**Introduction**

- Riboflavin is known as vitamin B$_2$, riboflavin was the second B complex vitamin to be discovered.
- Riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).
- The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metalloflavoproteins.
Both classes of enzymes are involved in a wide range of redox reactions, e.g. succinate dehydrogenase and xanthine oxidase.

During the enzymatic reactions which involving the flavoproteins, the reduced forms of FMN and FAD are formed, FMNH$_2$ and FADH$_2$, respectively.

Physiological role of B$_2$ is being the precursor of riboflavin-5'-phosphate (FMN & FAD).
Chemistry

- Riboflavin is chemically known as 7,8-dimethyl-10 (1'-D-ribityl) isoalloxazine. In pure form it is a yellow-orange, water-soluble compound.
- In tissues there is a broad distribution of flavin but little is present as free riboflavin.
- B₂ is stable to heat, acid, and oxidation, however, destruction by light, especially ultraviolet light, as in sunlight. Thus, foods containing even moderate amounts of riboflavin, example, milk needs to be protected from sunlight. Only a little of the B₂ in foods is lost in the cooking process.
Chemistry (cont.)

- In order to maintain the biological activity:
  - **positional 7 & 8 must be substituted with more than a hydrogen**;
  - **the imine group in position 3 must be unsubstituted**;
  - **a ribityl group on position 10**

- Fluorescence is **pH dependent** (best measured between pH 4-8), maximal fluorescence occurs at 556 nm (**yellow-green**).

- The majority is found in flavocoenzymes [mainly in FAD and lesser amounts in FMN].
Chemistry (cont.)

Flavocoenzymes are largely *covalently* associated within diverse flavoproteins:

- Histidine & cysteinyl residue of *succinate dehydrogenase* [inner mitochondrial membrane]
- *Monoamine oxidase* [outer mitochondrial membrane]
- *L-gulonolactone oxidase* [liver microsomes of animals], but some exist as 8α-linked FAD.
Metabolism --- Absorption

- Flavonproteins digest by gastric HCl and intestinal enzymes, then released FMN & FAD.
- FMN and FAD then hydrolyzed by FMN phosphatase and FAD pyrophosphatase, respectively.
- Free vitamin B2 is easily absorbed from the small intestine into the blood, which transports it to the tissues.
**Metabolism --- Absorption**

- Free form absorbed by a **specific, saturated, active transport system** in proximal small intestine.
- Free form of B₂ is quickly trapped at the site by phosphorylation of FMN. Flavokinase needs ATP. Thus, riboflavin enters the portal circulation by free riboflavin. Absorption is enhanced by bile salts.
- Animal source represents better absorption, however, **divalent metals (Cu²⁺, Zn²⁺, Fe²⁺, Mg²⁺)** may chelate riboflavin and FMN.

**Metabolism -- Transportation**

- Free riboflavin and FMN bind with **globulin, fibrinogen and albumin** (weakly H-bond) in plasma.
- **Specific binding protein** --- specific riboflavin-binding protein (RfBPs) in plasma of laying hen, pregnant cow & pregnant mice & rats, stimulated by **estrogen**, 32 KD phosphoglycoprotein.
- Synthesis of FMN & FAD are regulated by **ACTH, aldosterone and thyroid hormone** which elevating activity of flavokinase.
Metabolism -- Tissue Distribution

- Predominantly as FMN (60-95%), FAD (5-22% in all tissues, but 37% in kidney)
- The greatest \([B_2]\) was found in liver, kidney & heart, total body reserve of \(B_2\) in adult for 2-6 wks.
- Riboflavin is not stored in the body, except for a small quantity in the liver and kidneys, so it is needed regularly in the diet.
- Free riboflavin is mainly converted into FAD and FMN in the small intestine, liver, heart and kidney cells by successional phosphorylation and dephosphorylation.

Metabolism -- Excretion

- Excess intake is eliminated in the urine, which can give it a yellow-green fluorescent glow, commonly seen after taking B complex 50 mg or 100 mg supplements.
- Excretion products of riboflavin include free riboflavin, \(7\alpha-\) & \(8\alpha\)-hydroxymethylriboflavin (microsomal cytochrome P450 enzymes involved), 10-hydroxyethylflavin, lumiflavin, 10-formylmethylflavin, 10-carboxymethylflavin and lumichrome etc. metabolites.
**Catabolism**

- Catabolism of B₂ (FAD & FMN) are catabolized by intracellular enzymes FAD-pyrophosphatase [FAD → FMN] & FMN-phosphatase [FMN → Riboflavin]
- Catabolism of riboflavin (riboflavin hydroxylation on 7α, 8α-positions by Cytochrome P450 system)
- Intestinal bacteria produce varying amounts of riboflavin (*hint: some people may utilize B₂ and minimize the degree of riboflavin deficiency, even with diets low in riboflavin intake.*)
Riboflavin functions as the precursor or building block for two coenzymes (dihydrolipoyl dehydrogenase [glycation] & succinate dehydrogenase [TCA cycle]) that are important in energy production.

As the catalytically operating moiety within bound flavocoenzymes, riboflavin participates in oxidation-reduction reactions in numerous metabolic pathways and in energy production via the respiratory chain.
Functions -- **Coenzyme (cont.)**

- Flavoproteins participate in both one- and two-electron transfers.
  - **One-electron** transfers (e.g. NADH, succinate) (glycation and TCA cycle)
  - **Two-electron** transfers (e.g. Fe-S proteins, heme proteins) (respiratory chain)
- FMN and FAD are the two coenzymes that act as hydrogen carriers to help make energy as adenosine triphosphate (ATP) through the metabolism of carbohydrates and fats.

1. FAD

- **2e⁻**
- FAD - yellow
- 2 e⁻ reduction
- **1 e⁻**
- FAD⁺ stabilized by extensive delocalization of lone electron
Riboflavin is instrumental in cell respiration, helping each cell utilize oxygen most efficiently. FMN & FAD operate in pyridine nucleotide-dependent and independent dehydrogenations, reactions with sulfur-containing compounds, hydroxylation, oxidative decarboxylation, deoxygenation, and reduction of $O_2$ to hydrogen peroxide following abstraction of hydrogen from substrates.
Examples

- The oxidases transfer \( H \) to \( O_2 \) to form \( H_2O_2 \), e.g.
  - **Xanthine oxidase** use a variety of purines as its substrate, converting hypoxanthine to xanthine which is then convert to uric acid & superoxide (\( O_2^- \));
  - **Succinate dehydrogenase** involves the mechanism in mitochondrial respiration and ATP synthesis.
  - **Acyl CoA dehydrogenase** catalyzes fatty acid oxidation, which needs the FAD linked. In fatty acid synthesis requires the presence of FMN-linked enzymes.
  - **Pyridoxine (pyridoxamine) 5'-phosphate oxidase** (FMN-dependent) is essential for conversion of the two form of vitamin B\(_6\) to its functional coenzyme, pyridoxal-5'-phosphate (PLP). *(vitamin-vitamin interaction)*
Examples

The oxidases transfer H to O₂ to form H₂O₂, e.g.

- Sphinganine oxidase involves the sphingosine synthesis requires FAD.
- Aldehyde oxidase using FAD converts aldehyde, such as pyridoxal (vit B₆) to pyridoic acid (PA) and retinal to retinoic acid.
- Monoamine oxidase (FAD dependent) involved in neurotransmitters (dopamine) and other amines (histamine).
- Choline catabolism requires FAD for choline dehydrogenase, mono methylglycine dehydrogenase and dimethylglycine dehydrogenase.
- Glutathione reductase needs FAD (GSSG → 2GSH)
Choline Synthesis

\[ \text{Choline} \rightarrow \text{P-Choline} \rightarrow \text{CDP-Choline} \rightarrow \text{Ptd-Choline} \]

- Ethanolamine: \( \text{ME} \)
- dimethylglycine: \( \text{DMG} \)
- choline: \( \text{CH}_3 \)
- CDP: \( \text{CDP} \)
- DMAE: \( \text{DMAE} \)
- Ptd: \( \text{Ptd} \)
- HMG: \( \text{HMG} \)
- Choline dehydrogenase: \( \text{ChDH} \)
- dimethylglycine dehydrogenase: \( \text{DMGDH} \)
- mono-methylglycine dehydrogenase: \( \text{MmGDH} \)

EA = Ethanolamine, \( \text{ME} = \) Monomethylglycine, \( \text{DM} = \) dimethylglycine, \( \text{P} = \) phosphonyllycine

\[ \text{choline} \rightarrow \text{Ptd-choline} \]
Figure 4. Regeneration of GSH under normal and riboflavin-deficient conditions. The diagram represents 2 major pathways for the formation of reduced glutathione in erythrocytes—reduction of GSSG via the glutathione reductase pathway and de novo biosynthesis via glutamylcysteine synthetase and glutathione synthetase. Bold arrows emphasize the predominant pathways, thin arrows represent pathways that are operating below maximal levels, and the dotted arrow indicates diminished enzymatic activity.
Riboflavin Deficiency

- Riboflavin deficiencies are rare in the United States & Taiwan due to the presence of adequate amounts of the vitamin in eggs, milk, meat and cereals.
- Riboflavin deficiency is often seen in chronic alcoholics due to their poor dietetic habits.
- Severe riboflavin deficiency is rare and often occurs with other B vitamin deficiencies.
Symptoms of $B_2$ deficiency

Symptoms of riboflavinosis include:

- sensitivity or inflammation of the mucous membranes of the mouth; cracks or sores at the corners of the mouth, called cheilosis; a red, sore tongue (glossitis) hyperemia edema of the oral mucosa;
- eye redness or sensitivity to light, burning eyes, eye fatigue, or a dry, sandy feeling of the eyes;
- fatigue and/or dizziness;
- seborrheic dermatitis with a dry yet greasy or oily scaling;
眼瞼發炎、角膜周圍毛細血管增生，充血(維生素B2)
Symptoms of $B_2$ deficiency

- Symptoms of **riboflavinosis** include:
  - nervous tissue damage (peripheral neuropathy), decreased sensitivity to touch, temperature, vibration and position may occur in the hands and feet;
  - normocytic anemia associated with pure red cell hypoplasia of the bone marrow; hair loss, weight loss, general lack of vitality;
  - retarded growth in infants and children.

Factors contributing to deficiency

- **Inadequate diet**
  - whom with lactose intolerant and can’t drink milk
  - those with malabsorption disorders, diarrhea and irritable bowel syndrome
- **Alcoholics**
- **Photography given to infants with hyperbilirubinemia often leads to $B_2$ deficiency**
- **Systemic infections**
- **Oral contraceptive agent**
- **Diuretics**
Therapeutic Uses

- Riboflavin supplements are used to treat or prevent riboflavin deficiency.
- Riboflavin is also used therapeutically to ameliorate ariboflavinosis resulting from diverse causes i.e.
  - inadequate dietary intake
  - decreased assimilation
  - rare genetic defects in the formation of specific flavoproteins
  - hormonal disorders
  - use of certain drugs.

Therapeutic Uses

- Riboflavin is also used for many kinds of stress conditions, fatigue, and vitality or growth problems.
- For people with allergies and chemical sensitivities, riboflavin-5’-phosphate may be more readily assimilated than riboflavin.
Therapeutic Uses (remedy)

- Anemia
- Migraine: 400 mg riboflavin for relieved migraine
- Carpal tunnel syndrome: Riboflavin may help to relieve the symptoms of carpal tunnel syndrome usually combined with vitamin B6
- Neonatal jaundice: phototherapy for infants with high bilirubin
- Other uses:
  - Skin problems i.e., acne, dermatitis, eczema and ulcers
  - eye problems such as cataracts;
  - muscle cramps and stress

Diet Recommendations

- The RDA of vit B$_2$ is based on weight, state of metabolism and growth, and protein and calorie intake.
- 1989 US RDA suggests 0.6 mg/1000 kcal, 1998 US DRI RDA for adult men and women over aged 19 years for individual intake for B$_2$ is 1.3 mg and 1.1 mg/day.
- The RDA range from 0.4 mg/d for early infants to 1.3 mg/d for women and 1.5 mg/d for men.
- An additional 0.3 mg/d is recommended during pregnancy and 0.5 mg/d for lactation.
Food Sources

- Small amounts of riboflavin are present in most plant and animal tissues.
- The richest sources of riboflavin include milk (daily products) and organ meats such as liver, kidney and heart. Yeast, eggs and vegetables are also rich sources. Flour and cereals are enriched with riboflavin.

Toxic effects of excess intake

- Riboflavin intake of many times the RDA is without demonstrable toxicity. Nevertheless, the photosensitizing properties of riboflavin raise the possibility of some theoretical, potential risks.
- High doses of riboflavin are not well absorbed so the risk of toxicity is very low. Possible reactions to excess intakes include itching, numbness, sensations of burning or pricking, and sensitivity to sunlight.
**Interactions with other nutrients & drugs**

- Riboflavin is necessary for the activation of vit B₆ and is also necessary for the conversion of tryptophan to niacin.
- Sulfa drugs, anti-malarial drugs, estrogen, cathartic agents and alcohol may interfere with riboflavin metabolism.
- High doses of riboflavin can reduce the effectiveness of the anticancer drug methotrexate. Some antibiotics and phenothiazine drugs may increase riboflavin excretion.

**Clinical assessment**

- **Urinary riboflavin & its metabolites.** High doses of riboflavin can produce urine discoloration, which can affect urine analysis results.
- **Negative nitrogen balance**
- **Erythrocyte Glutathione Reductase (ERG) (EC 1.6.4.2) & Pyridoxine Oxidase (EC 1.1.1.65)**
- **Microbiological assays:** *Lactobacillus casei*, *Leuconostoc mesenteroides* & *Tehrahymena pyriformia.*
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<th>adequate</th>
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Source: from data reported by Bates 1993; Sauberlich. et al. 1974.