

MEDCH 562 Fall 2018

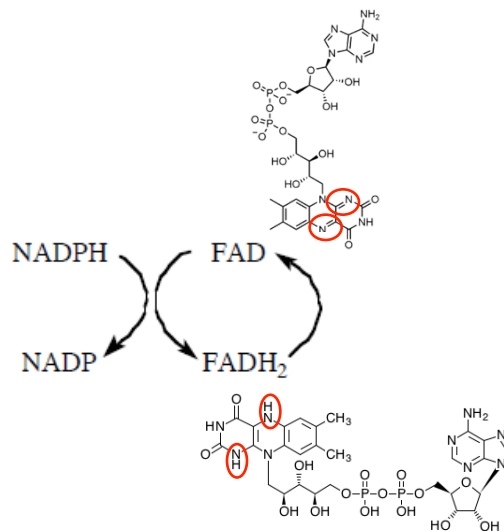
Water Soluble Vitamins – Problem Set 2

- 1) Cytochrome P450 Reductase (CPR) is an enzyme responsible for the reduction of a large portion of the cytochrome P450 (CYP) enzymes.
 - a. What cofactor is required for CPR activity, and from what vitamin is the cofactor derived?
 - b. Do you expect a purified solution of CPR to be colored, and if so what color would it be (you may assume that all enzyme is in its **oxidized** state)?
 - c. Suppose CYP3A4 and excess NADPH are added to the solution. What color change (if any) would you expect as a result (ignoring any color contribution from the 3A4)?

a. Flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN) are both cofactors which are required for CPR activity. These cofactors are derived from riboflavin (B2).

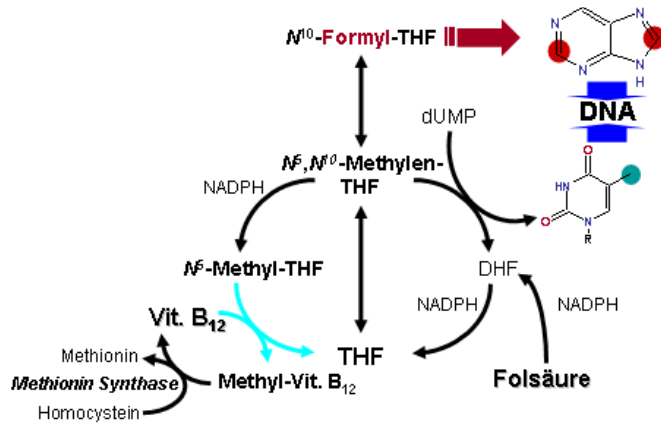
b. FAD and FMN cofactors are widely conjugated following oxidation; the conjugation alters the absorbance of the molecule giving it a yellow color. Therefore, a solution of pure oxidized enzyme should be yellow

c. NADPH can reduce FAD or FMN, thus the cofactors should be converted to their colorless forms.



- 2) What are the main types of reactions that use folates as a cofactor?

- Methylation reactions where folate as cofactor provides a single carbon. One example is the folate mediated conversion of B12 to methyl-B12, a process crucial to homocysteine metabolism.
- Folate is also important to the synthesis of nucleic acids. One example is the N₅-N₁₀ methylene-THFA mediated formation of deoxythymidine monophosphate from deoxyuridine monophosphate.



- 3) What is one of the most important concerns with folate deficiency (especially for pregnant women or women who may become pregnant)?

Folic acid deficiency can result in teratogenesis. In pregnant women this can lead to birth defects such as neural tube defects or orofacial clefts.

- 4) How does Methotrexate affect folates? Describe the therapeutic strategy of “Leucovorin rescue”.

Methotrexate inhibits DHFA reductase preventing formation of THFA, which is the active coenzyme form of folic acid. By supplementing certain patients with leucovorin, it is possible to offset the toxic effects of methotrexate. This is because leucovorin treatment increases N5-formyl THFA, which is readily converted into THFA and other forms of THFA.

- 5) What makes high dose folate supplements risky?

Supplementation with folate (B9) can mask hematological symptoms of pernicious anemia (B12 deficiency). Pernicious anemia can result in neurological damage if untreated.

- 6) How does Vitamin B₁₂ deficiency promote folate deficiency, in biochemical terms?

Vitamin B12 is required for conversion of 5-methyl THFA to THFA. Therefore, B12 deficiency can lead to depletion of THFA and a buildup of 5-methyl THFA (megaloblastic anemia).

- 7) What two classes of enzymes use Vitamin B₁₂ as a cofactor? What are their main functions?

Methionine synthase: uses B12 (5-methyl cobalamin) to convert homocysteine to methionine.

Methyl malonyl CoA mutase: uses B12 (5-deoxyadenosyl cobalamin) in the metabolism of odd chain fatty acid, such as methyl malonyl CoA.

- 8) How is Vitamin B₁₂ absorbed? In cases of B₁₂ deficiency, what are the major routes of administration and what are the rationalizations for each?

Hydrochloric acid in the stomach liberates B12 bound in peptide bonds to food, possibly allowing for absorption in the digestive system. This has been rationalized by observing pernicious anemia in patients taking proton pump inhibitors, which are known to reduce excessive stomach acid.

Intrinsic factor (glycoprotein, IF) has also been implicated in B12 absorption. This has been rationalized by observing lack of IF in patients with pernicious anemia.

Oral administration has limited effect in compensating for poor B12 absorption by patients with low IF or gastric acid. In general these patients are treated with intramuscular (IM) injections of vitamin B12

- 9) What type of chemical reactions is Coenzyme A (CoASH) used for and what vitamin is converted to Coenzyme A?

Pantothenic acid (B5) is converted to Coenzyme A by addition of β -mercaptoethylamine. CoA can form a very high energy thioester bond. The thioester of CoA can participate in an acyl transfer reaction, a process which is important to the metabolism of fatty acids, amino acids, and carbohydrates.

- 10) What vitamin deficiency can be induced by consumption of raw egg whites? Why? (Bonus: How do biochemists leverage this effect for research?)

Raw egg whites are rich in Avidin, a protein with unusually high affinity for biotin (B7). Overconsumption of egg whites can lead to nearly irreversible sequestration of B7, limiting absorption and causing deficiency.

Bonus: Biochemists have taken advantage of the strong biotin-avidin interaction to aid in a process called a pull down. In the pull down, biotinylated tags are attached to protein targets in a mixture through a cleavable linker. Next, the mixture is treated with small beads functionalized with the avidin protein, interaction between the beads and the biotinylated target lead to immobilization of the tagged protein target. Following immobilization, the researcher can wash away all non-targeted cellular debris leaving behind only the tagged protein target bound to the bead. As the biotin-avidin interaction is too strong, the researcher must cleave the linker to liberate the target protein from the bead.

- 11) How to check vitamin B12 deficiency?

Methyl malonic acid levels in urine can be measured to assess for B12 deficiency. It is also possible to use radiolabeled B12 (schillings test).

- 12) What disease commonly occurred in the "corn belt" of the US in the 1900's? What could have remedied this situation?

Pellagra was commonly observed in the "Corn Belt" due to the lack of available nicotinic acid and tryptophan in corn. Occupants of the region could have been given access to more diverse food rich in

nicotinic acid or tryptophan. Alternatively the use of zinc supplements could have been helpful, as low zinc is known to contribute to pellagra.

13) What enzymatic reactions does biotin mostly participate in?

Biotin is an important cofactor for carboxylase enzymes. Carboxylase uses the active carboxybiotin to perform carboxylation reactions important to lipid and carbohydrate biosynthesis.

14) What are the major functions of Vitamin C in the cell? Which stereoisomer can serve as a co-factor of enzymes?

Ascorbic acid (vitamin C) is known to be an important electron donor in biological redox reactions. Vitamin C is important to a variety of biosynthetic pathways however, only the L-isomer is capable of acting as a cofactor.

15) What Nobel Prize winner claimed that mega-dosing (multiple grams/day) of Vitamin C was "How to Live Longer and Feel Better"? Do you agree or disagree with the efficacy of high intake of Vitamin C in prevention of disease?

Linus Pauling suggested that vitamin C could improve health and extend life. Contemporary research has not conclusively demonstrated any therapeutic benefits to "mega-dosing" Vitamin C. Furthermore, vitamin C absorption becomes increasingly inefficient as an individual approaches 500mg/d, so it is unlikely that "mega-dosing" conveys any tangible benefit.

16) How to check folate deficiency?

Homocysteine levels may be elevated, also macrocytic red blood cells and megaloblasts in bone marrow are often observed in individuals with folate deficiency (megaloblastic anemia)

17) Why do alcoholics frequently suffer from folate deficiency?

Alcohol is understood to interrupt enterohepatic circulation of folate.

18) The tolerable Upper Limit dose is the:

- a. highest dose of a vitamin at which no toxicity or insufficiency is observed.
- b. the lowest dose of a vitamin at which benefits are observed with supplementation.
- c. 1.2 times the RDA.
- d. none of the above.

19) If the EAR for a vitamin is 0.5 mgs/day then the RDA is:

- a. 0.6 mg/day
- b. 0.5 mg/day

- c. 0.75 mg/day
- d. 1.0 mg/day
- e. none of the above

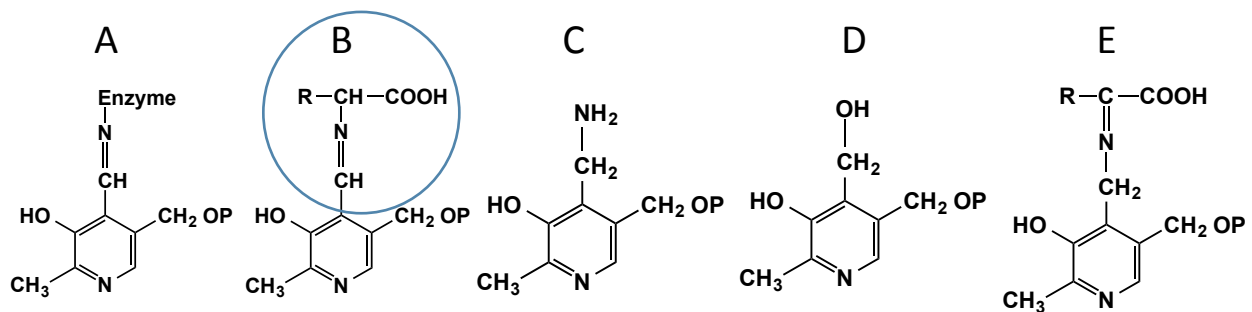
20) Which of the following statement is false?

- a. Vitamin C is not well retained in our body
- b. Overdosing of vitamin C likely causes toxicity**
- c. Overdose of vitamin A likely causes toxicity
- d. Vitamin B1 and C needs regular replacement

21) Rice polishings containing which vitamin and is responsible for beriberi resulting from overconsumption of polished rice in Ancient Asia?

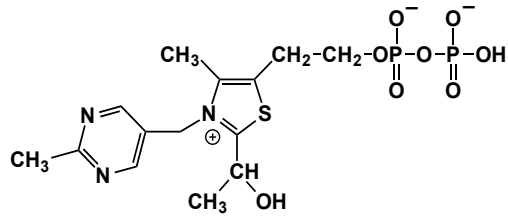
- a. Vitamin C
- b. Vitamin A
- c. Vitamin B1**
- d. Vitamin D

22) Which imine form of pyridoxal below yields an amino acid upon hydrolysis [P= HPO₃]:



23) Which of the following are possible fates for the hydroxyethyl TPP form of thiamine:

- a. decarboxylation to yield CO₂ and pyruvate
- b. deprotonation to a carbanion followed by attack at an aldehydic carbon to transfer two carbons to a sugar**
- c. attack by ethanol to reduce systemic ethanol levels and benefit alcoholics
- d. reduce dehydroascorbate back to vitamin C



24) Which vitamin need is proportional to calorie intake?

- a. Riboflavin
- b. Folic acid
- c. Thiamin
- d. B12

25) Tryptophan is the biosynthetic precursor to which vitamin? F And which vitamin is involved in this transformation? C

- a. Thiamin
- b. Riboflavin
- c. Pyridoxal
- d. Folic acid
- e. B12
- f. Niacin

26) Match the test for deficiency of a vitamin, with the vitamin:

C Vitamin B₂

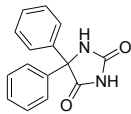
A Vitamin B₁₂

- a. urine methylmalonic acid level
- b. transketolase assay in red blood cells
- c. erythrocyte glutathione reductase activity
- d. erythrocyte transaminase activity

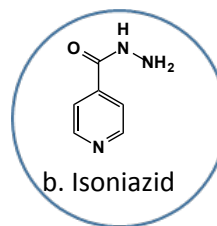
27) To lower the plasma level of homocysteine, the best way is to supplement with:

- a. Thiamine
- b. Thiamine, riboflavin and B₆
- c. Vitamin B₆, folic acid and cobalamin
- d. Thiamine, riboflavin and niacin

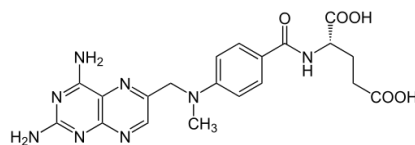
28) In principle, which drugs or vitamins below could possibly result in iatrogenic Vitamin B₆ deficiency if used chronically, based on the structures shown:



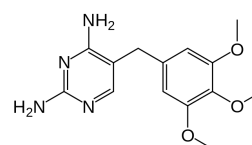
a. Phenytoin



b. Isoniazid



c. Methotrexate



d. Trimethoprim

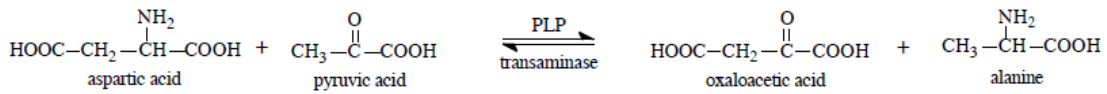
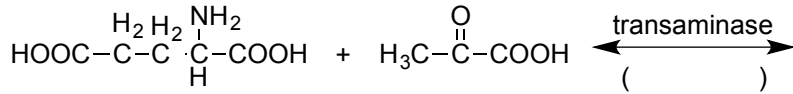
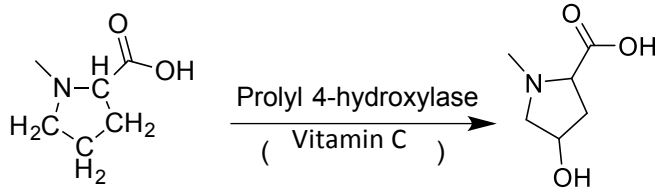
29) Choose the correct description about Leucovorin:

- a. is contraindicated in levo-DOPA therapy
- b. can cause vitamin B₆ deficiency
- c. can allow ordinarily lethal dose of methotrexate to be used against tumor
- d. act as an antibiotic by inhibiting bacterial DHFA reductase

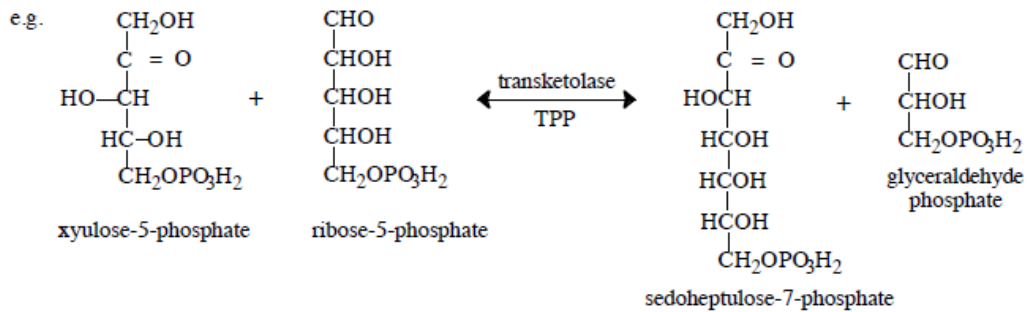
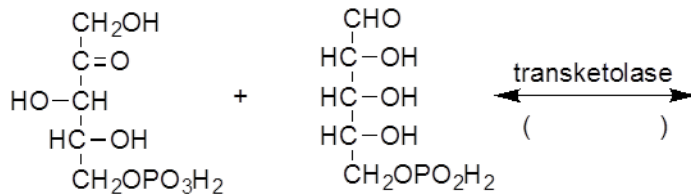
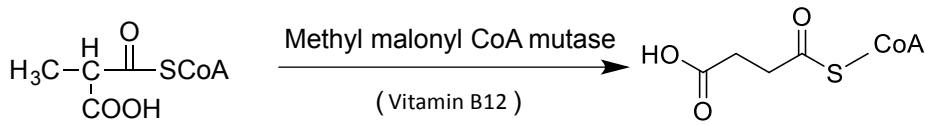
30) An elderly patient with pernicious anemia and low gastric HCl would most likely benefit from:

- a. oral supplements containing vitamin B₁₂.
- b. oral supplements containing vitamin B₉.
- c. IM injections of vitamin B₁₂.
- d. transdermal patches of containing vitamin B₆.

31) Please write the products for the following transformations and specify which vitamins participated as a cofactor.



(vitamin B6, PLP)



(Vitamin B1, phosphorylated TPP)