WATER SOLUBLE VITAMINS

B- Complex group of Vitamins

THIAMINE (VITAMIN B1)

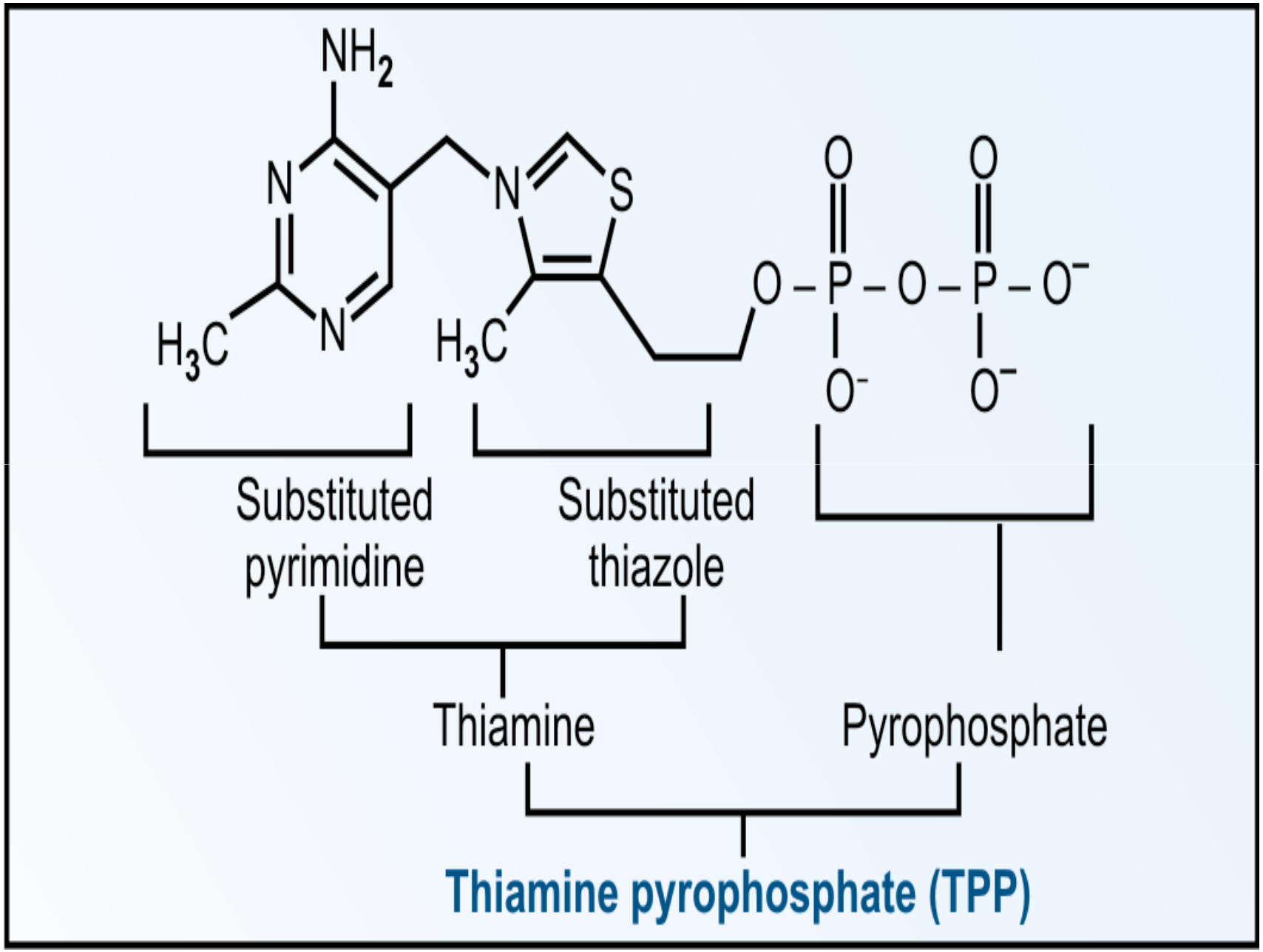
- Also called as vitamin B1.
- Sources:
- A leurone layer of cereals(food grains) is a rich source of thiamine

Whole wheat and unpolished handpound rice have better nutritive value than completely polished refined foods. When the grains are polished, aleurone layer is usually removed.

Yeast is also very good source. Thiamine is partially destroyed by heat.

➢ Structure:

- Thiamine contains a substituted pyrimidine ring connected to a substituted thiazole ring by means of mathylene bridge.
- The vitamin is then converted to its active co-enzyme form by addition of two phosphate groups, with the help of ATP. It is catalysed by thiamine pyrophosphotransferase.


NH2
N
N
S
H3C
N
H3C
O
O
O – P – O – P – O⁻
O⁻
O⁻
Substituted pyrimidine
Substituted thiazole
Thiamine
Pyrophosphate
Thiamine pyrophosphate (TPP)

➢ Physiological role :

1. Pyruvate dehydrogenase: The co-enzyme form is thiamine pyrophosphate (TPP).

- It is used in oxidative decarboxylation of alpha keto acids e.g. pyruvate dehydrogenase catalyses the breakdown of pyruvate, to acetyl CoA, and CO2.

2. Alpha ketoglutarate dehydrogenase :An analogous biochemical reaction that requires TPP is the oxidative decarboxylation of alpha ketoglutarate to Succinyl CoA and CO2.

3. Transketolase: The second group of enzymes that use TPP as co-enzyme are the transketolases, in the HMP shunt pathway of glucose.

4. The main role of thiamine (TPP) is the carbohydrate metabolism. So, the requirement of thiamine is increased along with higher intake of carbohydrates.

- Deficiency Manifestations:

a. Beriberi:

- It is a Singhalese word, meaning "Weakness".Early symptoms are anorexia, dyspepsia, heaviness and weakness.Subjects feel weak and get easily exhausted.

b. Wet beriberi:

- Here cardiovascular manifestations are prominent.

- Edema of legs, face, trunk, and serous cavities are the main features.
- Palpitation, breathlessness and distended neck veins are observed. Death occurs due to heart failure.

c. Dry beriberi:

- CNS manifestations are prominent.
- Walking becomes difficult. Peripheral neuritis with sensory disturbance leads to complete paralysis.

d. Infantile beriberi:

- It occurs in infants born to mothers suffering from thiamine deficiency.
- Restlessness and sleeplessness are observed.

e. Wernicke- Korsakoff syndrome:

- It is also called as cerebral beriberi.
- Clinical features are those of cerebral encephalopathy (ophthalmolpegia, nystagmus, cerebral ataxia) plus psychosis.
- It is seen only when the nutritional status is severely affected.

f. Polyneuritis:

- It is common in chronic alcoholics. Alcohol utilization needs large doses of thiamine. Alcohol inhibits intestinal absorption of thiamine, leading to thiamine deficiency.

➢ Biochemical parameters:

- In thiamine deficiency, blood thiamine 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:

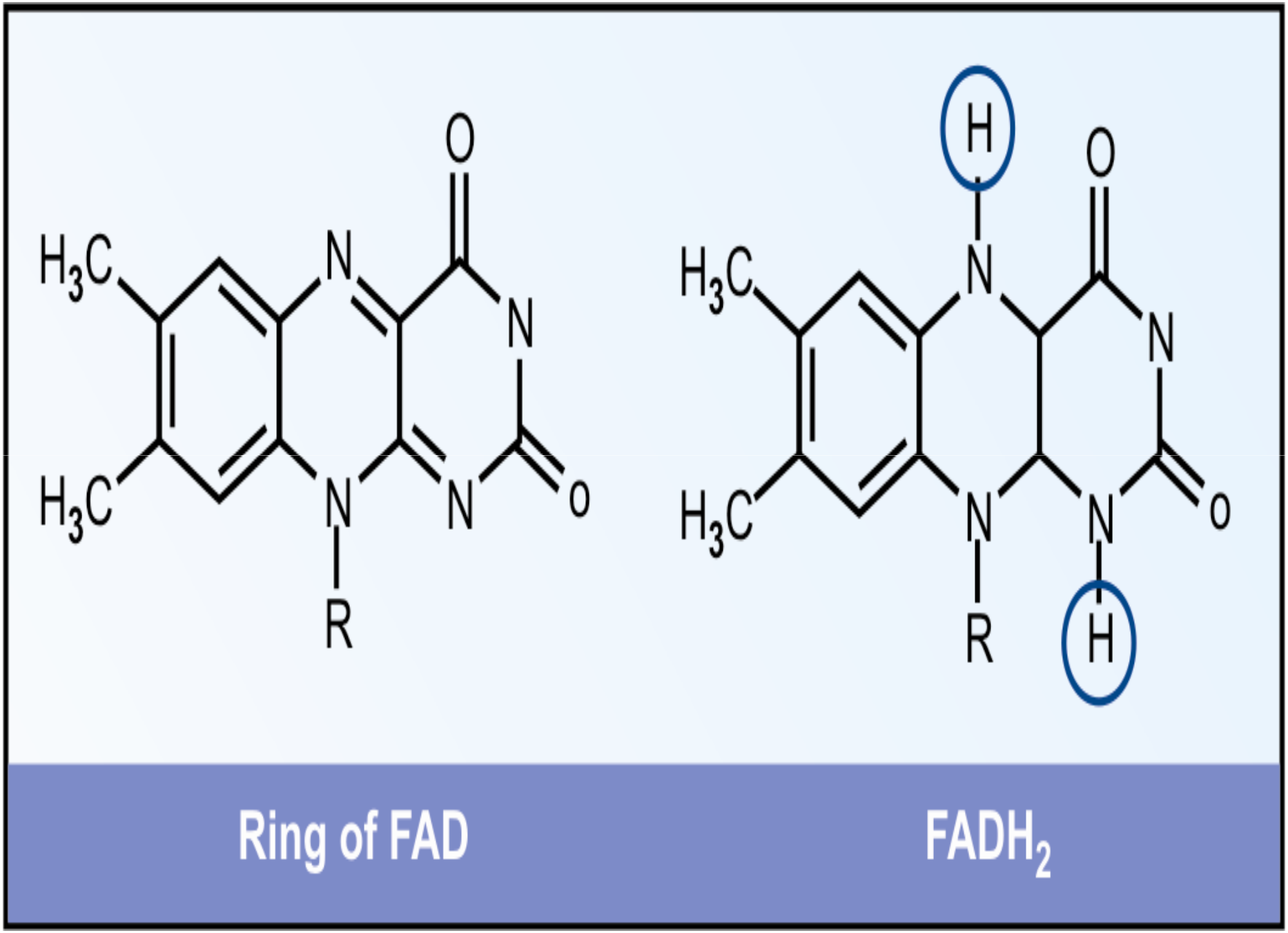
- It depends on calorie intake(0.5mg/1000 calorie). Requirement is 1-1.5 mg/day.
- Thiamine is useful in treatment of beriberi, alcoholic polyneuritis, neuritis of pregnancy and old age.

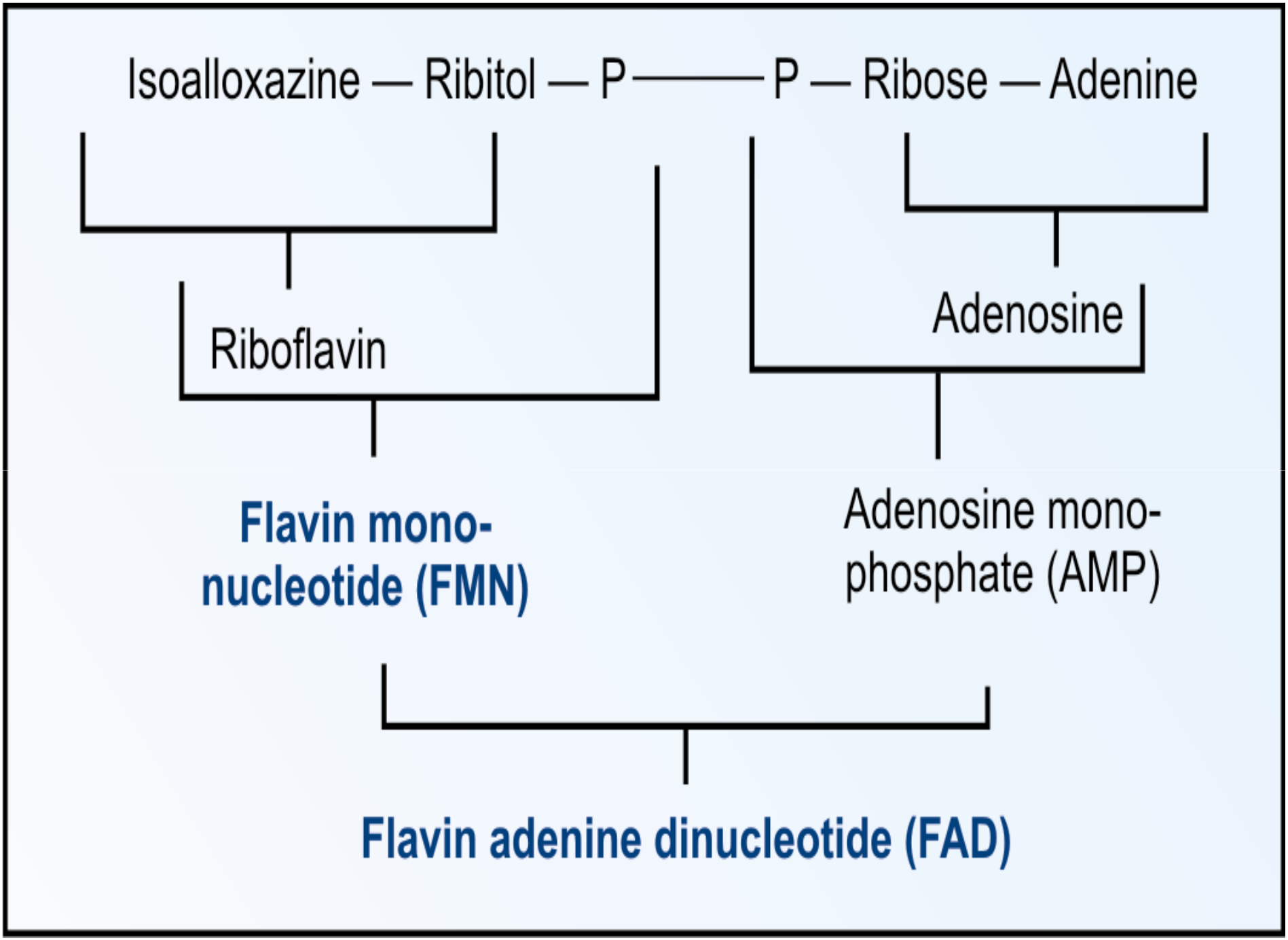
RIBOFLAVIN (VITAMIN B2)

- Riboflavin has a dimethyl isoalloxazine ring to which a ribitol is attached. Ribitol is the alcohol of ribose sugar.
- Riboflavin is converted to its active co-enzyme forms (FMN and FAD) with the help of ATP. Riboflavin is heat stable.

Co-enzyme Activity of Riboflavin

- Riboflavin exists in tissues tightly bound (but not covalently) with enzymes. Enzymes containing riboflavin are called flavoproteins. The two coenzymes are FMN and FAD.


H3C
H3C
N
N
N
O
O
R
H
H3C
H3C
N
N
N
O
O
R
H
Ring of FAD
FADH2


Isoalloxazine — Ribitol — P
P — Ribose — Adenine
Riboflavin
Adenosine
Flavin mono-nucleotide (FMN)
Adenosine mono-phosphate (AMP)
Flavin adenine dinucleotide (FAD)

FAD Accepts Hydrogen

- During the oxidation process, FAD accepts two hydrogen atoms from substrate. In turn, FAD is reduced to FADH2. The two nitrogen atoms of isoalloxazine nucleus accept the hydrogen atoms.

FMN-dependent Enzymes

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

NAD+ ⟶ FMN ⟶ CoQ

FAD-dependent Enzymes

- FADH2 when oxidised in the electron transport chain will generate 2 ATP molecules. For example,
- Succinate to fumarate by succinate dehydrogenase
- Acyl CoA to alpha-beta unsaturated acyl CoA by acyl CoA dehydrogenase
- Xanthine to uric acid by xanthine oxidase
- Pyruvate to acetyl CoA by pyruvate dehydrogenase
- Alpha ketoglutarate to succinyl CoA by alpha ketoglurate dehydrogenase.

Riboflavin Deficiency

Causes

- Natural deficiency of riboflavin in man is uncommon, because riboflavin is synthesised by the intestinal flora. Riboflavin deficiency usually accompanies other deficiency diseases such as beriberi, pellagra and kwashiorkor.

Manifestations

- Symptoms are confined to skin and mucous membranes like Glossitis,Magenta coloured tongue, Cheilosis, Angular stomatitis Circumcorneal vascularisaation. Proliferation of the bulbar conjunctival capilaries is the earliest sign of riboflavin deficiency.

Dietary Sources of Riboflavin

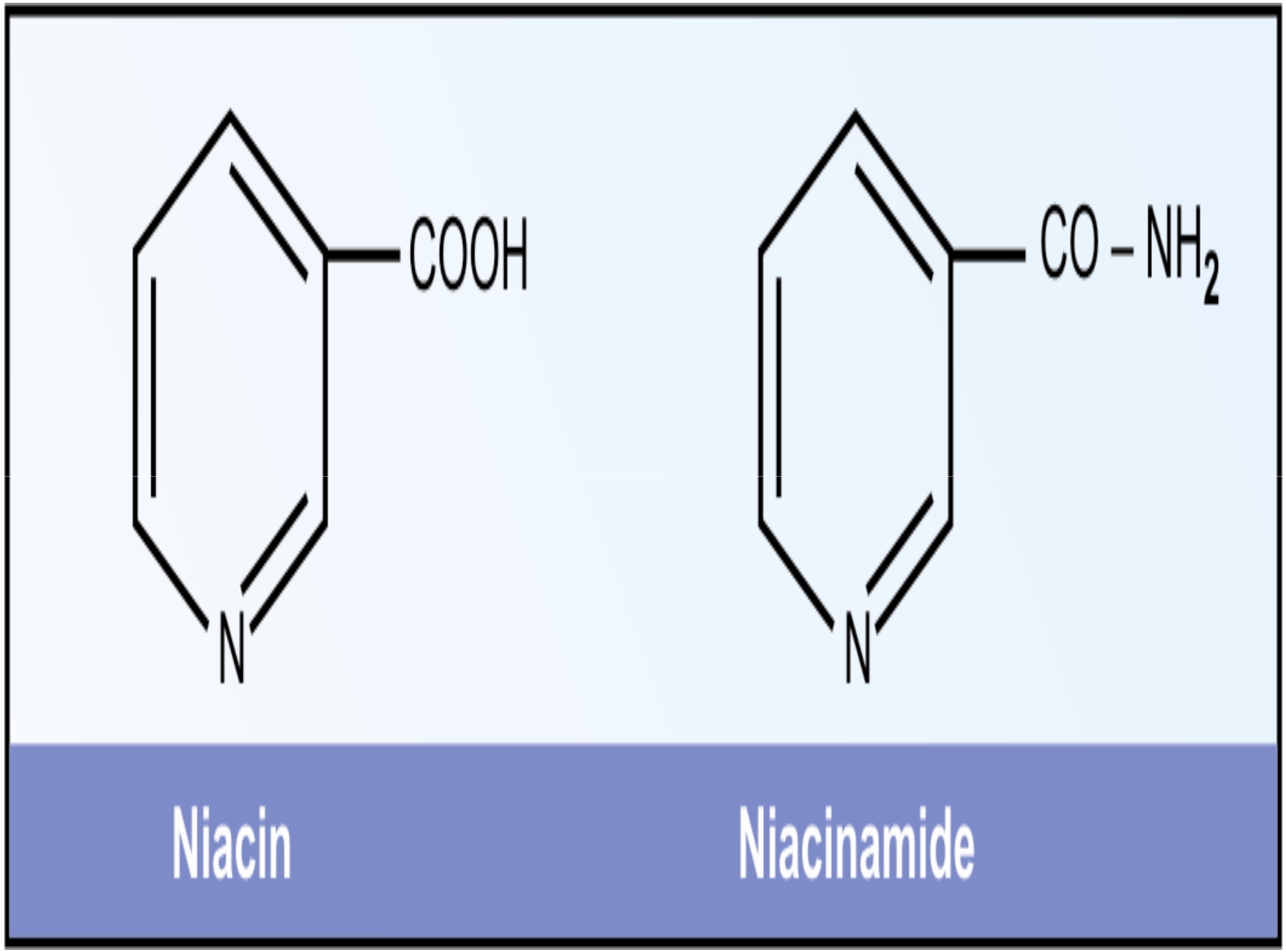
- Rich sources are liver, dried yeast, egg and whole milk. Good sources are fish, whole cereals, legumes and green leafy vegetables.

Daily Requirement

- Riboflavin is concerned mainly in the metabolism of carbohydrates and requirement is related to calorie intake.
- Adults on sedentary work require about 1.5 mg/day.
- During pregnancy, lactation and old age, additional 0.2 to 0.4 mg/day are required.

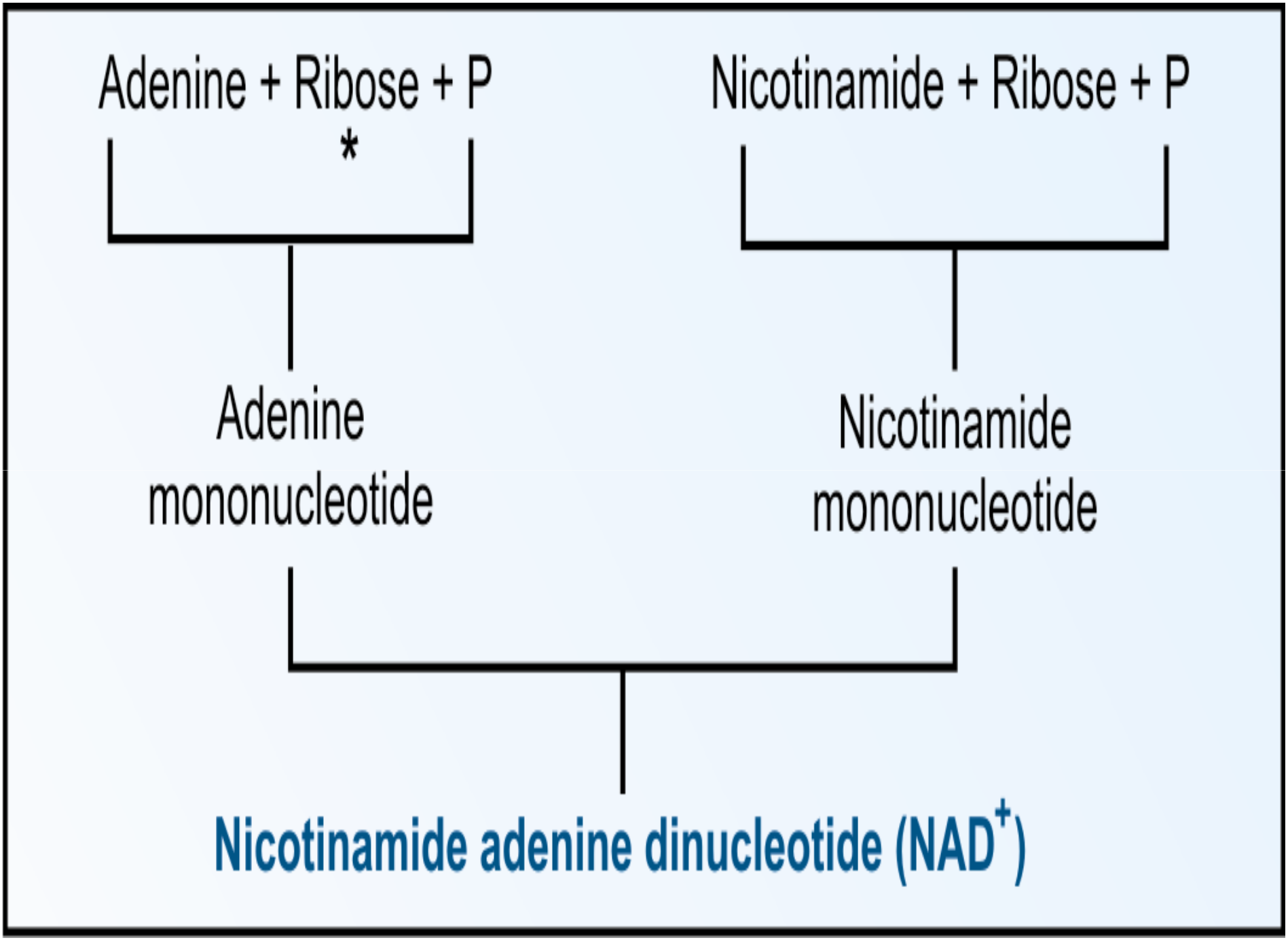
NIACIN

- Niacin and nicotinic acid are synonyms. It is also called as pellagra preventing factor of Goldberger.
- Nicotinic acid is a vitamin; but, nicotine is the potent poison from tobacco.
- Niacinamide is the active form of the vitamin, present in the tissues.
- Niacin is pyridine-3-carboxylic acid. Niacinamide is the acid amide.
- In NAD+ or NADP+, the reactive site is the carbon atom 4 and the nitrogen atom of the nicotinamide ring.


COOH
N
CO – NH2
N
Niacin
Niacinamide

Co-enzyme Forms of Niacin

- Niacin is converted to its co-enzyme forms, e.g. NAD+ and NADP+.
- The niacin is attached to a ribose phosphate to form a mononucleotide. It is then attached to AMP, to form the dinucleotide.
- The nitrogen atom of niacinamide contains one positive charge. In the case of NADP+, one more phosphoric acid is attached to the ribose of the AMP.

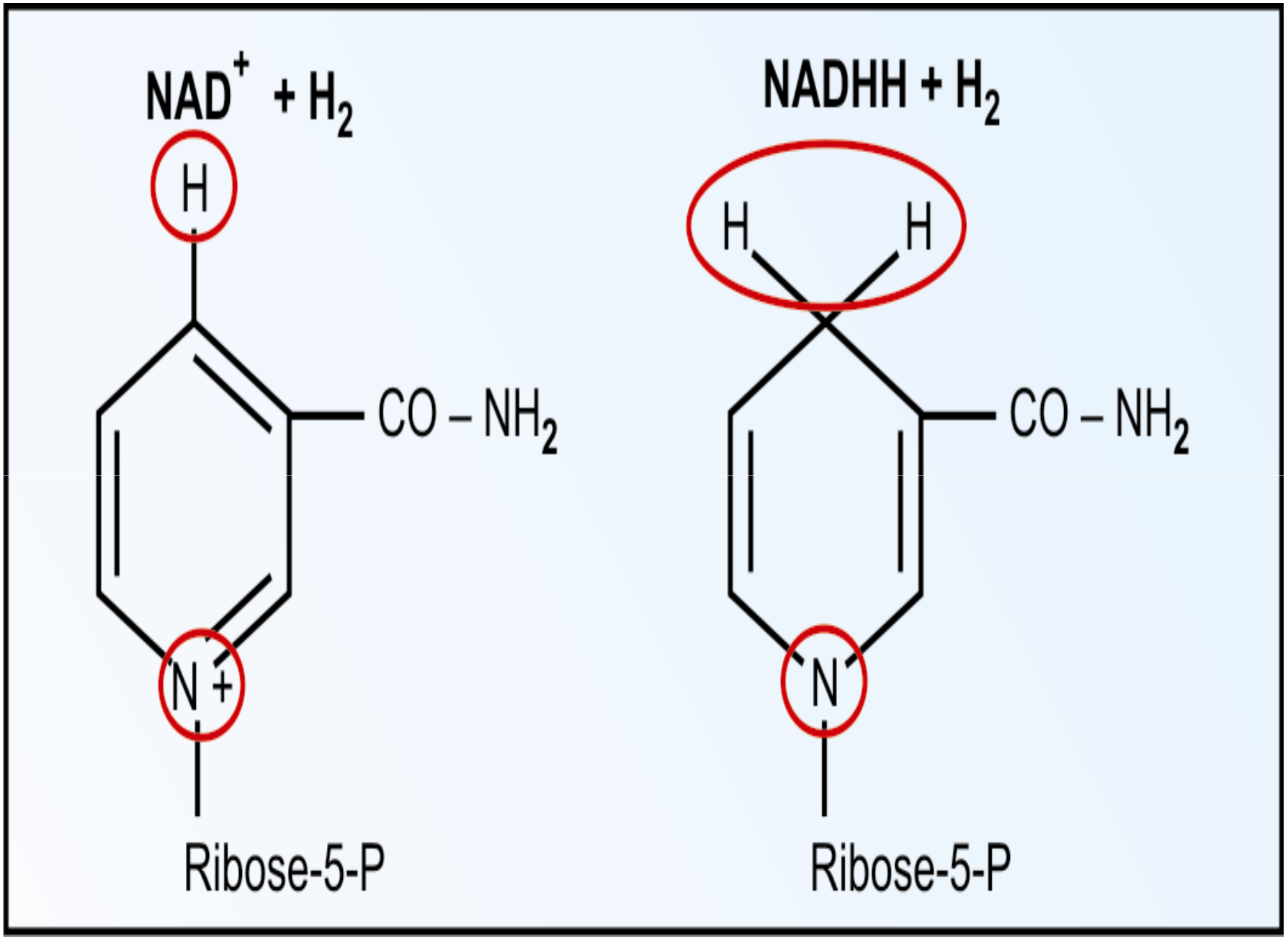

Adenine + Ribose + P
*
Nicotinamide + Ribose + P
Adenine mononucleotide
Nicotinamide mononucleotide
Nicotinamide adenine dinucleotide (NAD+)

One Hydrogen Atom and One Electron

- In the oxidised form, nitrogen of the nicotinamide residue has a positive charge. So the oxidised form of co-enzyme is usually written as NAD+.
- In the process of reduction, NAD+ accepts one hydrogen atom fully. The other hydrogen is ionized. Only the electron is accepted.

$$2H \longrightarrow H + H^{+} + e^{-}$$

- Thus NAD+ accepts one H atom and one electron, to form NADH. The hydrogen ion is released into the surrounding medium. During the oxidation of NADH, the reaction is reversed.


NAD+ + H2
H
CO – NH2
N +
Ribose-5-P
NADHH + H2
H
H
CO – NH2
N
Ribose-5-P

NAD+ Dependent Enzymes

- One NADH molecule is oxidised in the respiratory chain to generate 3 ATPs. But NADPH is used almost exclusively for reductive biosynthetic reactions.
- For example, Lactate dehydrogenase, Glyceraldehyde-3-phosphate dehydrogenase, Pyruvate dehydrogenase, Glutamate dehydrogenase.

NADPH Dependent Reactions

- Some enzymes can use either NAD+ or NADP+ as coenzyme, e.g. glutamate dehydrogenase.

Niacin Deficiency

Pellagra

- Deficiency of niacin leads to the clinical condition called pellagra.
- *Dermatitis:* In early stages, bright red erythema occurs, especially in the feet, ankles and face. Increased pigmentation around the neck is known as Casal's necklace. The dermatitis is precipitated by exposure to sunlight.
- *Diarrhea:* It may be mild or severe with blood and mucus. This may lead to weight loss. Nausea and vomiting may also be present.

- *Dementia:* It is frequently seen in chronic cases. Delerium is common in acute pellagra. Irritability, inability to concentrate and poor memory are more common in mild cases. Ataxia and spasticity are also seen.

Niacin is Synthesised from Tryptophan

- Quinolinate phosphoribosyl transferase is the rate limiting enzyme in the conversion of niacin to NAD+.
- About 60 mg of tryptophan is equivalent to 1 mg of niacin.

Causes of Niacin Deficiency

- *Dietary deficiency of tryptophan:* Pellagra is seen among people whose staple diet is maize (South and Central America). In maize, niacin is present; but it is in a bound form, and is unavailable. Pellagra is also seen when staple diet is sorghum (jowar or guinea corn) as in Central and Western India. Sorghum contains leucine in high quantities. Leucine inhibits the QPRT enzyme, and so niacin cannot be converted to NAD+ (Leucine pellagra).

- *Lack of 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 anti-tuberculous drug, which inhibits pyridoxal phosphate formation. So, there is block in conversion of tryptophan to NAD+.
- *Hartnup disease*: Tryptophan absorption from intestine is defective in this congenital disease. Morever, tryptophan is excreted in urine in large quantities. This leads to lack of tryptophan and consequently deficiency of nicotinamide.
- *Carcinoid syndrome*: The tumour utilises 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 are required.

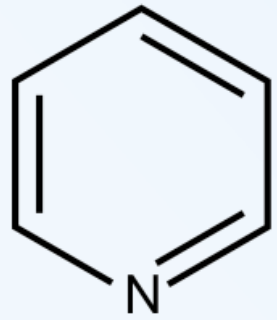
Therapeutic Use of Niacin

- 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.
- Nicotinic acid inhibits the flux of free fatty acids from adipose tissue; so acetyl CoA pool is reduced; and hence serum cholesterol is lowered.

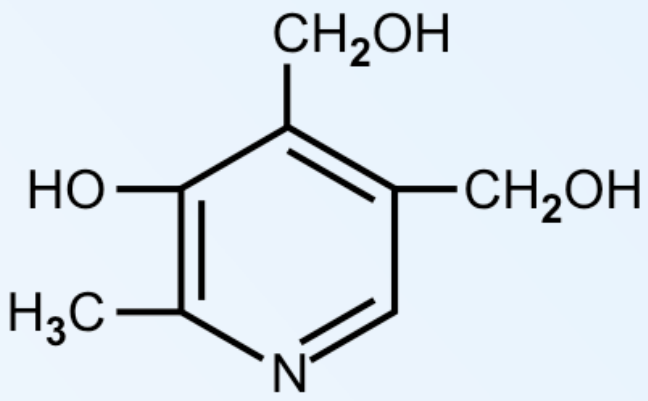
VITAMIN B6

Co-enzyme Form

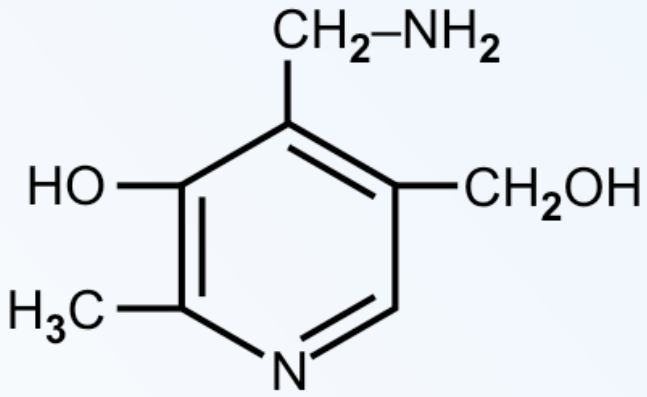
- Vitamin B6 is the term applied to a family of 3 related pyridine derivatives; pyridoxine (alcohol), pyridoxal (aldehyde) and pyridoxamine.
- Active form of pyridoxine is pyridoxal phosphate (PLP).
- It is synthesised by pyridoxal kinase, utilising ATP.



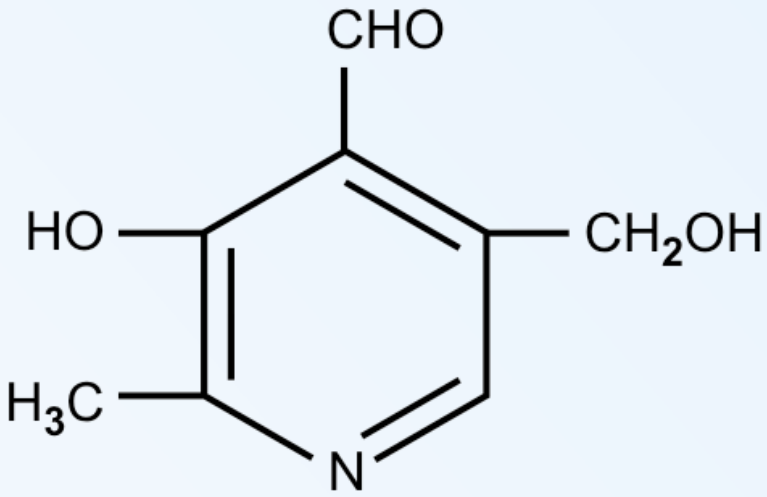
Pyridine



Pyridoxine



Pyridoxamine



Pyridoxal

Functions of Pyridoxal Phosphate

- The pyridoxal phosphate (PLP) acts as co-enzyme for many reactions in amino acid metabolism.

Transamination

- These reactions are catalysed by amino transferases (transaminases) which employ PLP as the co-enzyme. For example,

  Alanine + Alpha ketoglutarate → Pyruvate +

  Glutamic acid (Enzyme alanine transaminase)

Decarboxylation

- All decarboxylation reactions of amino acids require PLP as co-enzyme. For example,

1. Glutamate → GABA. It is an inhibitory neurotransmitter, and so in B6 deficiency, mainly in children, convulsions may occur.

2. Histidine → histamine, which is the mediator of allergy and anaphylaxis.
3. 5-hydroxy tryptophan → serotonin
4. Cysteine → taurine
5. Serine → ethanol amine

Sulphur containing amino acids

- PLP plays an important role in methionine and cysteine metabolism.

a. Homocysteine + Serine → Cystathionine. (cystathionine synthase)

b. Cystathionine → Homoserine + Cysteine (Cystathionase).

- Both these reactions require PLP. So in vitamin B6 deficiency homocysteine in blood is increased.
- Homocysteine level is correlated with myocardial infarction. So, pyridoxine is used in clinical practice to prevent coronary artery disease in homocysteinemia.

Heme synthesis

- ALA synthase is a PLP dependent enzyme. This is the rate limiting step in heme biosynthesis. So, in B6 deficiency, anemia may be seen.

Production of Niacin

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

  3-hydroxy kynurenine → 3-hydroxy anthranilic acid (enzyme is kynureninase).
- Kynureninase is a PLP dependent enzyme. So, in vitamin B6 deficiency niacin production is less. Kynurenine cannot be converted further, which is metabolised to xanthurenic acid and excreted in urine.

Glycogenolysis

- Phosphorylase enzyme (glycogen to glucose-1-phosphate) requires PLP. In fact, more than 70% of total PLP content of the body is in muscles, where it is a part of the phosphorylase enzyme.

Deficiency Manifestations of Pyridoxine

Neurological Manifestations

- In vitamin B6 deficiency, PLP dependent enzymes function poorly. So, serotonin, epinephrine, noradrenaline and GABA are not produced properly. Neurological symptoms are therefore quite common in B6 deficiency.

- 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. This is reversible with high doses of B6.

Dermatological Manifestations

- Deficiency of B6 will also affect tryptophan metabolism. Since niacin is produced from tryptophan, B6 deficiency in turn leads to niacin deficiency which is manifested as pellagra.

Hematological Manifestations

- In adults hypochromic microcytic anemia may occur due to the inhibition of heme biosynthesis. Impaired antibody formation as also reported.
- The metabolic disorders which respond to vitamin B6 therapy are xanthurenic aciduria and homocystinuria.

Effect of Drugs on Vitamin B6

1. INH : Isonicotinic acid hydrazide (isoniazid) is an antituberculosis drug. It inhibits pyridoxal kinase; reduces the formation of PLP and causes vitamin B6 deficiency.
2. Cycloserine : It acts as B6 antagonist.
3. Oral contraceptives : Mild vitamin B6 deficiency may be seen in women taking oral contraceptive pills.
4. Ethanol : It is converted to acetaldehyde, which inactivates PLP. So B6 deficiency neuritis is quite common in alcoholics.

Dietary Sources

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

Requirement of B6

- Vitamin B6 requirements are related to protein intake and not to calorie intake. It is recommended that adults need 1 to 2 mg/day. During pregnancy and lactation, the requirement is increased to 2.5 mg/day.

Toxicity of Vitamin B6

- Doses over 100 mg may lead to imbalance, numbness, muscle weakness and nerve damage.

PANTOTHENIC ACID

- Pantothenic acid contains beta alanine and D-pantoic acid in amide linkage.
- Synthesis of the CoA needes the expenditure of 4 high energy bonds.
- Pantothenic acid and beta mercapto ethanol amine are parts of co-enzyme A.

Co-enzyme Activity of Pantotheinc Acid

- The beta mercaptoethanol amine contains one thiol or sulfhydryl group. It is the active site where acyl groups are carried.So, the co-enzyme A is sometimes abbreviated as CoA-SH to denote this active site.
- The thioester bond in acyl-CoA is a high energy bond. These acyl groups are transferred to other acceptors, for example,

  Acetyl CoA + Choline → Acetylcholine + CoA
  (enzyme is acetylcholine synthase)

- Acyl groups are also accepted by the CoA molecule during the metabolism of other substrates, for example:

  Pyruvate + CoA + NAD+ → AcetylCoA+CO2+ NADH

  (Enzyme is pyruvate dehydrogenase)
- The important CoA derivatives are:

  a. Acetyl CoA

  b. Succinyl CoA

  c. HMG CoA

  d. Acyl CoA
- Co-enzyme A is an important component of fatty acid synthase complex. The ACP (acyl carrier protein) also contains pantothenic acid.

Deficiency of Pantothenic Acid

- Gopalan's Burning foot syndrome is manifested as paresthesia (burning, lightning pain) in loweer extremities, staggering gait due to impaired co-ordination and sleep disturbances.
- These deficiency manifestations are rare in human beings. The syndrome is seen during famine, in prison camps, in chronic alcoholics and in some renal dialysis patients.
- In experimental animals, deficiency has resulted in anemia, reduced steroidogenesis, dermatitis, fatty liver and adrenal necrosis.

Sources of Pantothenic Acid

- It is widely distributed in plants and animals. Morover, it is synthesised by the normal bacterial flora in intestines. So, deficiency is very rare. Yeast, liver and eggs are good sources.

Requirement of Pantothenic Acid

- RDA is assumed to be about 10 mg/day.

BIOTIN

Structure of Biotin

- It consists of an imidazole ring fused with a thiophene ring with a valeric acid side chain. The carboxyl group forms an amide linkage with the epsilon nitrogen of a lysine residue in the apo-enzyme.

Co-enzyme Activity of Biotin

- Biotin acts as co-enzyme for carboxylation reactions. Biotin captures a molecule of CO2 which is attached to nitrogen of the biotin molecule.
- The energy required for this reaction is provided by ATP. Then the activated carboxyl group is transferred to the substrate.

Biotin Requiring CO_2 Fixation Reactions

1. Acetyl CoA carboxylase

- This enzyme adds CO_2 to acetyl CoA to form malonyl CoA. This is the rate limiting reaction in 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

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

It provides the oxaloacetate, which is the catalyst for TCA cycle. It is an important enzyme in the gluconeogenic pathway.

Biotin-Independent Carboxylation Reactions

- Carbomoyl phosphate synthetase, which is the stepping stone for urea and pyrimidine synthesis.
- 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 great affinity to biotin. So, intake of raw (unboiled) egg may cause biotin deficiency. Biotin was originally named as anti-egg-white-injury-factor.

- Avidin is heat labile, and boiling of egg will neutralise the inhibitory activity. One molecule of avidin can combine with four molecules of biotin. It is curious that egg white contains avidin and egg yolk contains biotin.
- The affinity of avidin to biotin is greater than most of the usual antigen-antibody reactions. So, avidin-biotin system is commonly utilised for detection of pathogens in the ELISA test.
- DNA is generally labelled by radioactive nucleotides. Recently, biotin labelling of DNA is becoming more popular. Biotin is added to nucleotides, which will be incorporated into the newly synthesising DNA. The fixed biotin can be identified by reaction with avidin.
- Streptavidin purified from streptomyces avidinee, can bind 4 molecules of biotin.

Deficiency of Biotin

- Due to the prolonged use of antibacterial drugs.
- Biotin deficiency symptoms include dermatitis, atrophic glossitis, hyperesthesia, muscle pain, anorexia and hallucinations. Injection of biotin 100-300 mg will bring about rapid cure of these symptoms.

Requirement of Biotin

- About 200-300 mg will meet the daily requirements.

Sources of Biotin

- Normal bacterial flora of the gut will provide adequate quantities of biotin. It is distributed in plant and animal tissues. Liver, yeast, peanut, soybean, milk, egg yolk are rich sources.

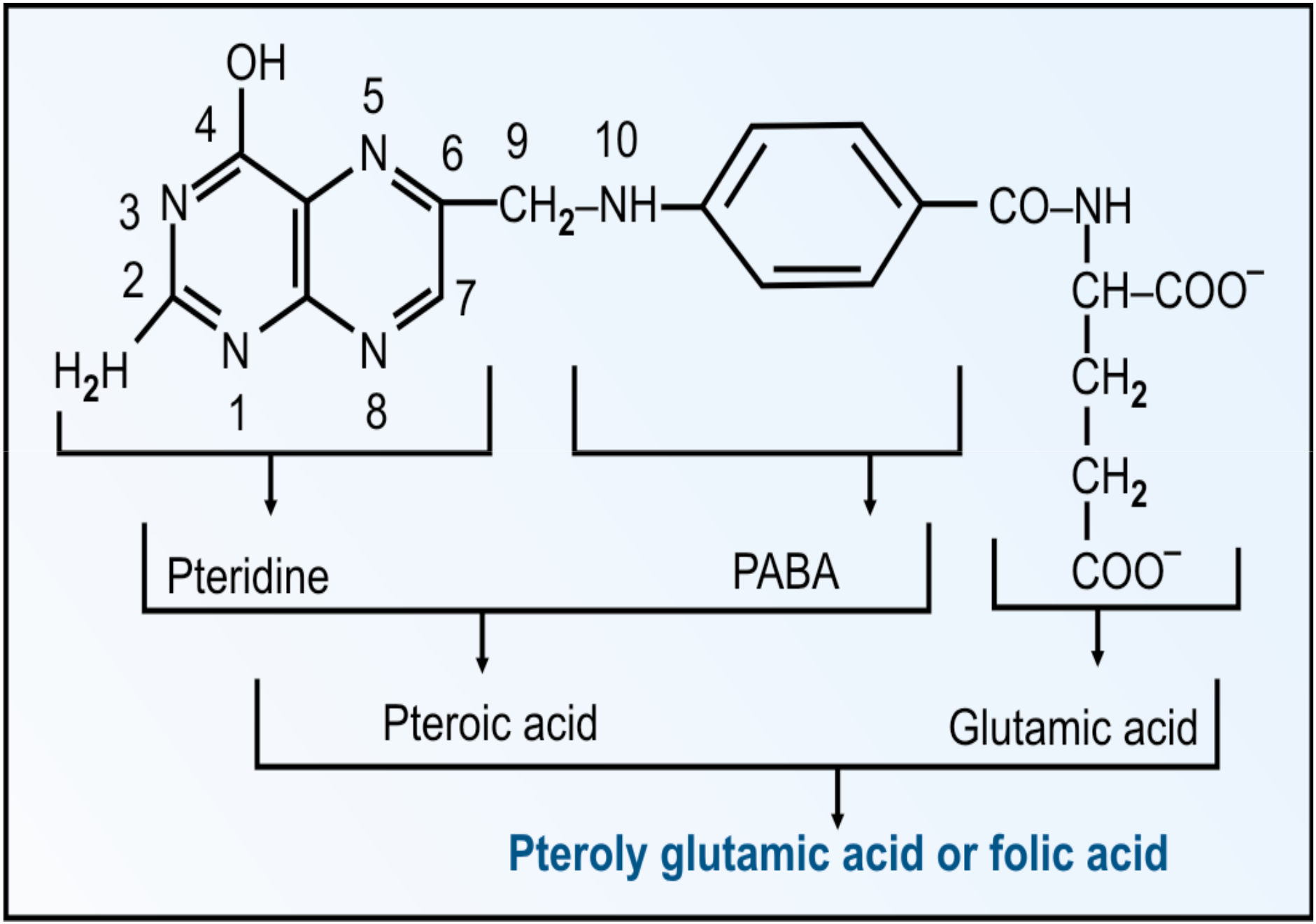
FOLIC ACID

Chemistry of Folic Acid

- Folic acid is abundant in vegetables. It is composed of three constituents. The pteridine group linked with para-aminobenzoic acid (PABA) is called pteroic acid. It is then attached to glutamic acid to form pteroylglutamic acid or folic acid.

Absorption of Folic Acid

- Folic acid is readily absorbed by the upper part of jejunum. In the blood, it is transported by beta globulins. It is taken up by the liver where the co-enzymes are produced. Folic acid is not stored in tissues.


OH
4
5
N
6
9
10
3
N
CH_2–NH
CO–NH
2
7
CH–COO^-
H_2H
N
N
CH_2
1
8
CH_2
Pteridine
PABA
COO^-
Pteroic acid
Glutamic acid
Pteroly glutamic acid or folic acid

Co-enzyme Functions of Folic Acid

- The folic acid is first reduced to 7,8-dihydro folic acid further reduced to 5,6,7,8-tetrahydro folic acid (THFA). Both reactions are catalysed by NADPH dependent folate reductase.
- The THFA is the carrier of one-carbon groups. One carbon compound is an organic molecule that contains only a single carbon atom. The following groups are one carbon compounds :

  a. Formyl (-CHO)

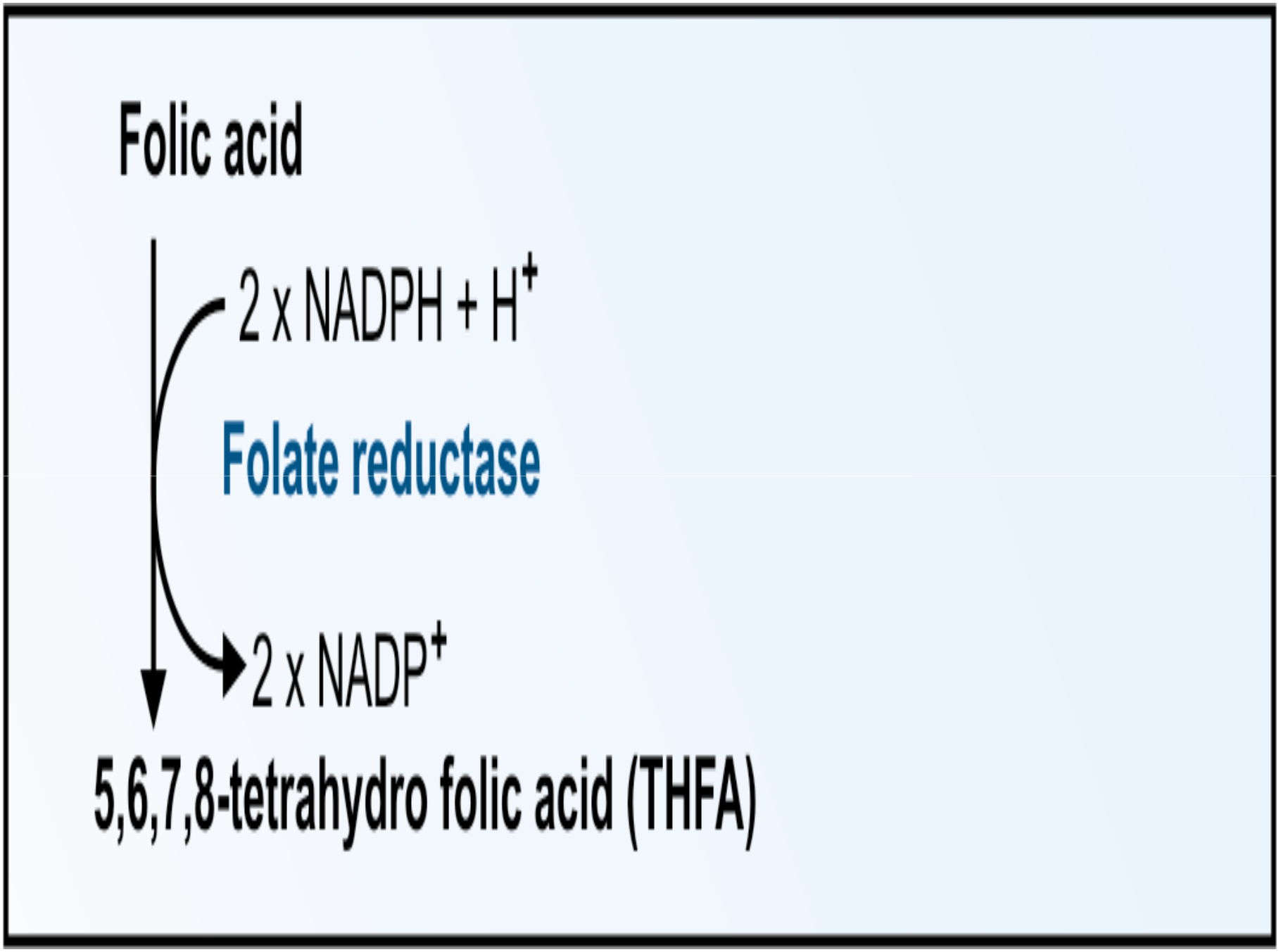
  b. Formimino (-CH=NH)

  c. Methenyl (-CH=)

  d. Methylene (-CH2-)

  e. Methy (-CH3).

- These one carbon compounds are attached either to the 5^{th} or to the 10^{th} or to both 5 and 10 nitrogen atoms of THFA.
- 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 choli9ne, epinephrine, creatine, etc.


Folic acid
2 x NADPH + H+
Folate reductase
2 x NADP+
5,6,7,8-tetrahydro folic acid (THFA)

Causes for Folate Deficiency

- Folic acid deficiency is very common in India, and is perhaps the most commonly seen vitamin deficiency.

Pregnancy : Folate deficiency is commonly seen in pregnancy, where requirement is increased.

Defective absorption : In sprue, celiac disease, gluten induced enteropathy, resection of jejunum and short-circuting of jejunum in gastroileostomy, absorption is defective.

Drugs: In the diet, folacins are mainly in polyglutamate form. Gastrointestinal enzymes in the gut remove the glutamate form of folic acid is absorbed.
Anticonvulsant drugs will inhibit the intestinal enzyme, so that folate absorption is reduced.

Hemolytic anemias : As requirement of folic acid becomes more, deficiency is manifested.

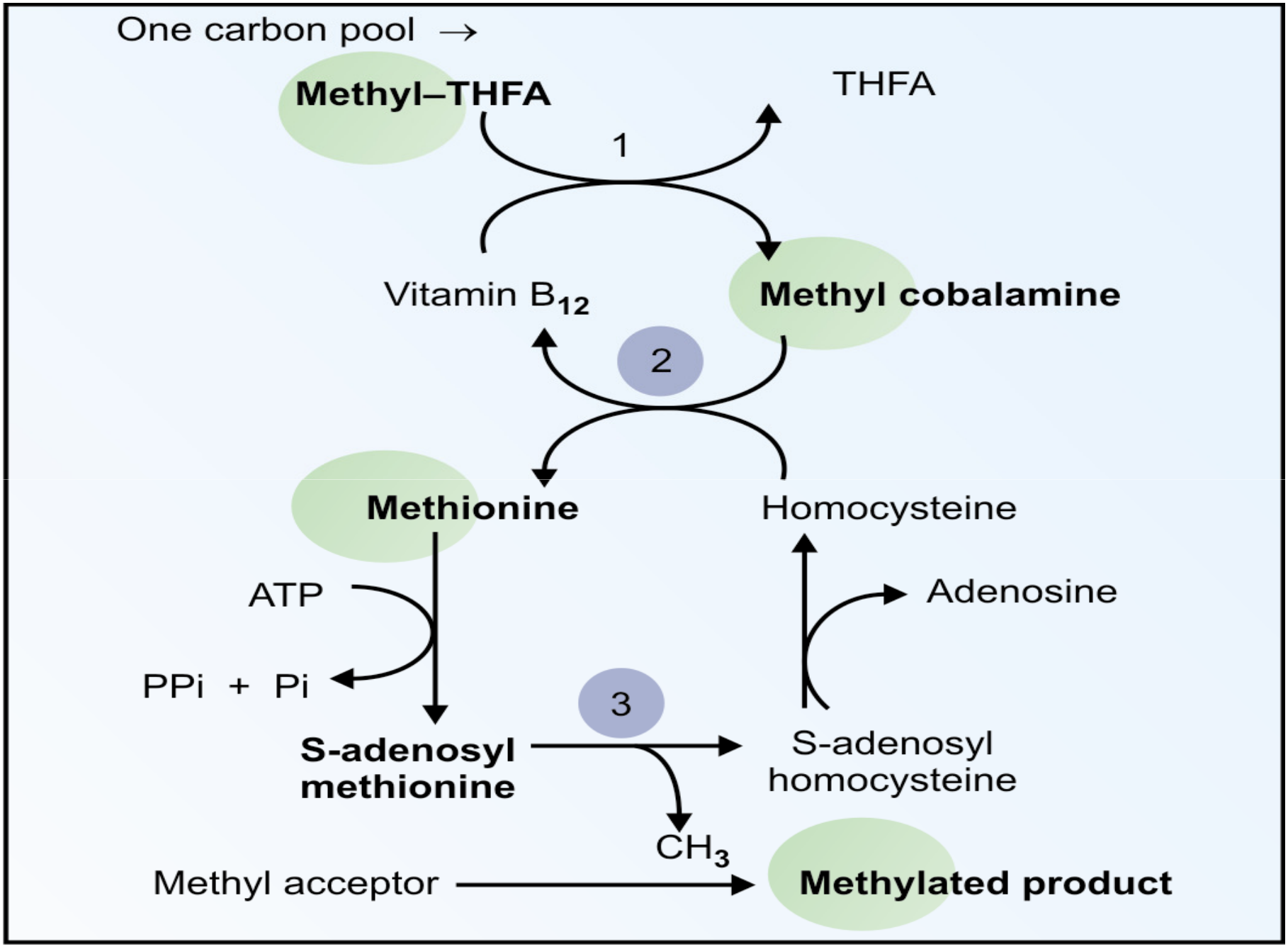
Dietary deficiency : Absence of vegetables in food for prolonged periods may lead to deficiency.

Folate trap : Vitamin B12 deficiency also lead to folate deficiency.

Deficiency Manifestations

Reduced DNA Synthesis:

- In folate deficiency, THFA is reduced and thymidylate synthase enzyme is inhibited. So, dUMP is not converted to dTMP.
- So, dTTP is not available for DNA synthesis. So cell division is arrested. Very rapidly dividing cells in bone marrow and intestinal mucosa are more affected.


One carbon pool →
Methyl–THFA
THFA
1
Vitamin B_{12}
Methyl cobalamine
2
Methionine
Homocysteine
ATP
Adenosine
PPi + Pi
3
S-adenosyl methionine
S-adenosyl homocysteine
CH_3
Methyl acceptor
Methylated product

Macrocytic Anemia :

- It is the most characteristic feature of folate deficiency. During RBC generation, DNA synthesis is delayed, but protein synthesis is continued. So, hemoglobin accumulates in RBC precursors.
- This 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 life span of RBC. Reduced generation and increased destruction of RBCs result in anemia.

- Leukopenia and thrombocytopenia are also manifested.
- The peripheral blood picture in folate deficiency is described as macrocytic, and in cobalamin deficiency as megaloblastic. In the B12 deficiency, there are additional neurological symptoms.

Hyper-homocysteinemia

- Folic acid deficiency may cause increased homocysteine levels in blood. Plasma homocysteine levels above 15 micromoles/L is known to increase the risk of coronary artery diseases. Providing adequate doses of pyridoxine, B12 and folic acid may lower the homocysteine levels.

Birth Defects

- Folic acid deficiency during pregnancy may lead to neural tube defects in the fetus. Folic acid prevents birth defects (fetal malformations such as spina bifida). So, intake of folic acid is a must from early pregnancy.

Cancer

- Folic acid is beneficial in prevention of cancer. Folate deficiency contributes to the etiology of bronchial carcinoma and cervical carcinoma.

Assessment of Folate Deficiency

- *Blood level :* Normal folic acid level in serum is about 20 nanogram/ml and about 200 microgram/ml of packed cells. The level is measured by radio-immunoassay.
- *Histidine load test or FIGLU excretion test :* Histidine is normally metabolised to formimino glutamic acid (FIGLU) from which formimino group is removed by THFA. So, in folate deficiency, FIGLU is excreted in urine.
- *AICAR excretion :* In the purine ring synthesis, the last step is the addition of C2 with the help of N10-formyl THFA. When this is blocked, the precursor, amino imidazole carboxamide ribosyl-5-phosphate (AICAR) accumulates and is excreted in urine.
- *Peripheral blood picture.*

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 of folic acid.

Recommended Daily Allowance (RDA)

- The requirement of free folate is 200 microgram/day. In pregnancy the requirement is increased to 400 microgram/day and during lactation to 300 microgram/day.

Folic acid Therapy

- 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

Sulphonamides:

- They have structural similarity with PABA. So, they competitively inhibit the enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid.
- Bacterial can synthesise folic acid from the components, pteridine, PABA and glutamate.
- When sulphonmides are given, such micro-orgtanisms cannot synthesise folic acid and so their growth is inhibited.
- As man connot synthesise folic acid, the entire molecule has to be supplied in the diet.

- The preformed folic acid connot enter into bacteria, but only into mammalian cells. So, sulphonamides are very good antibacterial agents, which do not affect the human cells.

Pyrimethamine :

- This antifolate agent is used against plasmodial infections (antimalarial drug).

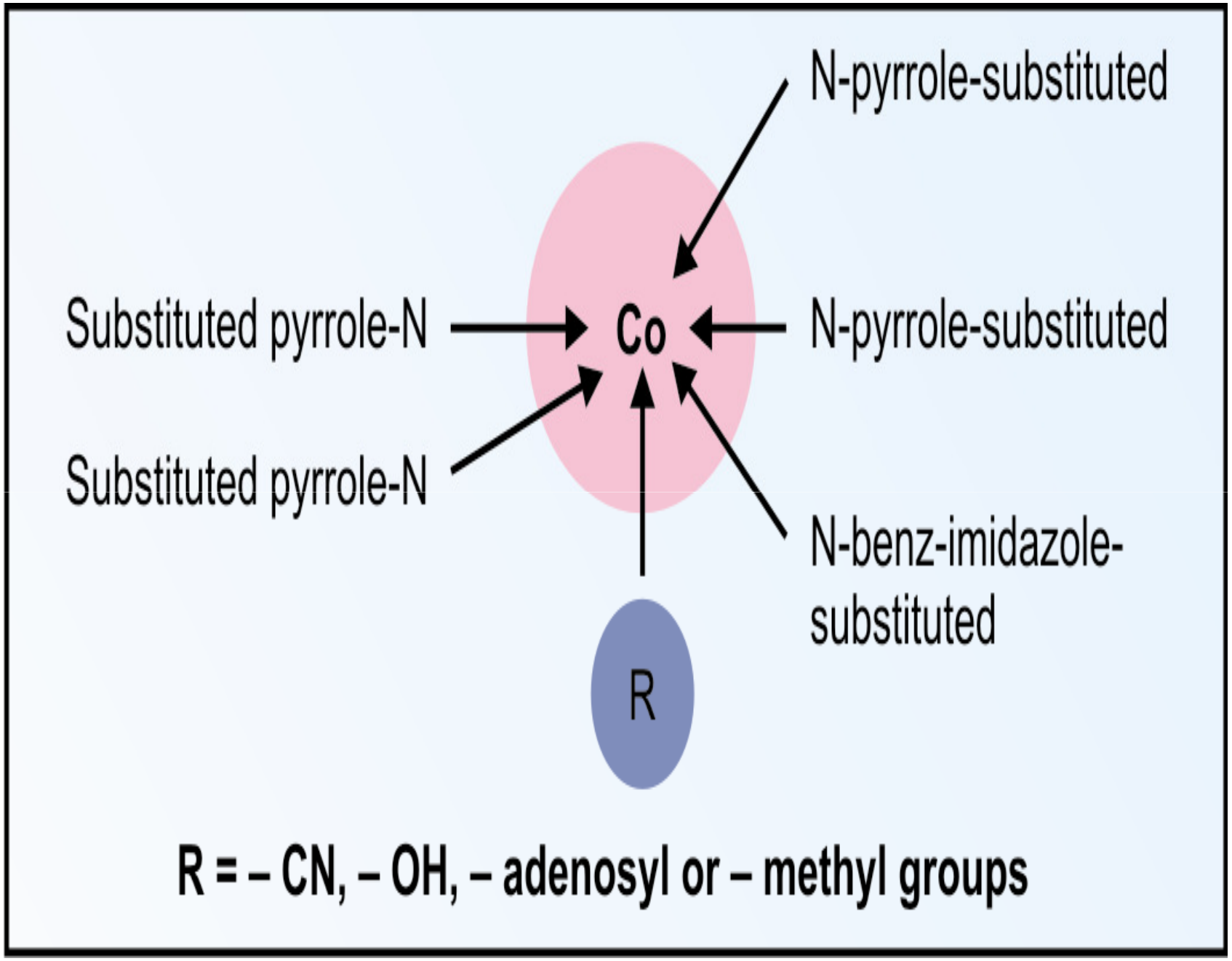
Aminopterin and Amethopterin :

- Aminopterin (4-amino folic acid) and amethopterin (methotrexate) (4-amino, 10-methyl folic acid) are powerful inhibitors of folate reductase and THFA generation.
- So, these drugs decrease the DNA formation and cell division. They are widely used as anticancer drugs, mainly for leukemias and choriocarcinomas. Folinic acid is given to rescue the patient from toxicity of methotrexate.

VITAMIN B_{12}

Chemistry

- It is water soluble, heat stable and red in colour. It contains 4.35% cobalt by weight. It contains one cobalt atom.
- Four pyrrole rings co-ordinated with a cobalt atom is called a **Corrin ring**. The 5^{th} valency of the cobalt is covalently linked to a substituted benzimidazole ring. This is then called cobalamin.
- The 6^{th} valency of the cobalt is satisfied by any of the following groups : cyanide, hydroxyl, adenosyl or methyl.


N-pyrrole-substituted
Substituted pyrrole-N
Co
N-pyrrole-substituted
Substituted pyrrole-N
N-benz-imidazole-substituted
R
R = – CN, – OH, – adenosyl or – methyl groups

Cyanocobalamin

- When cyanide is added at the R position, the molecule is called cyanocobalamin. During the isolation procedure, cyanide is added to get stable crystals. The CN group has no physiological function. Oral preparations of vitamin B_{12} are in this form.

Hydroxy cobalamin

- When hydroxyl group is attached at the R position, it is called hydroxy cobalamin or vitamin $B_{12a.}$ Injectable preparations are in this form.

Adenosyl cobalamin (Ado-B_{12})

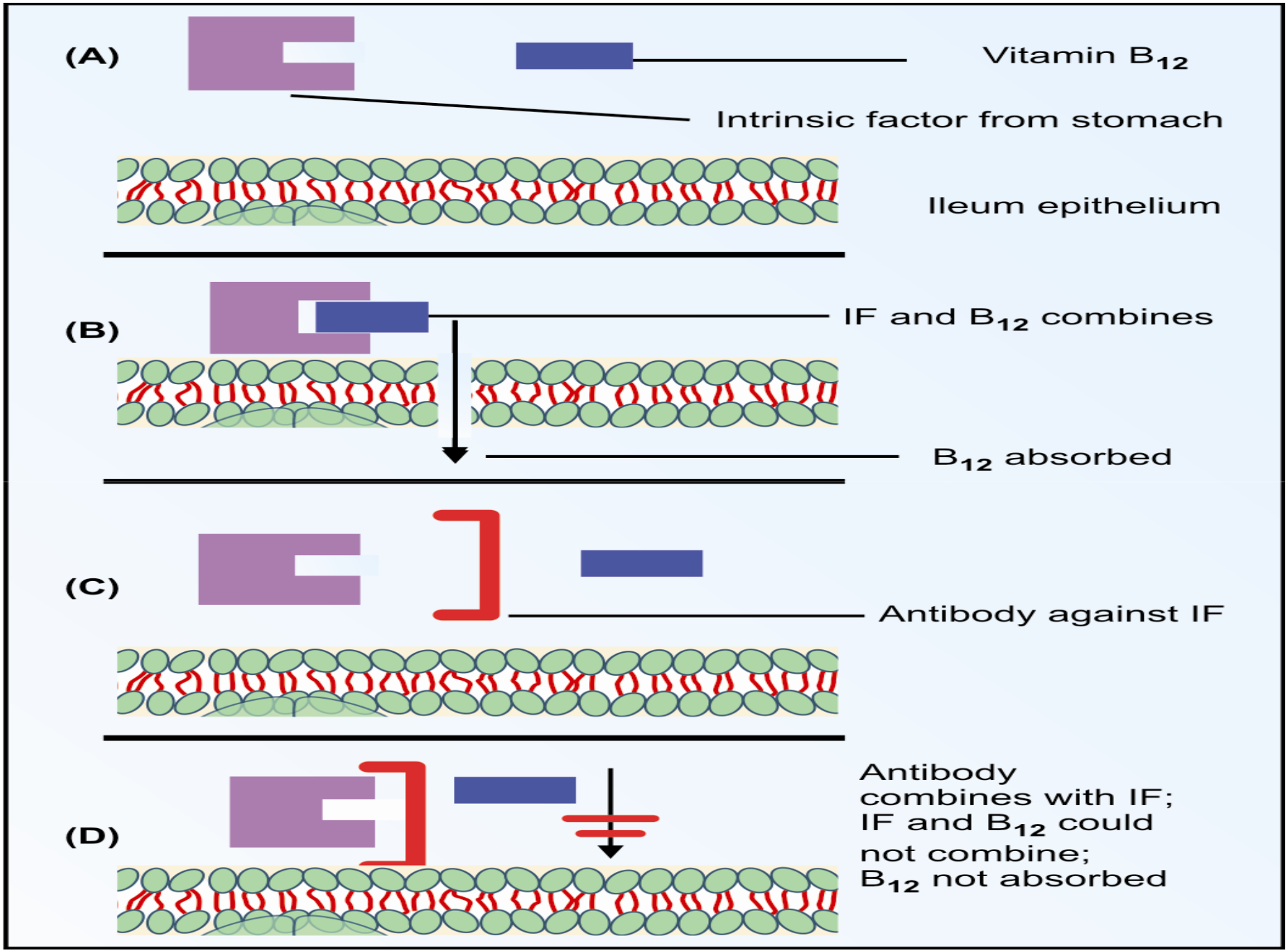
- When taken up by the cells, these groups are removed and deoxy adenosyl cobalamin or Ado-B_{12} is formed. This is the major **storage form**, seen in liver.

Methyl cobalamin

- When the methyl group replaces adenosyl group, it is known as methyl cobalamin. This is the major form seen in blood circulation as well as in cytoplasm of cells. The Ado-B_{12} and methyl B_{12} are the functional co-enzymes in the body.

Absorption of Vitamin B_{12}

- Vitamin B12 combines with the intrinsic factor of Castle. So, the B_{12} is otherwise known as extrinsic factor, that is, the factor derived from external sources.
- Intrinsic factor is secreted by the gastric parietal cells. It is a glycoprotein with a molecular weight of 50,000.
- One molecule of IF can combine with 2 molecules of B_{12}. This IF- B_{12} complex is attached with specific receptors on mucosal cells.
- The whole IF- B_{12} complex is internalised. It may be noted that B_{12} is absorbed from ileum, while folic acid is from jejunum.


(A)
Vitamin B_{12}
Intrinsic factor from stomach
Ileum epithelium
(B)
IF and B_{12} combines
B_{12} absorbed
(C)
Antibody against IF
(D)
Antibody combines with IF; IF and B_{12} could not combine; B_{12} not absorbed

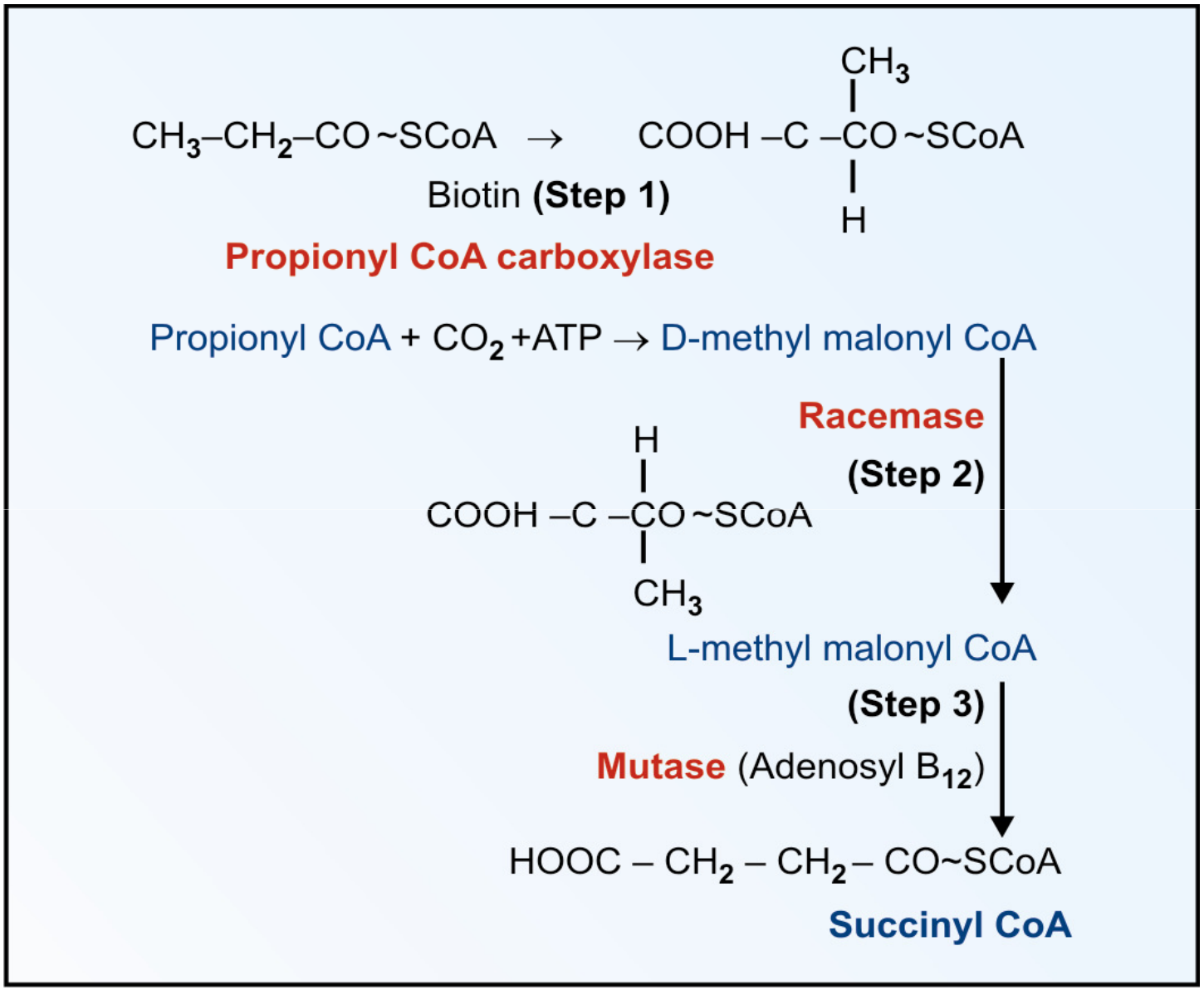
Transport and Storage

- In the blood, methyl B12 form is predominant. Transcobalamin, a glycoprotein, is the specific carrier.
- It is stored in the liver cells, as ado-B12 form, in combination with transcorrin.
- Generally B complex vitamins are not stored in the body, B12 is an exception.
- Whole liver contains about 2 mg of B12, which is sufficient for the requirement for 2-3 years. So, B12 deficiency is seen only years after gastrectomy.

Functional Role of B12

A. Methyl Malonyl CoA isomerase

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

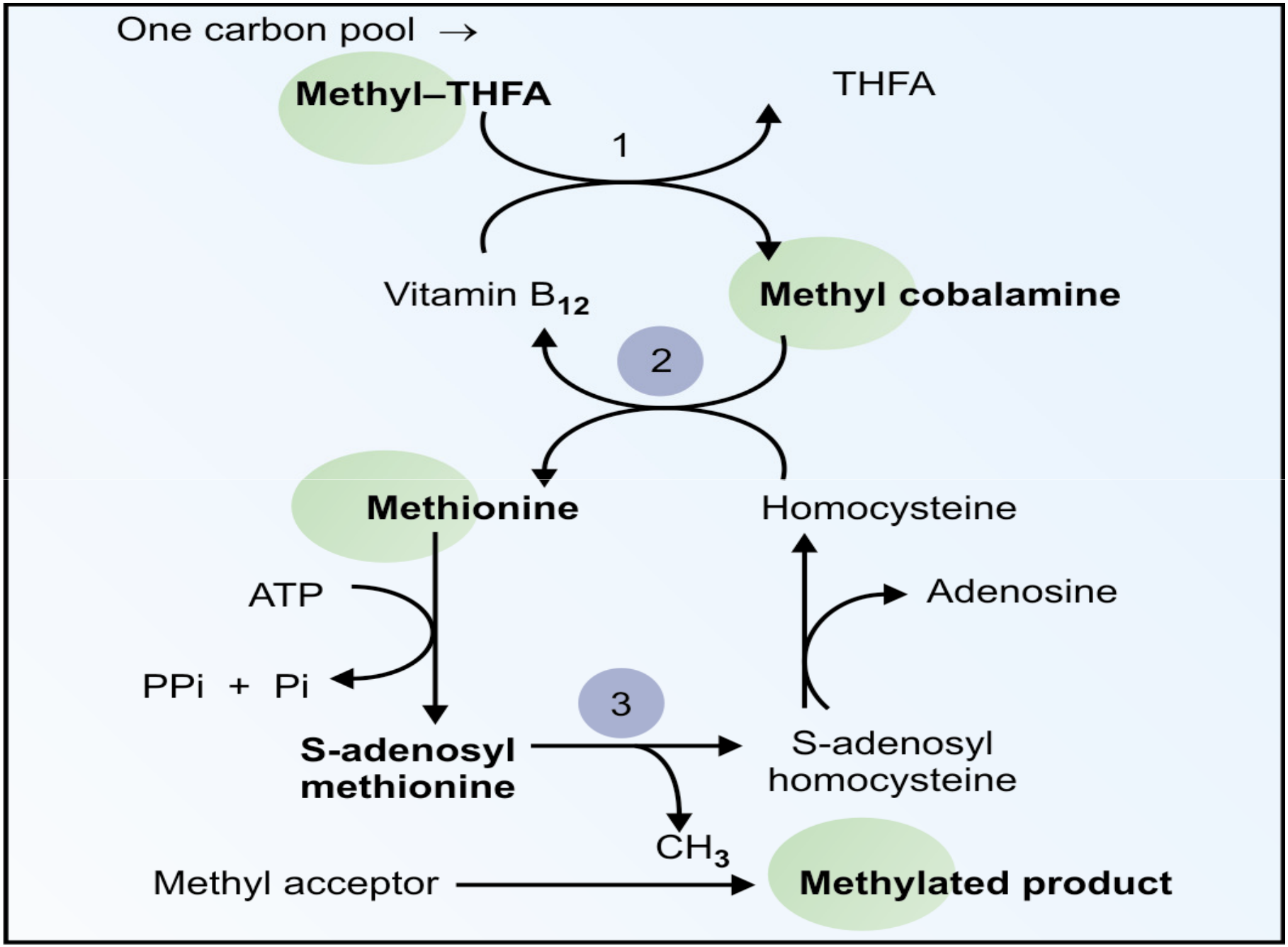

CH3–CH2–CO~SCoA →
COOH –C –CO~SCoA
CH3
H
Biotin (Step 1)
Propionyl CoA carboxylase
Propionyl CoA + CO2 +ATP → D-methyl malonyl CoA
Racemase
(Step 2)
COOH –C –CO~SCoA
H
CH3
L-methyl malonyl CoA
(Step 3)
Mutase (Adenosyl B12)
HOOC – CH2 – CH2 – CO~SCoA
Succinyl CoA

B. Homocysteine Methyl Transferase

- Transmethylation is catalysed by the enzyme methionine synthase or homocysteine methyl transferase. These enzymes need the activity of vitamin B12.

C. Methyl Folate Trap and Folate Deficiency

- The production of methyl THFA is an ireversible step. So, the only way for generation of free THFA is transmethylation.
- 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.


One carbon pool →
Methyl–THFA
THFA
1
Vitamin B_{12}
Methyl cobalamine
2
Methionine
Homocysteine
ATP
Adenosine
PPi + Pi
3
S-adenosyl methionine
S-adenosyl homocysteine
CH_3
Methyl acceptor
Methylated product

Causes of B12 Deficiency

Nutritional

- Nutritional vitamin B12 deficiency is very common in India, specially among vegetarians of low socio-economic group. The only group for B12 in vegetarian diet is curd/milk, and lower income group may not be able to afford it.

Decrease in Absorption

- Absorptive surface is reduced by gastrectomy, resection of ileum and malabsorption syndromes.

Addisonian Pernicious Anemia

- It is very rare in India, but common in European countries.
- It is manifested usually in persons over 40 years. It is an autoimmune disease with a strong familial background. Antibodies are generated against IF. So, IF becomes deficient, leading to defective absorption of B12.

Gastric Atrophy

- Atrophy of gastric epithelium leading to deficiency of IF and decreased B12 absorption is common in India.
- In chronic iron deficiency anemia, there is generalised mucosal atrophy. In about 40% cases of iron deficiency anemia, superadded gastric atrophy is seen.

Pregnancy

- Increased requirement of vitamin in pregnancy is another common cause for vitamin B12 deficiency in India.

Fish Tapeworm

- Although not seen in India, the fish tapeworm, *diphilobothrium latum* infection is common in Scandinavian countries where people eat live fish. This tapeworm has a special affinity to B12 causing reduction in available vitamin.

Deficiency Manifestations

Folate trap :

- Vitamin B12 deficiency causes simultaneous folate deficiency due to the folate trap. So all the manifestations of folate deficiency are also seen.
- Reduced DNA synthesis, macrocytic anemia, bith defects.

Megaloblastic anemia :

- In the peripheral blood, megaloblasts and immature RBCs are observed.

Abnormal homocysteine level :

- In vitamin B12 deficiency, homocysteine is accumulated, leading to homocystinuria. Homocysteine level in blood is related with myocaridial infarction. So, B12 and folic acid are protective against ischemic heart disease.

DEMYELINATION:

- In vitamin B12 deficiency , step 3 is also suppresed due to the non-availabilty of active methionine. Thefore methylation of phosphotidyl ethanolamine to phosphatidyl ethanolamine to phosphatidyl choline is not adquate. This leads to deficient formation of myelin sheaths of nerves, demyelination and neurological lesions.
- **Subacute combined degeration:**
- Damage to nervous system is seen in B12 deficiency.

- There is **demyelination** affecting cerebral cortex as well as dorsal column and pyrimidal 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 and positive Babinski's sign are seen.
- **ACHLORHYDRIA:**
- Absence of acid in gastric juice is associated with vitamin B12 deficiency.

Assessment of B12 deficiency:

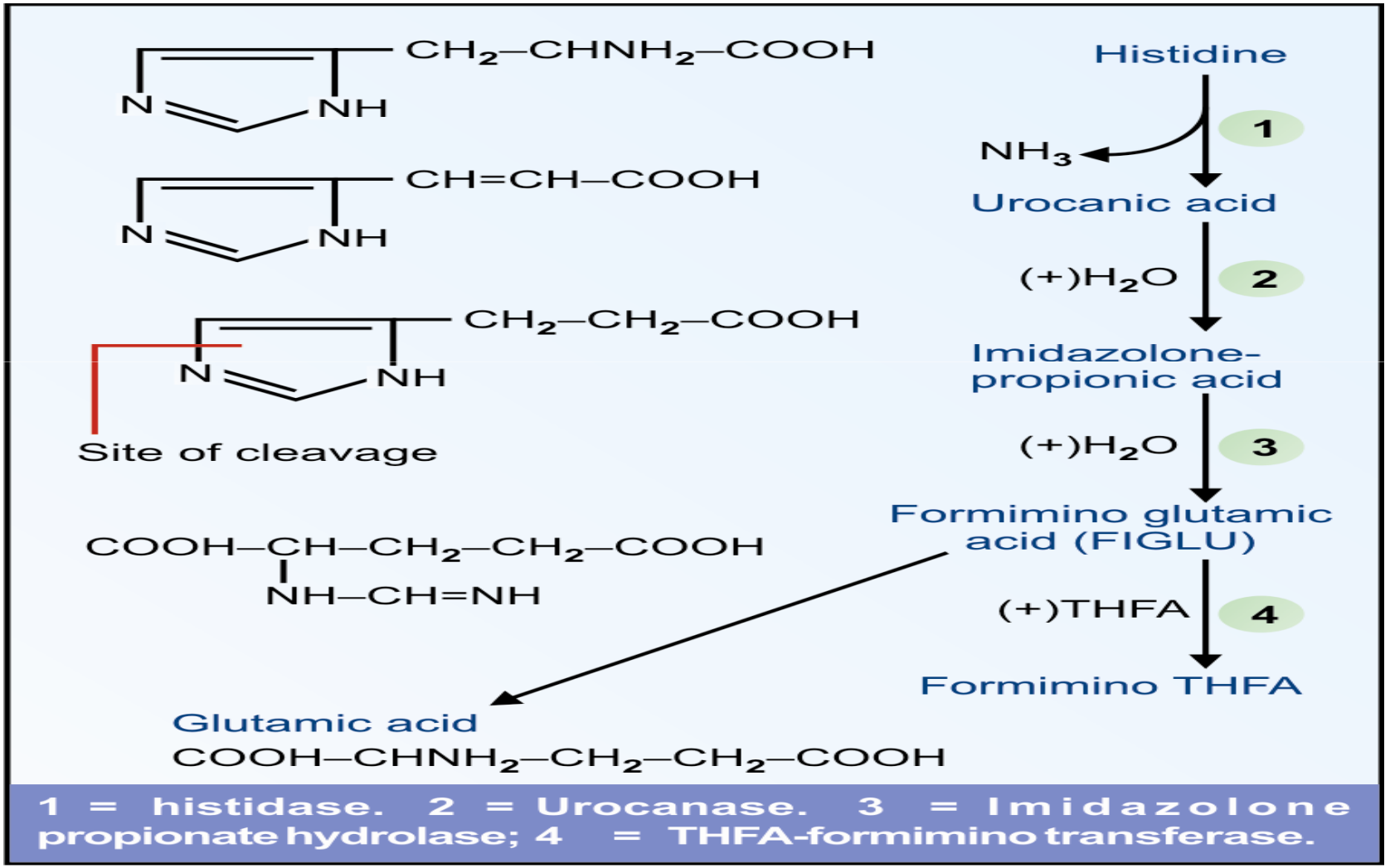
- Serum B12: It is quantitated by radio-immunoassay or by ELISA.
- Schilling test: one microgram radioactive labelled (Cobalt-60) vitamin B12 is given orally. In gastric atrophy cases, there is no absorption, hence the entire radioactivity is excreted in faeces and radioactivity is not observed in liver region.If the cause is nutritional deficiency, there will be enhanced absorption. Then radioactivity is noted in the liver region, with very little excretion in feces.
- Methly malonic acid: it is seen in urine.

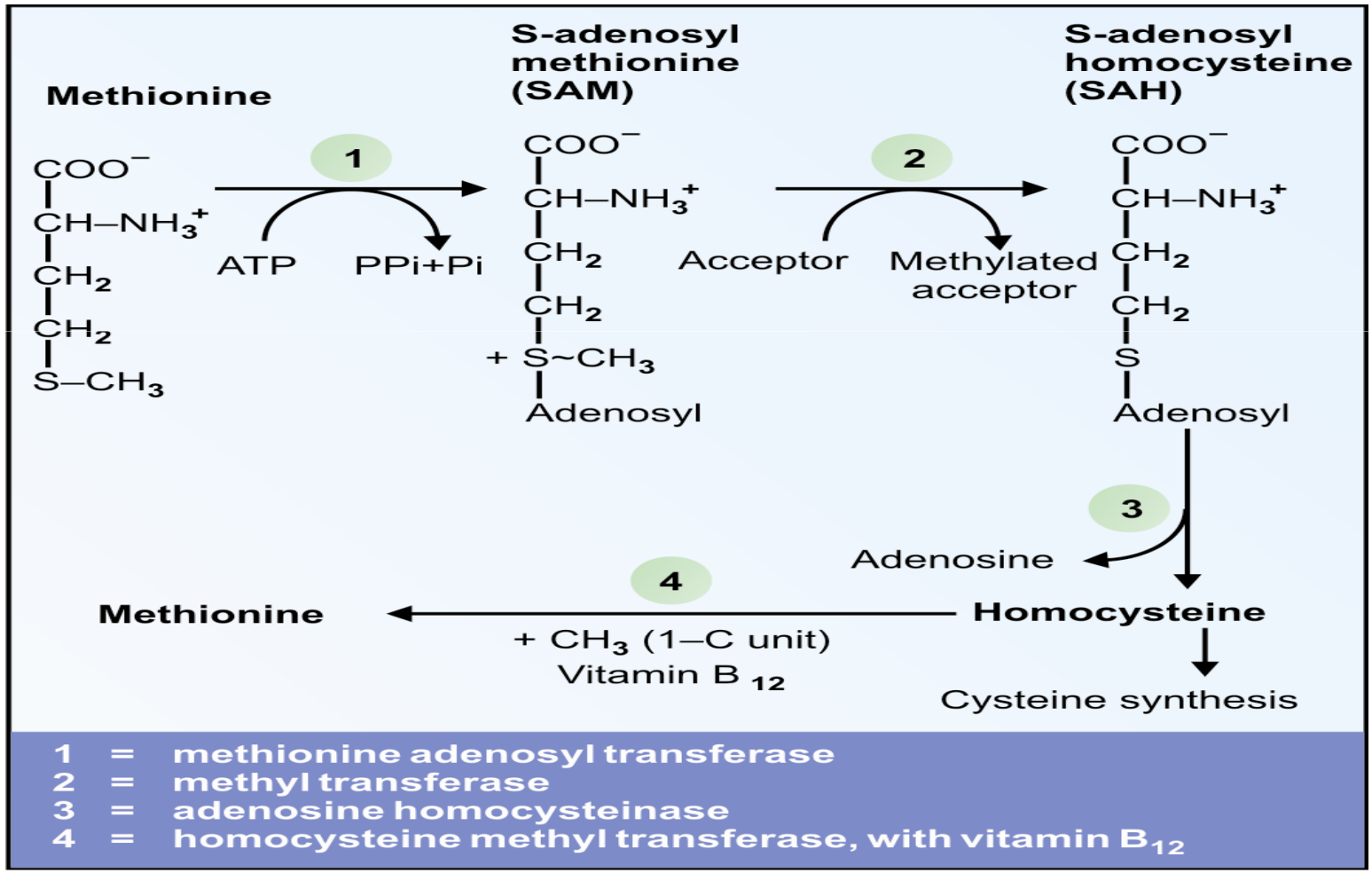
- FIGLU EXCRETION TEST:

Histidine is normally metabolised to formimino glutamic acid from which formimino group is removed by THFA. Therefore in vit B_{12} deficiency,FIGLU is excreted in urine.

Peripheral Smear: Peripheral blood and bone marrow morphology shows megaloblastic anemia .

- Homocysteinuria


CH2–CHNH2–COOH
N
NH
CH=CH–COOH
N
NH
CH2–CH2–COOH
N
NH
Site of cleavage
COOH–CH–CH2–CH2–COOH
NH–CH=NH
Histidine
1
NH3
Urocanic acid
(+)H2O
2
Imidazolone-propionic acid
(+)H2O
3
Formimino glutamic acid (FIGLU)
(+)THFA
4
Formimino THFA
Glutamic acid
COOH–CHNH2–CH2–CH2–COOH
1 = histidase. 2 = Urocanase. 3 = Imidazolone propionate hydrolase; 4 = THFA-formimino transferase.


Methionine
S-adenosyl methionine (SAM)
S-adenosyl homocysteine (SAH)
1
2
3
4
ATP
PPi+Pi
Acceptor
Methylated acceptor
Adenosine
Homocysteine
Methionine
+ CH3 (1–C unit)
Vitamin B 12
Cysteine synthesis
1 = methionine adenosyl transferase
2 = methyl transferase
3 = adenosine homocysteinase
4 = homocysteine methyl transferase, with vitamin B12

- **Treatment**:
- If megaloblastic anemia is treated with folic acid alone, the anemia may improve, but associated nervous lesions are aggravated. Hence , all macrocytic anemias are generally treated with folate and vitamin B12. therapeutic dose of B12 is 100 to 1000 microgram by intramuscular injections.

- **Requirement of vitamin B12:**
- Normal daily requirement is 1-2 microgram/day. During pregnancy and lactation, this is increased to 2 microgram/day. Those who take folic acid, should also take vitamin B12. Elderly people are advised to take B12 supplementation.
- DIETARY SOURCES:
- Vitamin B12 is not present in vegetables. Liver is the richest source. Curd is a good source, because lactobacillus can synthesise B12.