**Introduction**

- Dr. Gyorgy identified in 1934
  - a family of chemically-related compounds including pyridoxamine (PM) & pyridoxal (PL) and pyridoxine (PN).
  - The form most commonly form is pyridoxine HCl.
- Phosphorylation of 5’ position of B₆ (PM, PL, & PN), which make PMP, PLP, & PNP.
  - In animal, predominant of PMP & PLP;
  - In plant foods, a glucoside form in which glucose (5’-O- [β-glucopyranosyl] pyridoxine) may play as storage form of vitamin.
Pyridoxine (Vitamin B6) | Pyridoxal | Pyridoxamine

![Pyridoxine](image1)

Pyridoxal Phosphate

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bacterial alanine racemase

![Bacterial Alanine Racemase](image2)
Introduction

- Vitamin B₆ is one of the most versatile enzyme (>100 enzymes) cofactors.
- Pyridoxal is the predominant biologically active form (PLP). (More inborn error???)
- Pyridoxal phosphate (PLP) is a cofactor
  1. in the metabolism of amino acids and neurotransmitters;
  2. in the breakdown of glycogen;
  3. bind to steroid hormone receptors and may have a role in regulating steroid hormone action;
  4. in the immune system
## Chemistry

- **Name:** pyridoxine, pyridoxal & pyridoxamine, vitamin B$_6$
- **Structure:** 2-methyl-3-hydroxy5-hydroxy methyl pyridines.
- **Chemistry & physical property property:**
  - Vitamin B$_6$s are readily soluble in water,
  - stable in acid, unstable in alkali,
  - is fairly easily destroyed with UV light, e.g. sunlight.
- **Stability ---** pyridoxine > pyridoxal or pyridoxamine

## Bioavailability

- Complxed form B$_6$ $\rightarrow$ poorly digested
- B$_6$ can be condensed with peptide lysyl or cysteiny1 residues during food processing (e.g. cooking) $\rightarrow$ thus, reduced the utilization of B$_6$.
- Plants contain complexed forms $\Rightarrow$ bioavailability of B$_6$ in animal products tends to be greater than in the plant materials.
Vitamin B₆ is readily absorbed in the small intestine. Pyridoxine uptake is by passive diffusion rather than by active transport, mainly in jejunum & ileum.

Driving force for B₆ depends on the de- & rephosphorylation & protein binding. De- & Re phosphorylation of PLP, PNP is catalyzed by *alkaline phosphatase & pyridoxal kinase*, respectively.

*Figure 1: B6 metabolism and transport*
Metabolism --- Absorption

- Excess vitamin B₆ is excreted in the urine so adequate daily intake is essential.
- Phosphorylated pyridoxine and pyridoxamine are then oxidized to the common form.

Metabolism --- Transportation

- Once absorbed, it (PLP and small amount of free pyridoxal) is carried in the blood tightly bound to proteins [the erythrocytes (hemoglobin) & albumin in plasma] to all body cells.
- B₆ binds via the amino group of the N-terminal valine residue of the hemoglobin α chain. (Schiff-Base)
Metabolism --- **Transportation**

- *Pyridoxal* more readily crosses cell membranes than pyridoxal phosphate.
- After being taken into the cell, the B$_6$ vitamers are converted via a saturable two-step process (phosphorylation) to pyridoxal phosphate (PLP).
- The reactions are catalyzed by a *pyridoxal kinase* (an enzyme present in the cytoplasm of mucosal cell.)

Metabolism --- **Distribution**

- 70-80% of the vitamin B$_6$ in the body is located in muscle (*glycogen phosphorylase*); 10% of B$_6$ in the liver; the remainder is distributed among the other tissues.
- Small amount of vitamin B$_6$ stored in body (40-150 mg; 20-75 days needed to deplete), mainly as *PLP & PNP*. 
Metabolism --- Conversion

- When the vitamins are phosphorylated, transmural absorption decreases whereas uptake is unaffected.
- Phosphorylation thus serves as a means of control of the cellular PMP, PLP, & PNP levels. This called “metabolic trapping”, because non-phosphorylated vitamers cross membranes more easily their phosphorylated analogues.

**Figure**: The relationship of various chemical forms of vitamin B6.
Why phosphated form of $B_6$?

- Reason: to protect it from hydrolysis and provide storage of the vitamin
- Principle:
  1. phosphorylation/dephosphorylation;
  2. oxidation/reduction;
  3. amination/deamination
- The involved hepatic enzymes:
  - *pyridoxal kinase* (Zn depended) $\rightarrow$ produced phosphated vitamers
  - *alkaline phosphatase* $\rightarrow$ produced dephosphated vitamers
  - *pyridoxal phosphate oxidase* (FMN depended) e.g. PL $\rightarrow$ PLP
Catabolism of B₆s

- The binding of PLP to albumin protects the coenzyme from degradation in the blood.
- In the liver, dephosphorylated & oxidized by pyridoxal oxidase (FAD, FMN) & aldehyde dehydrogenase (NAD), respectively.
- PMP & PNP are oxidized by a FMN-dependent oxidase to form PLP.
- The forms of vitamin B₆ found in food are converted to active forms in the liver. Zinc, riboflavin, and niacin are necessary for this process.
- Fasting and reducing diets usually deplete the vitamin B₆ supply unless it is supplemented. Usually within 8 hours, much of the excess is excreted through the urine.

Excretion

- Bound B₆ vitamers are catabolized by FAD-dependent aldehyde (pyridoxal) oxidase as well as NAD-dependent aldehyde dehydrogenase to produce 4-pyridoxic.
- 4-Pyridoxic acid (PA) is the major excretory product, 50% in urine. It is also produced by the intestinal bacteria.
- Alcohol and its degradation product, acetaldehyde, displace PLP from proteins resulting in enhanced catabolism of the coenzyme.
- Amphetamines, chropromazine, oral contraceptives, and reserpine all increase B₆ loss. Oral contraceptives had been found to increase tryptophan use and thus increased B₆ use.
Functions

- Helping amino acid and protein metabolism
- Enabling the breakdown of glycogen to glucose
- Converting tryptophan to niacin (amino acid & vitamin interaction)
- Enabling red blood cell metabolism
- Helping the nervous system function efficiently
- Helping the immune system function efficiently

PLP is involved in over 100 enzymatic reactions. Some of the major functions are as follows:

- Aminotransferase reactions
- Decarboxylation reactions
- Site-chain cleavage reactions
- Dehydratase reactions
- Other functions
**Mechanism of Action**

- Metabolically active form of B₆ (PLP) serves as a coenzyme of numerous enzymes most of which are involved especially in *amino acid metabolism* and in *neurotransmitters* of central nervous system.

- **Cofactor of Many Enzymes:**
  - The largest group of vitamin B₆-dependent enzymes is *transaminase*, which use α-ketoglutarate as amino group acceptor.
  - Other PLP-dependent enzymes include *decarboxylases*, *racemases*, & enzymes that catalyze *amino acid side-chain alterations*.
  - PLP also serves as a coenzyme for the *phosphorylase* & as a *modulator of protein structure* (e.g. steroid hormone receptor, hemoglobin & other enzymes).

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[Diagram showing the metabolic pathways involving pyridoxine (vitamin B₆), including the conversion of homocysteine to methionine and the role of PLP in various metabolic reactions.]
**Metabolic Roles**

1. PLP involved in both *amino acids* biosynthesis & catabolism *(amino acid transamination)*
   - The response of *erythrocyte aspartate transaminatase* *(EAST)* to in vitro additions of PLP has been used as a biomarker of B₆ status
   - *Transsulfuration* of methionine to cysteine needs PLP *(cystathionine synthase & cystathionase)*, thus B₆ deprivation, individual shows homocysteinuria & cystathionuria.
Mechanism of transaminases

α-Amino acid $\rightarrow$ COO$^-$ (CH$_3$)$_2$N$^+$ Lys

\[ \text{Reamination} \]

External aldime

\[ \text{Quinonoid intermediate (resonance-stabilized cation)} \]

\[ \text{Ketimine} \]

\[ \text{Pyridoxamine phosphate (PMP)} \]
Serine, Glycine and Cysteine

Serine reacts with homocysteine, derived from methionine, to form cystathione. This reaction is catalyzed by cystathione β-synthase (CBS).

Cystathione is cleaved by cystathionase to yield cysteine and α-ketobutyrate which goes on to form succinyl CoA, a TCA cycle intermediate.

Both enzymes are homologous to aminotransferases and require PLP (vitamin B6).

Metabolic Roles

2. PLP involved in:
   - **Lipid metabolism**: Sphingolipid biosynthesis (by serine palmitoyltransferase) & arachidonic acid to prostaglandin E<sub>2</sub> & carnitine synthesis
   - **Gluconeogenesis**: the release of glycogen (glycogen phosphorylase) from the liver and muscles.
   - **Hemoglobin synthesis**: helps red blood cell production (the synthesis of porphyrin precursors to heme) [δ-aminolevulenic acid synthase]
   - **Niacin formation**: the conversion of tryptophan to niacin (kynureninase)
Glycogen Phosphorylase catalyzes phosphorolytic cleavage of the \( \alpha(1\rightarrow4) \) glycosidic linkages of glycogen, releasing glucose-1-phosphate as reaction product:

\[
glycogen_{(n \text{ residues})} + P_i \rightarrow glycogen_{(n-1 \text{ residues})} + \text{glucose-1-phosphate}
\]

This phosphorolysis may be compared to hydrolysis:

- Hydrolysis: \( R-O-R' + \text{HOH} \rightarrow \text{R-OH} + R'\text{-OH} \)
- Phosphorolysis: \( R-O-R' + \text{HO-PO}_3^{2-} \rightarrow \text{R-OH} + R'\text{-O-PO}_3^{2-} \)
Metabolic Roles

3. PLP involved in **Neurotransmitters synthesis:**
   - Conversion of *tryptophan to serotonin* (tryptophan decarboxylase needs PLP)
   - Conversion of *tyrosine to norepinephrine* (tyrosine decarboxylase) and acetylcholine that depend on PLP their metabolism.
   - γ-Aminobutyric acid (GABA) synthesis for brain as energy source
   - Synthesis of vasodilator and gastric secretagoge *histamine (histidine decarboxylase).*

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**Biosynthesis of Serotonin**

Tryptophan → **Bicpterin** → 5-Hydroxytryptophan

**Tryptophan hydroxylase**

5-Hydroxytryptamine (Serotonin)

5-HTP decarboxylase

Monoamine Oxidase

FAD (B₆)

Cu²⁺

Dehydrogenase

NAD⁺ (B₃₄)

5-Hydroxyindoleacetate (excreted in urine)
Biosynthesis of the catecholamines

Tyrosine hydroxylase

Phenylalanine \[ \rightarrow \text{Tyrosine} \]

Tyrosine \[ \rightarrow \text{DOPA} \]

\[ \text{Cu}^+ \]

DOPA decarboxylase

\[ \text{DOPA} \rightarrow \text{Dopamine} \]

Ascorbate \[ \rightarrow \text{Cu}^{++} \]

\[ \rightarrow \text{O}_2 \]

\[ \rightarrow \text{H}_2\text{O} \]

\[ \rightarrow \text{CO}_2 \]

Thyramine \[ \rightarrow \text{Melain} \]

\[ \text{SAH} \]

\[ \text{SAM} \]

Epinephrine \[ \rightarrow \text{Norepinephrine} \]

\[ \text{Pyridoxine} \]

\[ \text{35} \]

GABA Shunt

\[ \text{GABA} \rightarrow \text{GABA-Shunt} \]

\[ \rightarrow \text{GABA-Transaminase} \]

\[ \rightarrow \text{Glutamic acid} \]

\[ \rightarrow \text{GABA} \]

\[ \rightarrow \text{GABA Synthetase} \]

\[ \rightarrow \text{GABA} \]

\[ \rightarrow \text{GABA Dehydrogenase} \]

\[ \rightarrow \text{Gluconate} \]

\[ \rightarrow \text{Krebs Cycle} \]

\[ \rightarrow \text{Paminate} \]

\[ \text{Pyridoxine} \]

\[ \text{36} \]
Histamine Synthesis and Metabolism

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>ENZYME</th>
<th>INHIBITORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-histidine</td>
<td>histidine decarboxylase (HD)</td>
<td>brosseresine, α-hydrastinine, α-methylhistidine</td>
</tr>
<tr>
<td></td>
<td>histamine methyltransferase (HMT)</td>
<td>taurine, metoprine</td>
</tr>
<tr>
<td></td>
<td>diamine oxidase</td>
<td>aminoguanidine, semicarbazide-sensitive amine oxidases (SSAOs)</td>
</tr>
</tbody>
</table>

Table 48.1 Major Neurotransmitters

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Structure</th>
<th>Functional Class</th>
<th>Secretion Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td><img src="image" alt="Structure" /></td>
<td>Excitatory or inhibitory to skeletal muscles, excitatory or inhibitory at other sites</td>
<td>CNS, PNS, neurotransmitter neuromuscular junction</td>
</tr>
<tr>
<td>Biogenic Amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropeptide</td>
<td><img src="image" alt="Structure" /></td>
<td>Excitatory or inhibitory</td>
<td>CNS, PNS</td>
</tr>
<tr>
<td>Dopamine</td>
<td><img src="image" alt="Structure" /></td>
<td>Generally excitatory, may be inhibitory at some sites</td>
<td>CNS, PNS</td>
</tr>
<tr>
<td>Serotonin</td>
<td><img src="image" alt="Structure" /></td>
<td>Generally inhibitory</td>
<td>CNS</td>
</tr>
<tr>
<td>Amino Acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABA (gamma aminobutyric acid)</td>
<td><img src="image" alt="Structure" /></td>
<td>Inhibitory</td>
<td>CNS, neurotransmitter neuromuscular junction</td>
</tr>
<tr>
<td>Glycine</td>
<td><img src="image" alt="Structure" /></td>
<td>Inhibitory</td>
<td>CNS</td>
</tr>
<tr>
<td>Glutamate</td>
<td><img src="image" alt="Structure" /></td>
<td>Excitatory</td>
<td>CNS, neurotransmitter neuromuscular junction</td>
</tr>
<tr>
<td>Aspartate</td>
<td><img src="image" alt="Structure" /></td>
<td>Excitatory</td>
<td>CNS</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>(a very diverse group, only two of which are shown)</td>
<td>Excitatory</td>
<td>CNS, PNS</td>
</tr>
<tr>
<td>Substance P</td>
<td><img src="image" alt="Structure" /></td>
<td>CNS, PNS</td>
<td></td>
</tr>
<tr>
<td>Met-enkephalin (an endorphin)</td>
<td><img src="image" alt="Structure" /></td>
<td>Generally inhibitory</td>
<td>CNS</td>
</tr>
</tbody>
</table>
**Metabolic Roles**

- **PLP involved in Nucleic acids & immune system:**
  - The synthesis of nucleic acids process modulates via 1-carbon metabolism, e.g. *serine hydroxymethyl-transferase (SHMT)*;
  - PLP Inhibits *thymidylate synthease* through this process $B_6$ could impair DNA synthesis.
Pyridoxine 41

(SHMT = serine-hydroxymethyltransferase)

Reactions with other proteins - 7 effects
“Vitamin B-6” PH, PL, PM
Sulfur Amino Acids
Protein Methylase
Carnitine Synthesis
Lipid Metabolism
Cellular Structure
Neurotransmitter Synthesis
Nervous System function

Amino Acid Metabolism

Lysine (Schiff Base)
Glycogen Phosphorylase
Transamination
Home Binding
Home Synthesis
1-Carbon Metabolism
Nucleic Acid Synthesis
Hormone metabolism
Immune function
Tryptophan Metabolism
Niacin formation
Zinc absorption?

Figure 1. Cellular processes in which pyridoxal 5'-phosphate (PLP) acts as a coenzyme or binds with proteins and modifies the action of the protein. Home binding refers to PLP binding to hemoglobin.

Pyridoxine 42
Primary deficiency of vitamin B₆ is rare, secondary deficiency may result in certain situations, including malabsorption, alcoholism, some medications, cigarette, and smoking.

Frank deficiencies are rare, but subclinical deficiencies may exist, especially in women, heavy smoker, alcoholics and the elderly.

Symptoms of vitamin B₆ deficiency include:

- Skin inflammation and irritation, glossitis (sore or inflamed tongue), cheilosis (cracking and scaling of the lips), angular stomatitis, extensive dandruff and anemia.
- Confusion, depression, irritability and nervousness, fatigue and sleepiness, convulsions, water retention.
Assessment

- **4-Pyridoxic acid (4-PA):** main biologically inactive metabolite of B₆ in urine, but not in blood & tissue.
- **Plasma PLP:** the predominant vitamer in plasma & the most frequently used index of B₆ status in human, decreases with age, exhibiting highest conc. in new born & early children.
- ↑ protein intake, ↑ alkaline phosphatase & ↑ smoking, will increase B₆ requirement.
**Assessment**

- *Erythrocyte transaminase index* (PLP-dependent enzyme activity):
  - Erythrocyte Asparate aminotransferase (EAST or EAAT index)
  - Erythrocyte Alanine aminotransferase (EALT index)
  - With or without B₆ added if EAST-index > 2.2 → deficiency
  - Normal EAST index < 1.5; EALT index < 1.25

![Chemical Reaction Diagram]
Some indicators for assessing vitamin B₆ status

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma pyridoxal 5'-phosphate (PLP)</td>
<td>Major vitamin B₆ form in tissue and reflects liver PLP; changes fairly slowly in response to vitamin intake</td>
</tr>
<tr>
<td>Urinary vitamin B₆ catabolite excretion</td>
<td>Excretion rate of vitamin and particularly 4-pyridoxate reflects intake; 5-pyridoxate appears with excess intake</td>
</tr>
<tr>
<td>Erythrocyte aminotransferase activity coefficients</td>
<td>Enzymes for aspartate and alanine reflect PLP levels; large variation in activity coefficients</td>
</tr>
<tr>
<td>Tryptophan catabolites</td>
<td>Urinary xanthurenate excretion, especially after a tryptophan load test</td>
</tr>
<tr>
<td>Other</td>
<td>Erythrocyte and whole-blood PLP, plasma homocysteine</td>
</tr>
</tbody>
</table>

Methods of analysis

- Microbiological
- Enzymatic
- HPLC assay for various nutritional & food status
- PLP is one of the most measurement.
Requirements

- Vitamin B₆ intake is determined primarily by **protein intake**.
- The RDA for adults is a minimum of 2 mg of B₆ per 100 grams of protein consumed. In children, it ranges from 0.6-1.2 mg per 100 grams of protein.

Sources

- The richest sources of vitamin B₆ are chicken, fish, liver, kidney, pork, eggs, milk, wheat germ and brewer's yeast.
- Other good sources include brown rice, soybeans, oats, whole wheat products, **peanuts** and **walnuts**.
Supplements

- Vitamin B₆ is available as pyridoxine HCl and pyridoxal-5’-phosphate. The latter is the more active form and may be best for those with liver disease who cannot convert pyridoxine to pyridoxal-5’-phosphate.
- Anyone at risk of deficiency or who is suffering from a condition possibly linked to vitamin B₆ deficiency is likely to benefit from supplements.

Toxicity

- Large doses of vitamin B₆ (over 2,000 mg) can cause nerve damage.
- The tolerable upper intake level (UL) for vitamin B₆ from dietary sources and supplements combined is 100 mg per day. Symptoms of vitamin B₆ toxicity include: muscle incoordination, numbness of the hands and feet, impaired reflexes, abnormal plasma amino acid levels.
- Night restlessness, vivid dreams, sun sensitivity and an acne-like rash may also occur with high doses.
**Therapeutic Use**

- B<sub>6</sub> supplements are used to treat deficiency symptoms. They have also been widely used in the treatment of a number of other health conditions.
  - Asthma
  - Cardiovascular disease
  - Mood disorders
  - Premenstrual syndrome and estrogen therapy
  - Nausea of pregnancy
  - Carpal tunnel syndrome
  - Convulsions
  - Immune system
  - Other uses

**Interactions with nutrients**

- Vitamin B<sub>6</sub> requires **riboflavin, zinc** and **magnesium** for its normal function in the body.
- Vitamin B<sub>6</sub> deficiency may result in low blood levels of **vitamin C**, increased excretion of **calcium, zinc** and **magnesium**, and reduced **copper** absorption.
- More B<sub>6</sub> is utilized with an increased intake of the amino acid methionine.
**Interactions with drugs**

- Drugs that influence needs for $B_6$ are *oral contraceptives*, *isoniazid* (for tuberculosis), *hydralazine* (for high blood pressure), *amphetamines*, *reserpine* (for high blood pressure), and some *antibiotics*.
- *Alcohol* increases breakdown of the biologically active form of vitamin $B_6$ and long-term use may cause liver damage, which interferes with the conversion of vitamin $B_6$ to the active form.
- *Levodopa*, some antidepressants, and *penicillamine* may alter vitamin $B_6$ requirements.

**Cautions**

- $B_6$ supplements should not be taken by those taking *anticonvulsants* or *levodopa* for Parkinson's disease as they may alter the effectiveness of these medications.
- $B_6$ may lower blood sugar and should be used with caution by diabetics.