoxamine** **Pyridoxal-5'-phosphate**

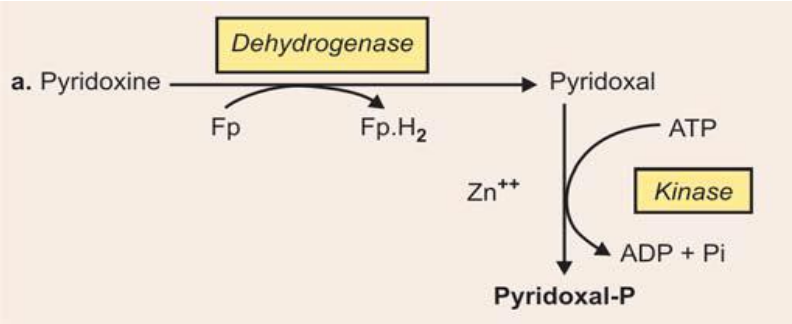
Biological 'Active' Forms

Biological 'active' forms of the vitamin B6 are **pyridoxal-PO_4**, and **pyridoxamine-PO_4**

The active forms are the phosphorylated derivatives; phosphorylation involves the hydroxymethyl group $–CH_2OH$ at position 5 in the pyridine ring. These forms occur in nature largely in combination with protein (apoenzyme).

Formation of pyridoxal-P: Phosphorylation takes place in liver, brain and other tissues with the help of ATP, Zn^{++} and an enzyme *pyridoxal kinase.*

Two pathways shown below:



Biosynthesis: Vitamin B6 can be formed by many microorganisms and probably also by plants. ***Human beings cannot synthesize the vitamin, hence has to be provided in the diet. Intestinal bacteria can synthesize the vitamin.***

Metabolism

Absorption: Dietary vitamin B6 is readily absorbed by the intestine.

Excretion

1. Pyridoxal and pyridoxamine are excreted in urine in small amounts 0.5 to 0.7 mg daily.
2. Major urinary metabolite, about 3 mg daily is the biologically inactive form 4-pyridoxic acid.

Pyridoxic acid

Occurrence and food sources: The vitamin is distributed widely in animal and plant tissues. Rich sources of the vitamin are yeast, rice polishings, germinal portion of various seeds and cereal grains and egg-yolk. Moderate amounts are present in liver, kidney, muscle, fish. ***Milk is a poor source.*** Highest concentration occurs in royal jelly (bee).

Metabolic Role: Pyridoxal phosphate acts as a coenzyme, it is **principally involved with metabolism of amino acids.**

1. It acts as a coenzyme for the enzyme transaminases or (aminotransferases) in transamination reactions of amino acids.
2. It acts as coenzyme for the enzyme decarboxylases in decarboxylation reaction. Amino acids are decarboxylated to form corresponding amines. **Examples:**

   Tyrosine ----------► Tyramine + CO_2

   Histidine ----------► Histamine + CO_2

   Glutamic acid ----------► G A B A + CO_2
3. **Acts as coenzyme for deaminases (dehydrases):** Catalyzes non-oxidative deamination of OH-amino acids viz., serine, threonine, etc.
4. **Transulfuration:** It takes part in transulfuration reactions involving transfer of –SH group, e.g.

   Homocysteine + Serine ----------► Homoserine + cysteine.
5. **As coenzyme for desulfhydrases:** It catalysis non-oxidative deamination of cysteine in which H_2S is liberated.
6. **Pyridoxal-P** is required as a coenzyme in the biosynthesis of arachidonic acid from linoleic acid.
7. **Intramitochondrial FA biosynthesis:** Required as a coenzyme with condensing enzyme for chain elongation of FA in intramitochondrial FA synthesis.
8. **Transport of K^+:** Vitamin B6 has been reported to promote transport of K^+ across the membrane from exterior to interior.
9. **Biosynthesis of CoA-SH (Coenzyme A):** Vitamin B6 is involved in synthesis of coenzyme A from pantothenic acid. In B6 deficiency, coenzyme A level in Liver is reduced.
10. **In porphyrin synthesis:** Pyridoxal-P is required for the biosynthesis of heme. **In B6-deficiency heme synthesis suffers and leads to anemia.**

11. **Immune response:** In vitamin B6 deficiency, immune response is impaired.

Clinical Aspects: **No deficiency disease has been described.** But following clinical manifestations are attributed to vitamin B6 deficiency.

1. **Epileptiform convulsions in infants:** have been attributed to pyridoxine deficiency.
2. **Pyridoxine responsive anemia:** A hypochromic microcytic anemia called as sideroblastic anemia, with high serum Fe level and hemosiderosis of liver, spleen and bone marrow may occur with B6-deficiency.
3. **Isonicotinic acid hydrazide treatment in tuberculosis:** A syndrome resembling vitamin B6 deficiency has been observed in humans during the treatment of tuberculosis with high doses of tuberculostatic drug Isonicotinic acid hydrazide or Isoniazid (INH).

Therapeutic uses: Vitamin B6 has been found empirically to be of value in treatment of: Nausea and vomiting of pregnancy ("morning sickness"), radiation sickness, muscular dystrophies, treatment of hyperoxaluria, and recurring oxalate stones of kidney, and mild forms of pyridoxine deficiency have been reported to occur sometimes in women taking oral contraceptives containing estradiol.

Recently it has been observed that a **higher intake of vitamin B6 may decrease the risk of Parkinson's disease.** It could lower Parkinson's disease risk by protecting brain cells from damage caused by free radicals.

Pyridoxine Status vs Hormone Dependent Cancer. Increased sensitivity to steroid hormones action may be important in the development of hormone-dependent cancer of the breast, prostate and uterus; hence pyridoxine status may affect the prognosis.

Daily requirement: It has been difficult to establish definitely the human requirement of vitamin B6 due to the fact that

a. quantity needed is not large

b. bacterial synthesis in intestine provides a portion of the requirement.

There is evidence that ***requirement of Vitamin B6 is related to dietary protein intake***, as it is involved as coenzyme in many metabolic reactions of amino acid metabolism.

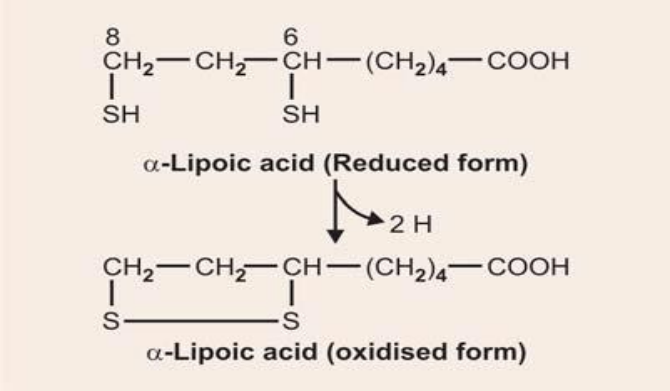
a. Adult : 2 mg/day

b. Infants : 0.3 to 0.4 mg/day

During second half of pregnancy: 2.5 mg/day In patients receiving antitubercular treatment with INH, requirement of vitamin B6 increases much.

Lipoic Acid (Thioctic Acid):

Chemistry: It is a ***sulphur containing*** fatty acid called 6,8-dithiooctanoic acid (α-lipoic acid or thioctic acid). It contains eight carbon and two sulphur atoms. Oxidized and reduced forms of the compound is shown below.



Metabolic Role of Lipoic Acid

It is recognized as an essential component in metabolism although it is active in extremely minute amounts.

1. **As a coenzyme of pyruvate dehydrogenase complex (PDH):** It is required along with other coenzymes in oxidative decarboxylation of pyruvic acid to acetyl-CoA.
2. **As a coenzyme of α-oxoglutarate dehydrogenase complex:**
3. **Lipoic acid is also required for the action of the enzyme sulphite oxidase:** Required for conversion of SO_2^- to $SO_4^=$. Hypoxanthine is also required for the action.

Clinical Aspect:

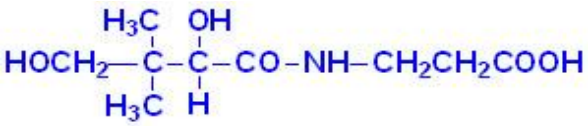
1. **Antioxidant Property:** Recently it has been shown that lipoic acid/or dihydrolipoic acid in large dosage 100 to 500 mg/day can act as an **antioxidant.** This property has been utilized in treatment of certain diseases (therapeutic uses).
2. May be useful in prevention of myocardial infarction and stroke.
3. Can prevent conditions like multiple sclerosis, Alzheimer's disease.
4. Helps in reducing the plasma low density lipoproteins (LDL).
5. Stimulates production of glutathione (G-SH).

Pantothenic Acid or Vitamin B5

Chemistry

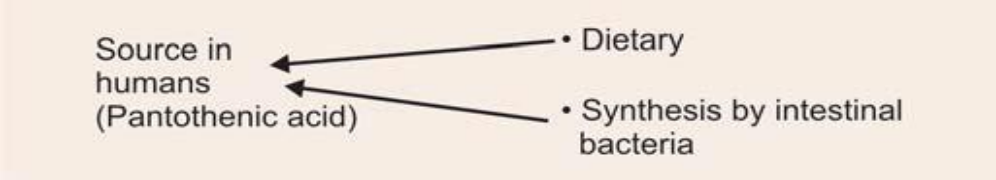
1. Pantothenic acid consists of β-alanine in peptide linkage with a dihydroxy dimethyl butyric acid ('Pantoic' acid).

β-Alanine + Pantoic acid ----------► Pantothenic acid



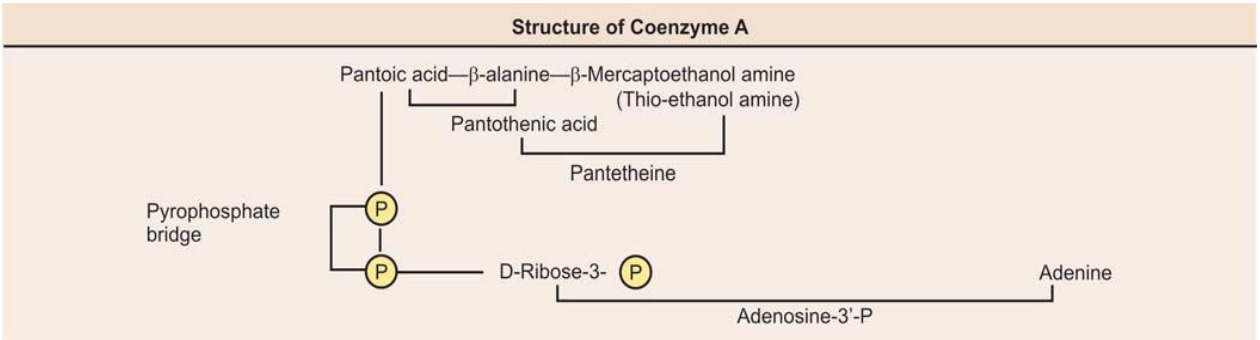
The inactive form of vitamin B_5 or pantothenic acid

2. The free acid is soluble in water and is hydrolyzed by acids/or alkalis. It is thermolabile and destroyed by heat.



Biological "Active" Form. In tissues, this vitamin is present almost entirely in the form of the coenzyme (coenzyme A) and largely bound to proteins (apoenzyme). It may be released from this combination by certain proteolytic enzymes, certain phosphatases, and a liver enzyme system.

Structure of Coenzyme A: Structure of coenzyme A can be represented schematically as follows:



Biosynthesis and Metabolism

1. ***Biosynthesis Pantothenic acid***
   a. In many microorganisms, including yeast pantothenic acid is synthesized by direct coupling of β-alanine and pantoic acid.
   b. Human tissues cannot synthesize pantothenic acid hence it has to be obtained from diet. In addition to dietary source, synthesis by intestinal bacteria supply fair amount of pantothenic acid.
2. ***Whole blood level:*** The concentration of pantothenic acid in whole blood is 15–45 µg/100 ml (average 30 µg%). It is present in all tissues in small amounts, the highest concentration occurring in liver (40 µg per gram wt) and kidney (30 µg/gm).
3. ***Excretion:***
   a. **Urine:** Under ordinary dietary conditions about 2.5 to 5 mg are excreted daily in the urine.
   b. **Sweat:** 3–4 µg/100 ml.
   c. **Milk:** Secretes 200 to 300 µg/100 ml.

Occurrence and Food Sources

It is widely distributed in plants, animal tissues and food materials.

1. ***Excellent food sources*** (100 to 200 µg/gm of dry materials): include kidney, liver, egg-yolk and yeasts, cereals and legumes.

2. Fair sources (35 to 100 µg/gm): Include milk, chicken, certain fishes and sweet potatoes.
3. Most vegetables and fruits are rather poor source.
4. Richest known source of pantothenic acid is Royal Jelly (also rich in Biotin and pyridoxine).

Metabolic Role

Pantothenic acid is essential to several fundamental metabolic reactions.

1. **Formation of active acetate (Acetyl-CoA):** It readily combines with acetate to form Acetyl-CoA or "Active" acetate, which is metabolically active.

$$CH_3—\overset{\overset{O}{\|}}{C}\sim S\text{-}CoA$$

Acetyl-CoA chemically is $CH_3—C\sim S\text{-}CoA$, the sulphur bond of acetyl-CoA is a high energy bond equivalent to that of the high energy PO_4 bond of ATP. In the form of active acetate, it participates in a number of important metabolic reactions, e.g.

   a. Utilized directly by combination with oxaloacetate (OAA) to form citric acid, which initiates TCA cycle.
   b. Acetylcholine formation.
   c. For acetylation reactions.
   d. Synthesis of cholesterol.
   e. Formation of ketone bodies.
   f. Acetyl-CoA and malonyl-CoA are used in the synthesis and elongation of fatty acids.

2. **Formation of active succinate (Succinyl-CoA):** Succinyl-CoA is involved in certain important metabolic reactions as follows:
   a. **Heme synthesis:** In heme synthesis, "active" succinate and glycine combines in the first step leading to heme formation. **Applied aspects:** Anemia may occur in pantothenic acid deficiency probably due to deficiency in formation of succinyl-CoA.
   b. **Degradation of ketone bodies by extrahepatic tissues:** Refer to Ketolysis.
3. **Role in lipid metabolism:**
   a. **Oxidation of Fatty Acid (β-oxidation):**
   b. **Biosynthesis of FA:**
4. **Role in Adrenocortical function:** Pantothenic acid appears to be involved in adrenocortical activity, being essential to the formation of adrenocortical hormones from "active" acetate and cholesterol. Pantothenic acid deficient animals have reduced levels of adrenal cholesterol.

Daily requirement: The human requirement of pantothenic acid is not known due to its widespread distribution.

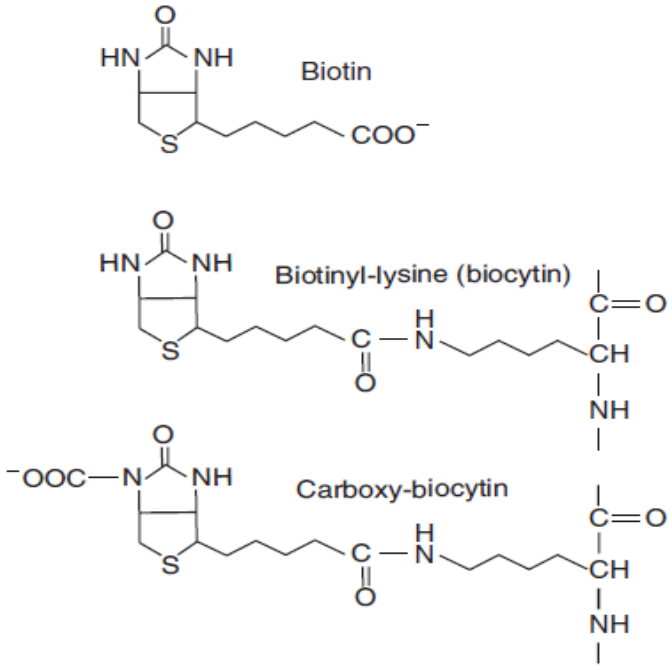
a. **For adults** it is recommended, a daily intake of 5 to 12 mg per 2500 cal.
b. **In infants :** 1 to 2 mg
c. **In children :** 4 to 5 mg.

Requirement Increases in presence of severe stress, e.g. acute illness, burns, severe injury, in pregnancy and lactation, and in growing children.

Biotin or Vitamin 7:

It also called vitamin H or Co-enzyme R or anti-egg-white injury factor.

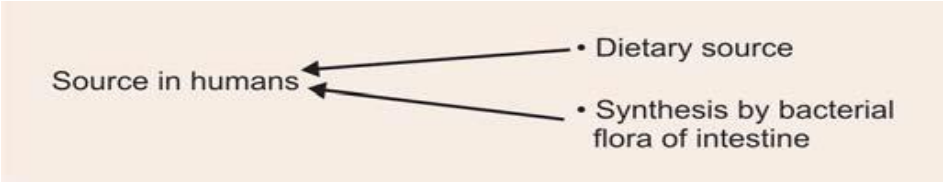
Chemistry: Biotin is a heterocyclic monocarboxylic acid; it is a ***sulphur-containing*** water-soluble B-vitamin.



Biotin, active form biocytin, and carboxy-biocytin.

It consists of two fused rings, one **imidazole** and the other **thiophene** derivative. Two forms with essentially identical biological activities: α**-Biotin** (egg-yolk) and β**-Biotin** (Liver)-differing only in the nature of the side chain. *Biotin is said to occur both as* free *form and* bound *form in tissues and foods.*

Egg-White Injury: Cooked egg white was not found to be toxic. **William and coworkers (1940)** demonstrated that egg-white injury was actually due to ***an antivitamin present in egg-white***. This ***substance*** is ***Avidin***, a basic protein, its ability to inactivate biotin.



Biosynthesis and Metabolism

1. Biotin can be synthesized by many bacteria, yeast, and fungi. In green plants, it may be formed in leaf and root.
2. **Coenzyme-R** is a growth essential for the nitrogen fixing organisms,

Storage: Biotin may be stored to a limited extent in the liver and kidneys.

Excretion: Excreted in urine, feces and milk. Normal adult on an adequate diet excretes 10 to 180 µg daily in the urine and 15 to 200 µg daily in the feces.

Occurrence and food sources: Widely distributed in plants and animal tissues. Occurs chiefly as:

a. Water-soluble form in most plant materials, except cereals and nuts,
b. Mainly in a water-insoluble form in animal tissues.

Foods rich in biotin include:

1. ***Animal sources:*** They are liver, kidney, milk and milk products and egg-yolk.
2. ***Vegetable sources:*** Include vegetables, legumes, and grains which are good sources.

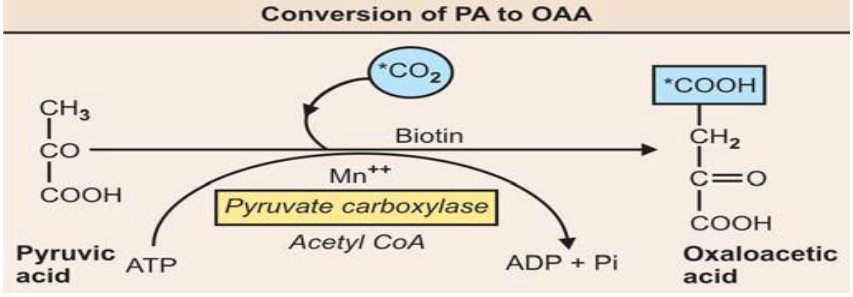
Human beings cannot synthesize the vitamin and hence it has to be supplied in diet. But bacterial flora in intestine can synthesize the vitamin and is a good source.

Metabolic Role

Biotin is the prosthetic group of certain enzymes that catalyze CO_2-transfer reaction (CO_2-fixation reaction). Biotin functions as the coenzyme for the enzyme called carboxylases, which catalyze the CO_2-fixation (Carboxylation). In this process, biotin is first converted to carboxybiotin complex by reaction with HCO_3^- and ATP. "CO_2-biotin complex'' is the source of "active" CO_2 which is transferred to the substrate.

Carbon dioxide fixation reactions

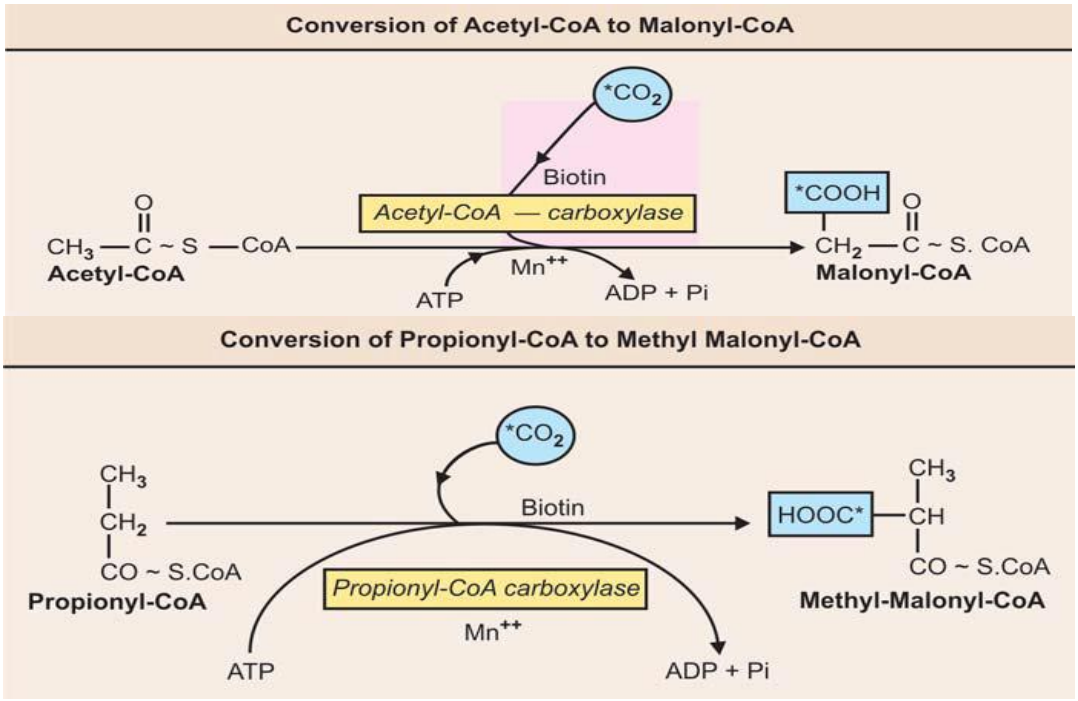
1. **Conversion of acetyl-CoA to malonyl-CoA:** In the first step of extra mitochondrial fatty acid biosynthesis, **see below.**
2. **Conversion of propionyl-CoA to methylmalonyl-CoA:** The enzyme catalyzing the reaction is **propionyl-CoA carboxylase, see below.**
3. **Conversion of pyruvic acid to oxalo-acetate:** The enzyme that catalyzes the reaction is **pyruvate carboxylase.**



Deficiency manifestations: Biotin deficiency may be induced ***in experimental animals:***

1. By inclusion of large amounts of raw-egg white in the diet.
2. By using sulphonamide drugs or broad-spectrum oral antibiotics for prolonged periods.

Human volunteers: Deficiency has been produced by excluding dietary biotin and feeding large amounts of raw egg white (30% of total calories). Such individuals developed following symptoms beginning after 5 to 7 weeks: Dermatitis of the extremities, pallor of skin and mucous membranes, anorexia and nausea, muscle pains and hyperaesthesia, depression, anemia, and hypercholesterolemia.



Role of biotin coenzyme in carboxylation reaction

Deficiency Diseases:

There is **no definite deficiency disease**. But following two conditions have been found to be related to biotin deficiency:

1. **Congenital:** A rare genetic deficiency of **holocarboxylase synthase** has been described. The enzyme helps to utilize biotin in metabolic role. The affected child cannot utilize biotin and develops biotin deficiency which is manifested as dermatitis, graying of hair, loss of hair (alopecia) and incoordination of movements. Urine shows high urinary lactate, β-OH-propionate and β-methyl crotonate due to the failure of corresponding enzyme activities.
2. **Acquired (Leiner's disease):** Leiner's disease in young infants has been investigated. The disease often occurs in breastfed infants, frequently in association with persistent diarrhea.

Mechanism: The low biotin content of human milk together with the poor absorption of biotin due to diarrhea appears to cause biotin deficiency.

Daily requirement: It is difficult to arrive at a quantitative requirement of this vitamin as it is ubiquitous and as intestinal bacteria synthesize and supply the vitamin.

a. Human adults : 25 to 50 µg daily
b. Infants : 10 to 15 µg daily; Children : 20 to 40 µg daily

Requirement Increases in

a. Pregnancy and lactation,
b. Oral antibiotic therapy for prolonged periods.