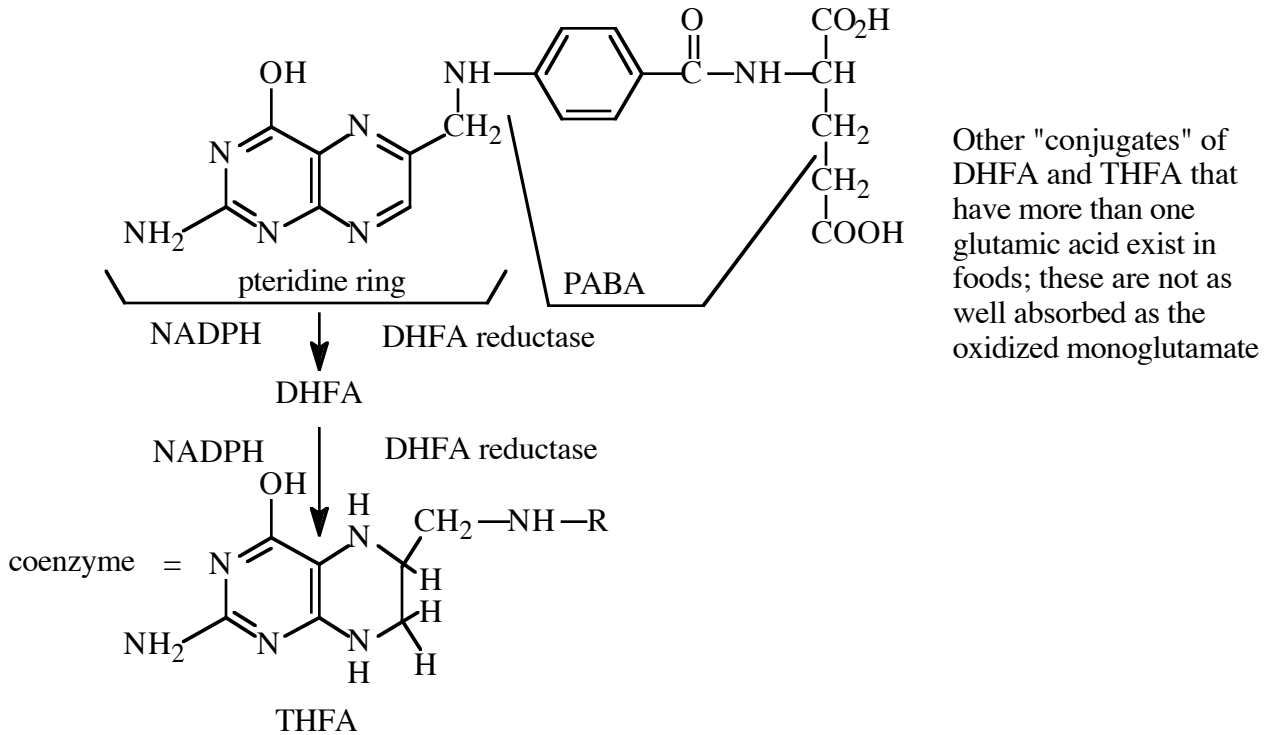


E. Folic Acid

1. Chemistry



2. Nomenclature

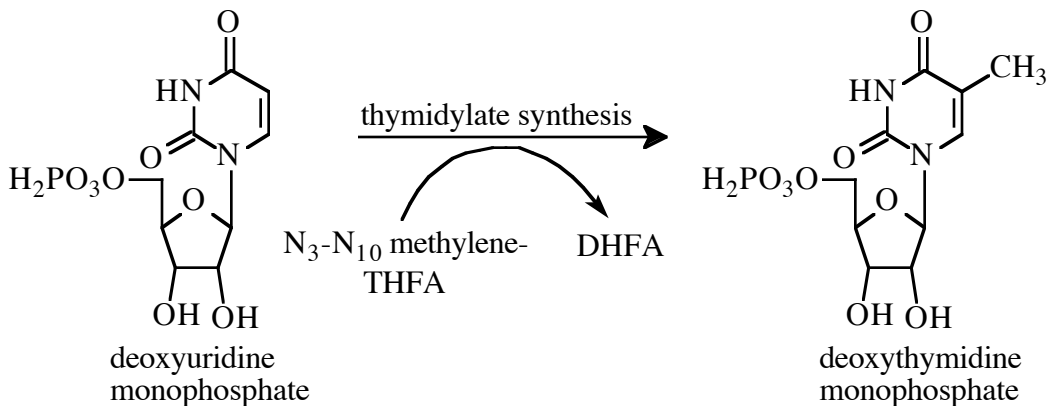
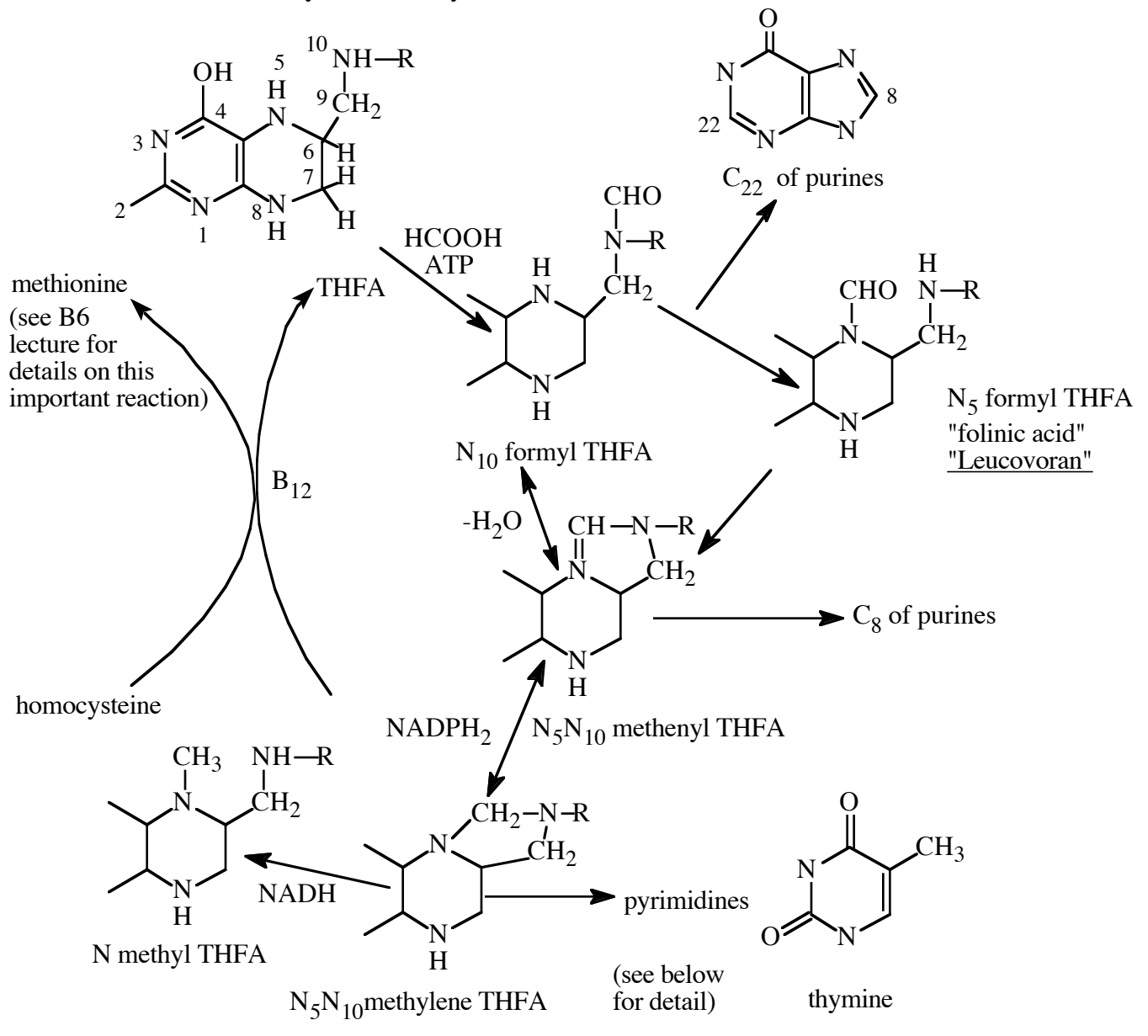
Folic acid is pteroyl monoglutamic acid. This fully oxidized form is not found naturally but is what is used in supplements. Reduced polyglutamates are found in animal and plant foods. Folic acid as a supplement or DHFA polyglutamates in foods are readily converted to the active fully reduced polyglutamates unless specific inhibitors are present (e.g. methotrexate).

Common use -- folates means all pteroglutamates having vitamin activity.

Absorption, transport, circulation, storage -- dietary folates are enzymatically cleaved by pancreatic and intestinal mucosal peptidases to the monoglutamate which is absorbed. Synthetic folic acid is well absorbed with a bioavailability of about 85%. Most of circulating folate is 5-methyl tetrahydrofolic acid (5-meTHFA). A considerable amount is excreted in the bile but most is reabsorbed. This continued enterohepatic circulation of folate is important for maintaining adequate levels and is interrupted by alcohol.

3. **Function**

The folate cycle and methylation



4. **Folate deficiency**

- Deficiency results in megaloblastic anemia. Symptoms include headache, fatigue, weight loss, anemia, nausea, anorexia, diarrhea, insomnia, irritability, forgetfulness. Signs are macrocytic red cells and megaloblasts in the bone marrow.
- Deficiency may result in teratogenesis with neural tube defects and possibly orofacial clefts. The importance of adequate folate intake at conception and for the first 3 weeks when the neural tube closes is obvious to few mothers. In 1992 the CDC, FDA, and NIH jointly recommended that "all women of childbearing age who are capable of becoming pregnant should consume 0.4 mg of folic acid per day as folic acid for the purpose of preventing neural tube defects." "Care should be taken to keep folate below 1 mg." "Enriched" grains now fortified with 140mg/100g to help decrease risk for birth defects.
- Deficiency may result in elevated homocysteine (HCS) which is associated with increased risk for coronary disease (and maybe the birth defects). Recent evidence indicates that a genetic polymorphism in the N5N10 methylene THFA reductase enzyme is involved. Those with a C to T substitution in the gene coding for this enzyme (13%) are at high risk due to an enzyme of higher K_m with resulting elevated HCS.
- Oral contraceptives and anticonvulsant drugs use may increase folate catabolism..
- Folate deficiencies are seen under conditions of poor nutrition, heavy alcohol ingestion and pregnancy and lactation.
- Alcohol decreases enterohepatic circulation of N5 methylTHFA.

5. **B₁₂ deficiency in relationship to folate**

B₁₂ deficiency results in a folate deficiency because folates are not recycled (see above folate cycle scheme). This is because 5-MeTHFA is not converted back to THFA in the absence of B₁₂. Megaloblastic anemia therefore results from a deficiency of B₁₂. Also, in a B₁₂ deficiency, neurological damage is observed due to lack of B₁₂ (see B₁₂ discussion) which is hard to detect. Therefore high dose folate supplements are risky in cases where there is a possibility of pernicious anemia (B₁₂ deficiency) because folate will mask hematological symptoms while neurological damage goes on unchecked. This is why preparations containing > 0.8 mg of folic acid are on Rx.

6. **Folate antagonists**

- a. Methotrexate -- ↓ DHFA reductase ∴ stops all in "S" phase cell cycle.

Note: "Leucovoran rescue" technique allows ordinarily lethal dose to be administered with consequent increased tumor kill.

- b. Trimethoprim --inhibits bacterial DHFA reductase and, combined with sulfamethoxazole (Bactrim® Roche, Septra® BW), which is a PABA antagonist, a "double hit" against bacterial folate metabolism is affected. There is usually very little effect on mammalian DHFA reductase.
- c. Alcohol-affects enterohepatic circulation of folates
- d. Nitrous oxide -- continued frequent inhalation has produced fatal megaloblastic hematopoiesis and a neuropathy similar to pernicious anemia. The N₂O oxidizes the cobalamin to create a B₁₂ deficiency with resulting folate deficiency.

- e. Phenytoin – suboptimal folate levels observed with long term therapy and rare megaloblastic anemia. BUT, folic acid (in high doses) decreases phenytoin levels with cases of seizures reported.
- f. Pyrimethamine- used for parasite infections (e.g. toxoplasma, malaria) as a parasite DHFAs reductase target. Folic acid supplements may reduce effect of drug. Leucovorin is OK.

7. Use

- a) Deficiency-use with oral contraceptives and during pregnancy and lactation.
- b) To prevent neural tube defects in the unborn, women contemplating pregnancy should take a supplement containing 0.4 mg folic acid, otherwise women of childbearing potential should assure that they are consuming at least 0.4 mg/day (I suggest a supplement).
- c) Cervical dysplasia, bronchial squamous dysplasia, and dysplasia of colonic tissue in ulcerative colitis patients -- studies show elevated risk is associated with low folate. More results needed but importance of folate is evident.
- d) Colon cancer and breast cancer- in moderate to heavy alcohol users, multivitamin use (folic acid) reduced risk but not in nondrinkers.
- e) Alzheimer's disease. Preliminary evidence shows low folate levels associated with increased risk.

8. Source

("Folic") leafy vegetables, fruit juices, beans; meats and most fruits are low.. "Enriched" flour now contains 140 µg/100 g of folic acid.

9. Stability

Labile to light, heat, storage. Should try to eat some "fresh" vegetables. Cooking losses are high (80-90%).

10. Dose

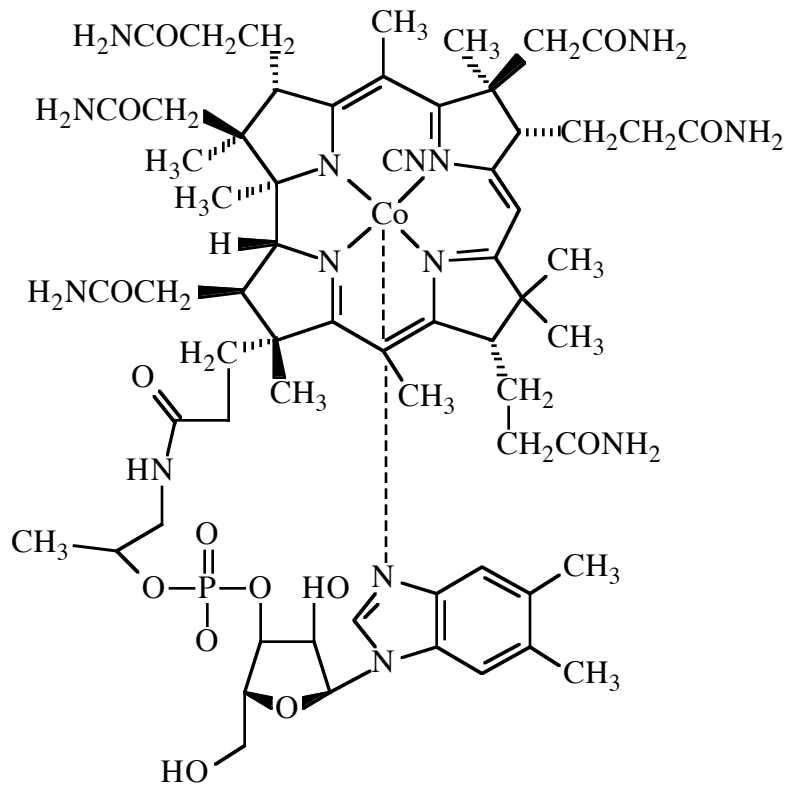
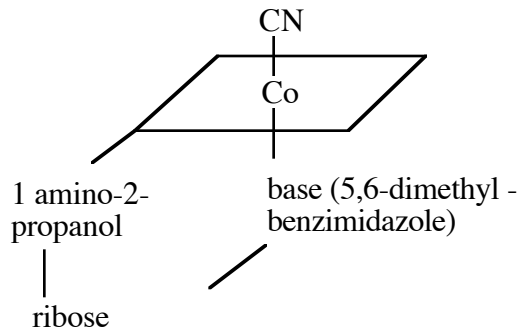
- Preparations containing greater than 0.8 mg are on Rx only.
- DV = 0.4 mg of folate
- The Food and Nutrition Board has set the requirement in DFE (dietary folate equivalents) (see supplemental table). 1 DFE=1µg of food folate; 1µg of supplement folic acid =1.7DFE. On an empty stomach it is 2DFE.

11. Toxicity: essentially non-toxic. UL=1000µg folic acid

F. Vitamin B₁₂

1. Structure -- synthetic material is cyanocobalamin

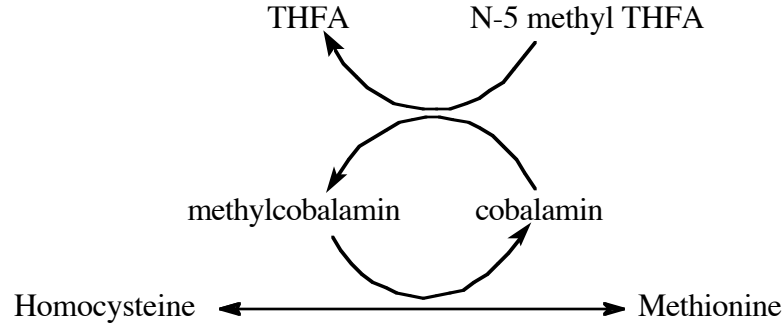
Coenzymes = 5 deoxyadenosine or methyl group replacing the CN; Hydroxy cobalamin is also active.



Vitamin B₁₂

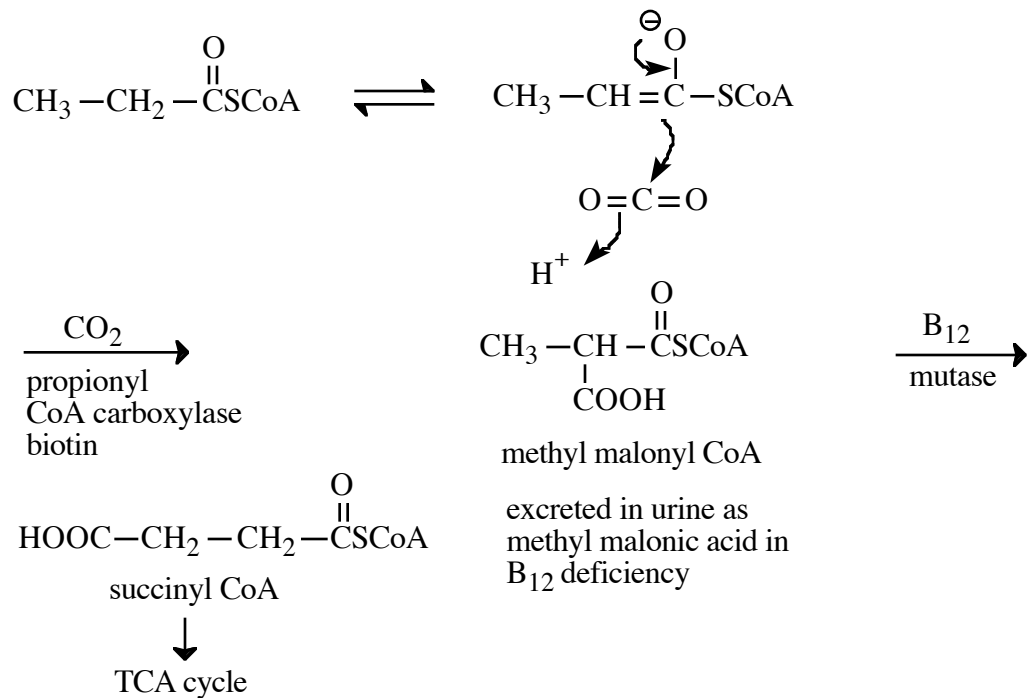
2. Function

a. Methyl transfer reactions



i.e. needed for recycling of THFA

b. Metabolism of odd chain fatty acids



c. Myelin synthesis

Mechanism of B_{12} involvement may relate to the buildup of methyl malonic acid with a resulting decrease in myelin synthesis. B_{12} deficiency results in demyelination and neurological damage.

3. Deficiency -- pernicious anemia

Symptoms related to inadequate myelin synthesis and megaloblastic anemia due to failure to recycle folates; i.e., numbness, poor coordination, poor memory, confusion, depression; at least 2-5 mg stored in liver and turnover is only 0.1% per day, therefore deficiency takes years to develop. Deficiency is rarely diet based, although vegans are at risk.

4. **Absorption** -- B₁₂ absorption

HCL in stomach splits B₁₂ from peptide links in food; intrinsic factor (a glycoprotein) secreted by stomach mucosa; required for transport of B₁₂ across ileum wall (also requires Ca⁺⁺ and a pH > 6 and releasing enzyme). Most of pernicious anemia is due to lack of synthesis of intrinsic factor and not due to dietary deficiency of B₁₂. A simple B₁₂ deficiency may be seen in older populations due to decrease in gastric HCl.

5. **Source** -- meats, especially liver and yeast; microorganisms are ultimate sources of B₁₂.

6. **Production** -- by fermentation.

7. **Stability** -- Stable at pH 4-7; labile to light.

8. **DV** = 6 µg.

9. **Use**

In pernicious anemia, give 100 µg IM q 4 weeks; Studies indicate that 1 mg/day P.O. will work also. A nasal solution 0.5mg/0.1ml) is marketed and is convenient. High IM doses are used for methylmalonic aciduria, an inborn error of metabolism.

Increasing interest in the role of B₁₂ in keeping folic acid levels up and homocysteine levels low. See folic acid lecture for the implications of high homocysteine and low folic acid.

Vegans should take supplements

Those over 65 should take a supplement (multivit) due to decreasing HCl needed for absorption of B₁₂ from foods

10. **Diagnosis:** Schillings test (labeled B₁₂) or better, measure methyl malonic acid in plasma. Accurate diagnosis is important for rational therapy of anemias.

11. **Preparations available**

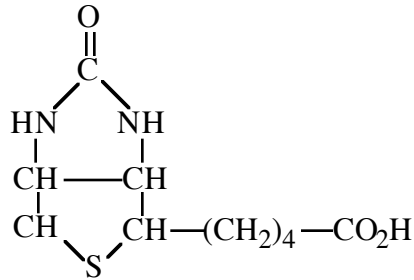
In case of pernicious anemia usually given IM because patients often become refractory to the intrinsic factor in oral preparations. If problem is lack of HCl, po doses are OK.

Long-acting form is hydroxycobalamin (Alpha redisol® MSD) -- dose is 100 µg/mo.

11. **Toxicity** -- essentially nontoxic

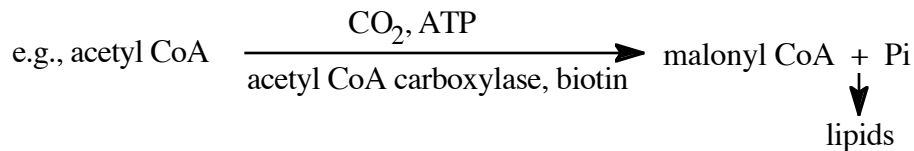
H. Biotin

1.



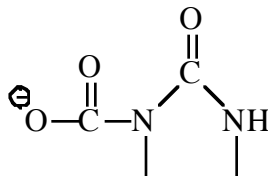
bound to enzymes through ϵ amino of lysine; dietary proteins are digested to lysine-biotin (biocytin) which is hydrolyzed by biotinase to release biotin. An inborn error with a defect in biotinase is known.

2. **Function** -- carboxylation reactions



e.g. pyruvate carboxylase- gluconeogenesis
e.g. propionyl-CoA carboxylase – see B12 lecture

carboxybiotin = active species



3. **Deficiency** -- rare; rash, hair loss, fatty deposits on face, depression.

4. **Requirement** -- avidin (protein from egg whites) can precipitate deficiency state → dermatitis, muscle pain, etc.; synthesized by intestinal flora.

5. **Stability** -- very stable.

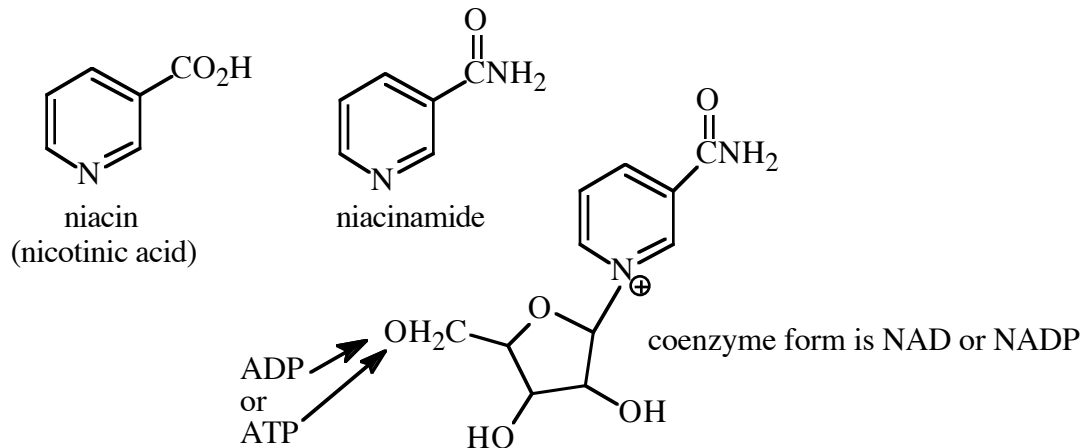
6. **Source** -- eggs, meat, nuts are rich sources.

7. **DV** = 0.3 mg.

8. **Use** -- rarely used alone; several biotin responsive inborn errors of metabolism are known, the most common being a defective biotinidase.

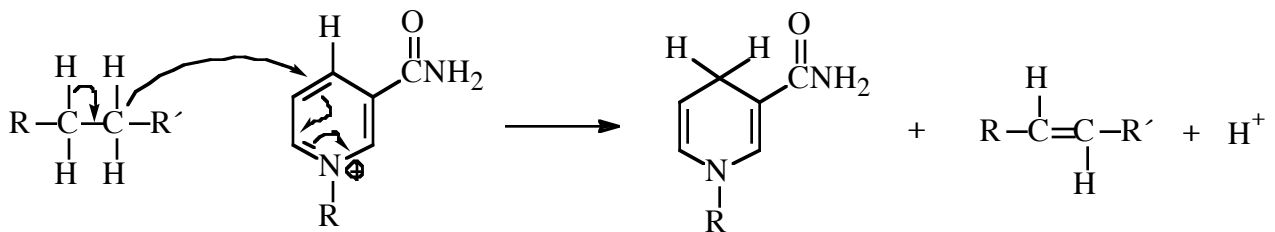
I. Niacin (Vitamin B₃)

1. Chemistry

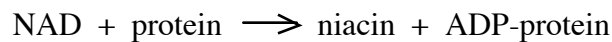


2. Function -- >150 enzymes require NAD or NADP.

a. redox and electron transport



b. ribosylation of proteins in cell signaling and DNA replication and repair

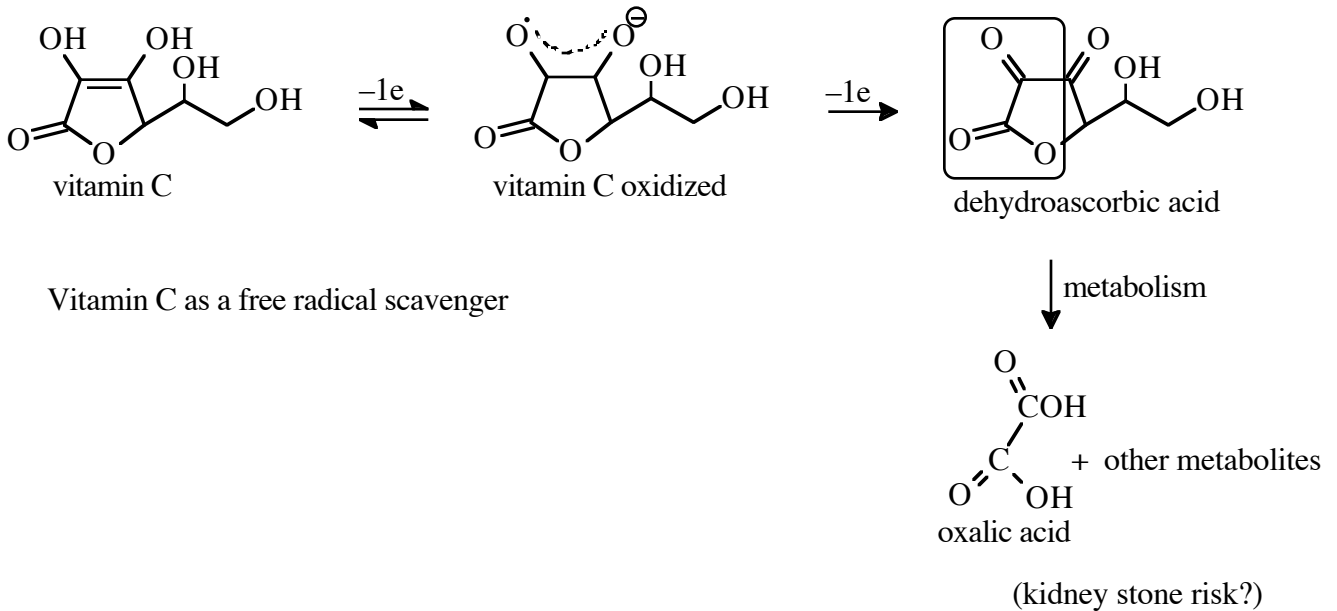


- Deficiency state** - Pellagra - "4D's" dermatitis, diarrhea, dementia, death; red tongue and pigmentation = common signs; seen in "corn belt" in U.S. during early 1900's, reason -- lack of available nicotinic acid and tryptophan in corn.
- Biosynthesis** -- See B₆ section for biosynthesis of niacin from tryptophan. It is estimated that 60 mg of tryptophan gives 1 mg of niacin. Isoniazid therapy can precipitate some symptoms of pellagra by binding up PLP and stopping the conversion of trypt to niacin.
- Source** -- meat, fish, whole grain cereals, peanuts. Ingested in foods as NAD or NADP then hydrolyzed in the intestinal mucosa.
- Stability** -- very stable, but much is lost if cooking water is discarded.
- Requirements** -- DV = 20 mg, but requirement will depend on tryptophan intake.
UL=35mg
- Use** -- Component of multivitamin preps also used in high doses for its pharmacological effects as described below.

- a. For improving serum lipids -- use Niacin (NA); Niacinamide is not effective; NA used in doses up to 10 q/d (usually 3-5g), will lower LDL 5-25%, triglycerides 20-50% and raise HDL 15-35%. Combines well with statin drugs. Side effects are significant, but decrease with time. Mechanism: exact mechanism is unknown but does lower production of VLDLP and activates lipoprotein lipase. Niacin and some sustained release products are OTC. The extended release product (Niaspan) is Rx.
 - b. Schizophrenia – use of high dose niacin has been popular but is of unproven efficacy.
 - c. Peripheral Vasodilator -- usefulness is questionable.
 - d. Diabetes—there is interest in niacinamide in high doses to prevent type 1 diabetes in high risk kids and for type 2 adults. Niacinamide may help protect pancreatic beta cells but results showing benefit are preliminary.
9. **Toxicity** (in doses over UL) -- peripheral vasodilation, flushing, GI upset, ulcers, diarrhea, impaired glucose tolerance, liver damage (reversible?) and increased gout. These effects are seen with high doses (gram quantities) and decrease the usefulness of this vitamin for treating hyperlipidemias. Hepatitis has been associated more with the sustained release preparations of NA but not as much with the “extended release” product (Niaspan). The vasodilation and GI upset decreases after a few weeks on the drug. Aspirin and NSAIDS help. Niacinamide in high doses has significant associated adverse effects also but not the flushing reaction.

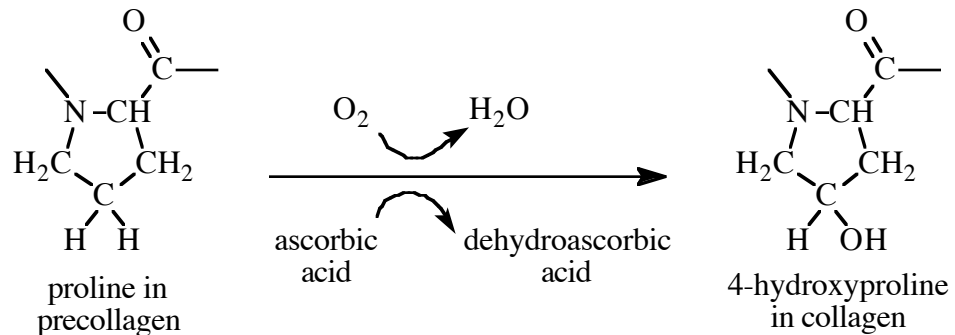
J. Vitamin C (ascorbic acid)

1. Structure

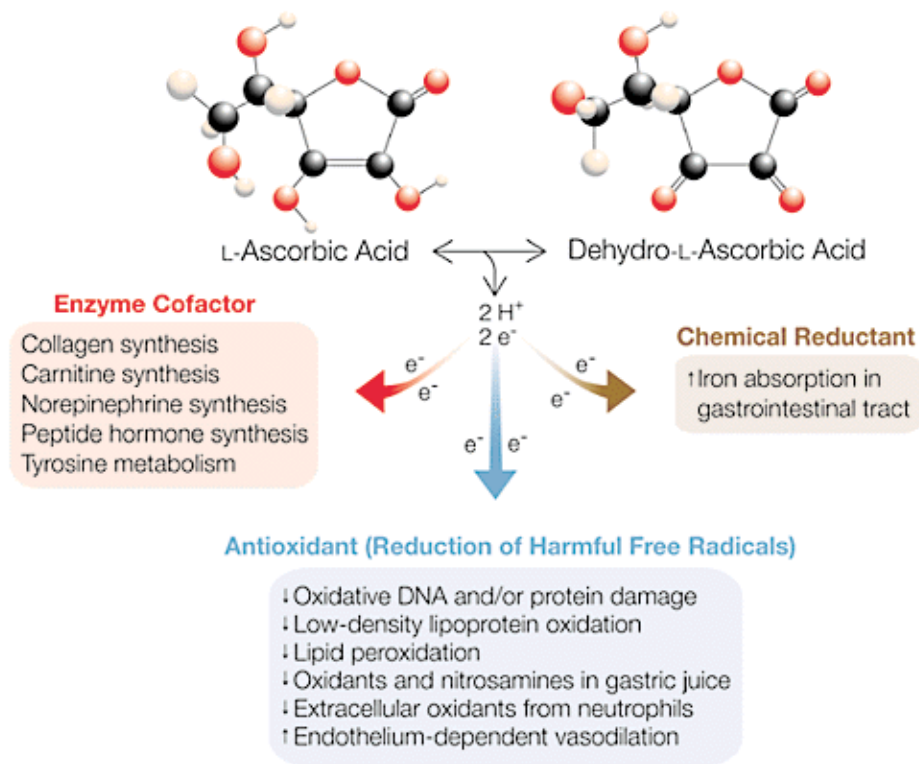


2. **Function** -- true coenzyme function is not well understood; ascorbic acid is an electron donor and facilitates the following reactions:

- a. Dopamine \rightarrow norepinephrine
- b. Proline \rightarrow hydroxyproline (this is a major component of collagen and many of the signs of scurvy are due to impaired collagen synthesis).



- c. Lysine hydroxylase \rightarrow collagen.
- d. Folic acid \rightarrow THFA (this explains the macrocytic anemia seen in scurvy).
- e. Involved in absorption of iron (keeps in ferrous form for better absorption).
- e. General water soluble antioxidant/free radical scavenger. We will discuss this aspect in more detail later.
 - a. Keeps LDL from being oxidized
 - b. Possible regeneration of reduced vitamin E
 - c. Prevent generation of mutagenic compounds in gastric juices and elsewhere



3. **Deficiency state** → Scurvy

Symptoms – hemorrhages, lassitude, weight loss, bone weakening, anemia, edema, tooth loss.

Biological lesion -- impaired collagen and connective tissue synthesis due to lack of hydroxyproline, hydroxylysine. Also low THFA.

4. **Utility of C**

- a. In surgery and fractures -- to increase collagen synthesis. Probably helps.
- b. Common cold prophylaxis -- The books by Linus Pauling ("Vitamin C and the Common Cold") and others have advocated that C has profound beneficial effects in preventing the common cold. Numerous clinical trials since 1970 show, at most, a slight beneficial effect. Ascorbic acid seems to cause a slight reduction in severity of colds, but the results are inconsistent from investigator-to-investigator. Gram quantities are not necessary, 100-500 mg/day will saturate tissues.
- c. Cancer --diets with >200mg/d vitamin C are associated with lower cancer risks, especially esophagus, stomach, colon and lung but studies using Vit C supplements have led to conflicting results.
- d. heart disease—low dietary levels and low blood levels are associated with an increased risk. Vitamin C supplements can modestly lower BP but no clear effect on outcomes in intervention studies. The benefit of supplements is unclear unless one is deficient in Vitamin C

- e. ataracts-higher intakes show lower risks but the benefit of intervention with supplements is not proven.
5. **Storage** -- is present in most tissues at low levels; there is a threshold level above which excess is excreted. This is about 200mg/d.
 6. **Source** -- richest sources are not citrus juices, but broccoli, brussel sprouts, peppers; other items high in ascorbate are citrus products, potatoes, and tomatoes. Cereal products, grains, and meats contain very little; if 5 fruits or vegetables are eaten daily, the intake would be 250 mg., average intake is about 75 mg./day.
 7. **Stability** -- in solution is relatively unstable being oxidized by air and being photolabile, therefore, much C can be destroyed in cooking foods.
 8. **Toxicity** -- Essentially nontoxic; in gram doses may increase oxalate urine concentrations and subsequent increased risk for urinary stones. Will make urine tests for sugar unreliable (false positive) because C will reduce the copper in Clinitest and Benedicts solution; In doses over 250mg/d can make false negative haemocult test for blood in stool; contraindicated when have iron overload.
 9. **Requirements** -- DV = 60 mg. but is this intake optimum for best health?? New RDA is 75mg for females and 90mg for males and 125mg for smokers. UL=2g/d
 10. **Bioavailability of various products.**
 AUC-PO/AUC-IV is true bioavailability
 studies should be done at steady state
 200 mg. = 80% bioavailability
 500 mg. = 63% bioavailability
 1250 mg. = 46% bioavailabililty
 at > 500 mg/d all of the absorbed ascorbate was excreted in the urine
 conclusions: best dose is 200-500 mg/d
 timed release products, Ester-C (calcium ascorbate) or esters of vitamin C are not worthwhile