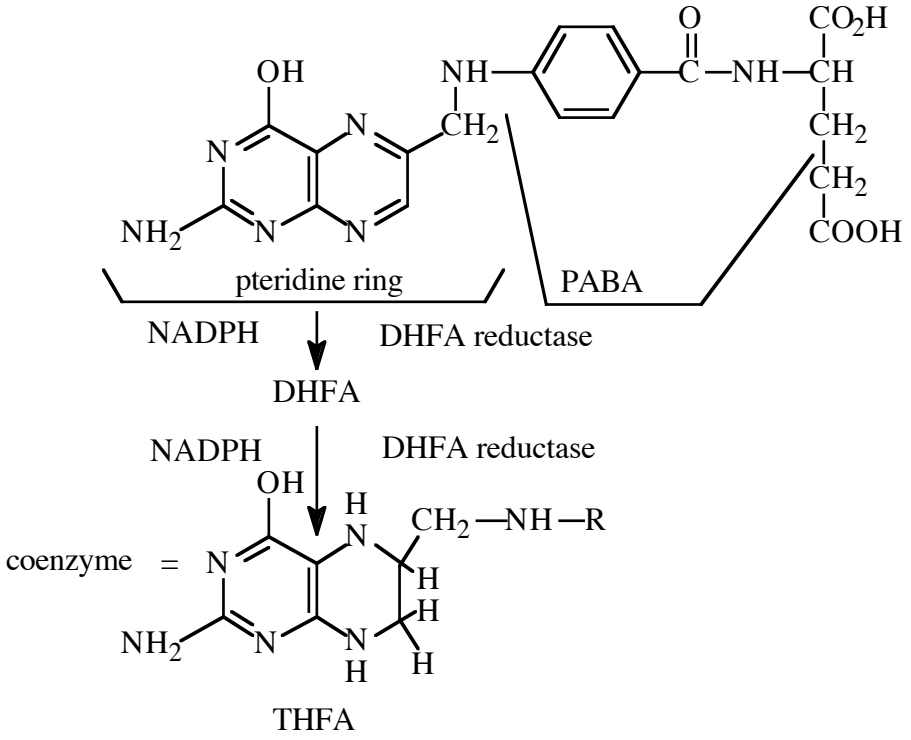
WATER SOLUBLE VITAMINS – PART 2

E. Folic Acid (B9)

1. Chemistry



Other "conjugates" of DHFA and THFA that have more than one glutamic acid exist in foods; these are not as well absorbed as the oxidized monoglutamate

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).

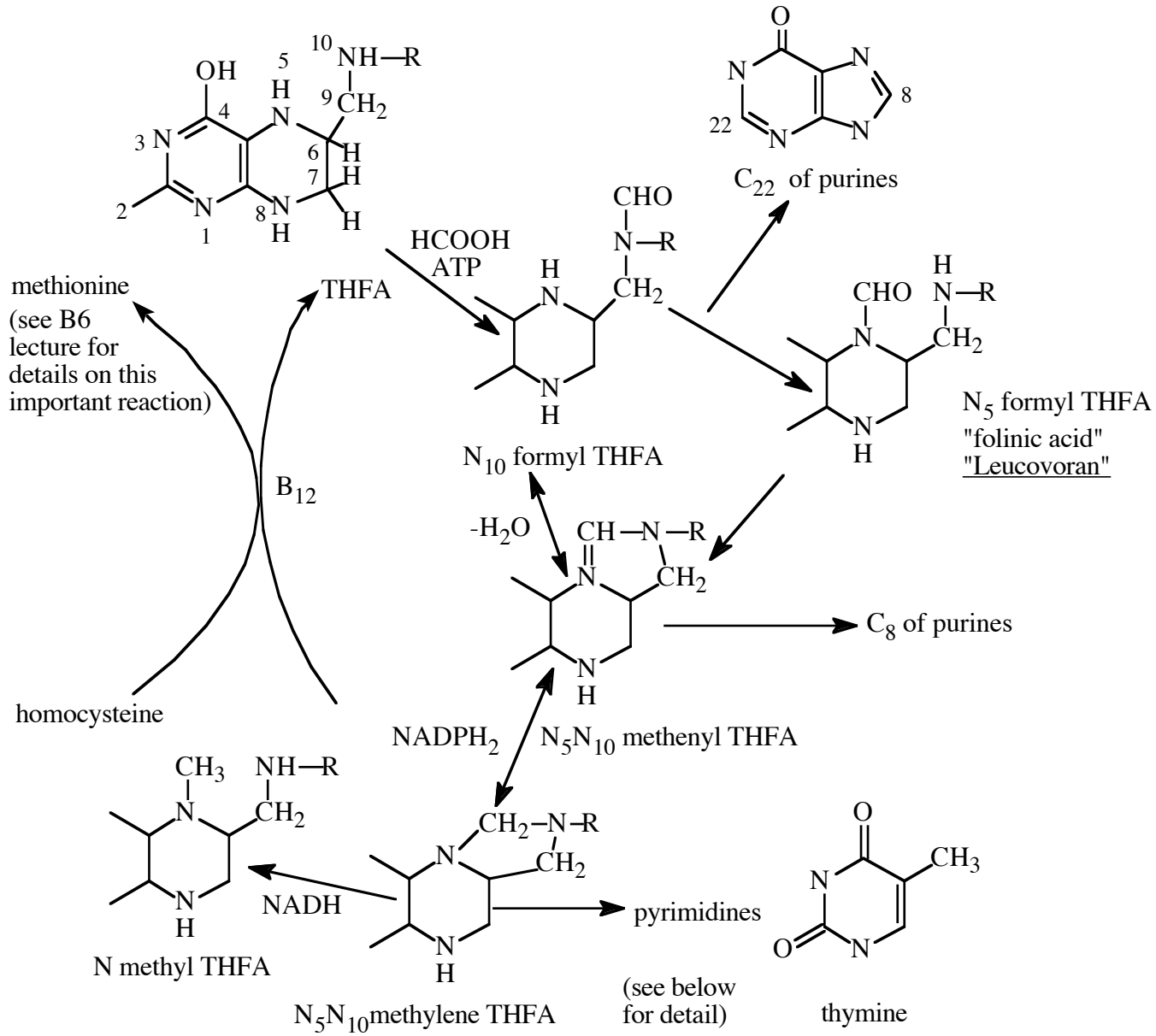
Common use – folates, means all pteroglutamates having vitamin activity.

3. Absorption, transport, circulation, storage

Absorption - dietary folates are enzymatically cleaved by pancreatic and intestinal mucosal peptidases to the monoglutamate that is absorbed. Synthetic folic acid is well absorbed with a bioavailability of about 85%. Deficiency in iron and VC can lead to impaired utilization of dietary folate.
Transport - most of circulating folate is 5-methyl tetrahydrofolic acid (5-MeTHFA). Most is free in plasma with some binding albumin. Cellular uptake requires transporters and receptors.
Tissue distribution – 5-10 mg total, half in liver, low in brain. 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.

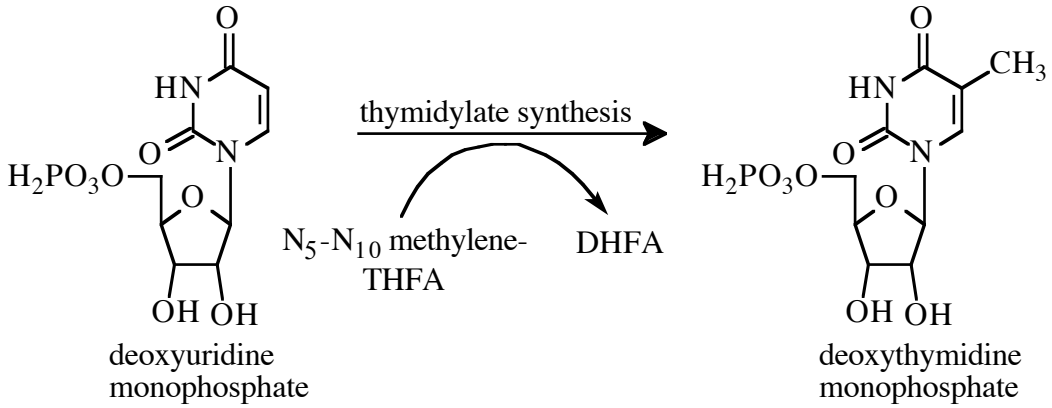
4. **Function** – closely related in function with vitamins B12 and B6 (methylation, homocysteine)

a. The folate cycle and methylation (single carbon metabolism): methionine and S-adenosylmethionine synthesis (SAM). SAM is methyl donor for > 100 enzymatic reactions.



MTHFR=methylene THFA reductase

b. Thymidine synthesis:



c. DNA methylation: epigenetic regulation

5. Folate deficiency

- Deficiency results in megabloblastic anemia. Symptoms include headache, fatigue, weight loss, anemia, nausea, anorexia, diarrhea, insomnia, irritability, forgetfulness. Signs are macrocytic red blood 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 (MTHFR) 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 (less active), which results in elevated HCS. This is the so-called TT genotype (homozygous of T allele).
- Elevated levels of HCS may also lead to increased risk of psychiatric and neurodegenerative disorders.
- Oral contraceptives and anticonvulsant drugs use may increase folate catabolism. This possibility seems to be behind the development of Beyaz, a combined oral contraceptive that includes progesterones AND 5-MTHF.
- Folate deficiencies are seen under conditions of poor nutrition, heavy alcohol ingestion and pregnancy and lactation.
- Alcohol decreases enterohepatic circulation of N5 methylTHFA.
- Folate level in erythrocytes is used to assess folate status.

6. B_{12} deficiency in relationship to folate

B_{12} 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 B_{12}. Megabloblastic anemia therefore results from a deficiency of B_{12}. Also, in a B_{12} deficiency, neurological damage is observed due to lack of B_{12} (see B_{12} discussion), which is hard to detect. Therefore, high dose folate supplements are risky in cases where there is a possibility of pernicious anemia (B_{12} 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.

7. <u>Folate antagonists</u>

a. Methotrexate -- inhibits DHFA reductase –> stops all in "S" phase cell cycle. Used for cancer, rheumatoid arthritis, psoriasis, asthma

Note: "Leucovorin 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 hematopoisis and a neuropathy similar to pernicious anemia. The N_2O oxidizes the cobalamin to create a B_{12} deficiency with resulting folate deficiency.

e. Phenytoin – suboptimal folate levels observed with long term therapy and rare megaloblastic anemia. BUT, folic acid (in high does) decreases phenytoin levels with cases of seizures reported.

f. Pyrimethamine- used for parasite infections (e.g. toxoplasma, malaria) as a parasite DHFA reductase target. Folic acid supplements may reduce effect of drug. Leucovorin is OK.

8. <u>Use</u>

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 folic acid, otherwise women of childbearing potential should assure that they are consuming at least 0.4 mg/day of synthetic folic acid (I suggest a multivitamin 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.

d) Colon cancer - low folate , high alcohol, and low methionine intakes as well as family history give an elevated risk. Long term use of multivitamins associated with lower risk (folate?).

e) Breast cancer – high dietary intake decreases risk

f) Alzheimer's disease. Preliminary evidence shows low folate levels associated with increased risk.

g) Coronary heart disease and stroke – elevated homocysteine associated with an increased risk but efficacy of folic acid, B12 and B6 to lower adverse outcomes are not well established. A modest effect may be evident (see B6 notes and discussion) especially for stroke. Those with preexisting disease show no benefit on outcomes for supplementation. Ongoing trials may clarify the situation.

9. Source

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

10. Stability

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

11. 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=1ug of food folate; 1ug of supplement folic acid =1.7DFE. On an empty stomach it is 2DFE.

12. Toxicity: essentially non-toxic. UL=1000ug folic acid

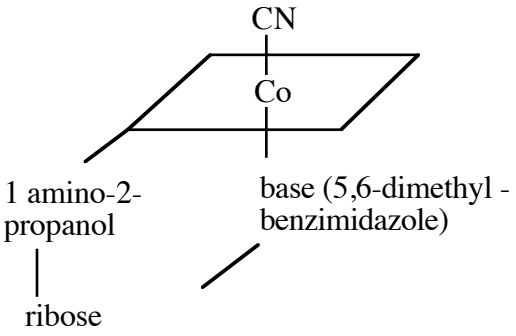
13. Patient Counseling/ patient use issues

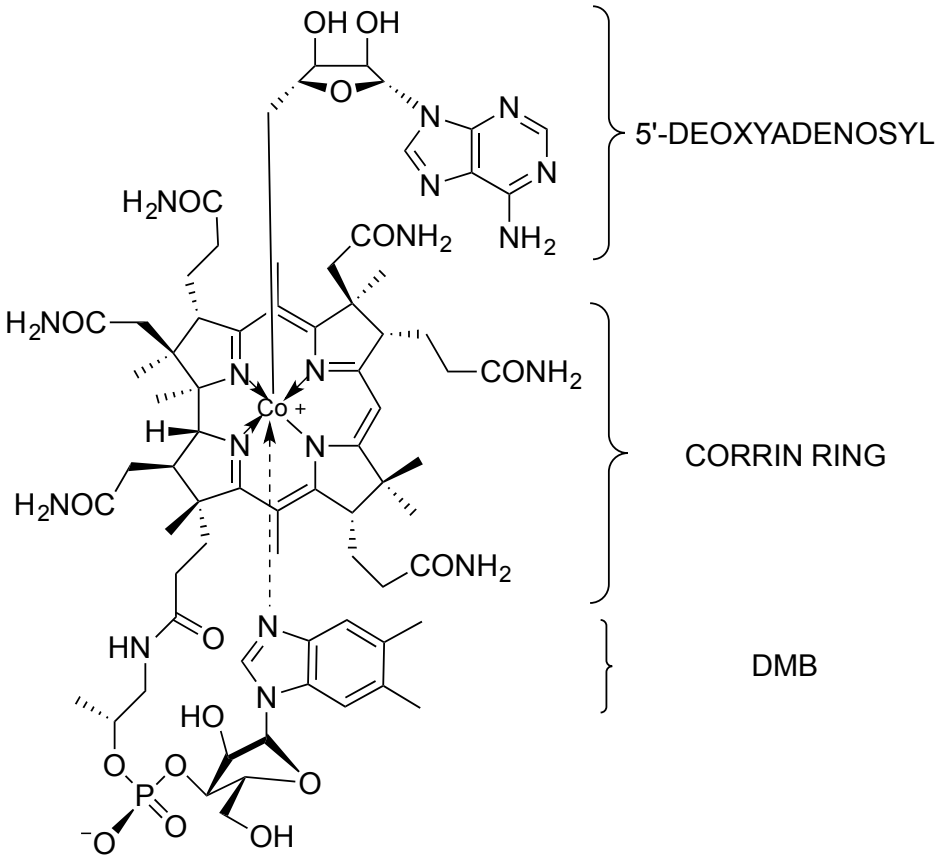
a. A very important vitamin because inadequate intakes can increase risks for cancers, heart disease and stroke.
b. Rarely needed as a single supplement. Use a multivitamin to get needed folic acid. Benefit seen at levels in most multivitamins (400ug/d) and the folic acid is very well absorbed. Folate rich foods (from which folate is less well absorbed) can supply the rest of intake.
c. Special benefit in alcoholics
d. Use for those with elevated HCS in doses 0.8mg and higher. Use together with B12 and B6.
e. Intake of 400ug/d of supplement (multivitamin) is very important for women of child bearing potential in order to help prevent birth defects.
f. Folic acid intake of >800ug/d is of concern in older populations due to the risk of masking pernicious anemia

F. Vitamin B_{12}

1. <u>Structure</u> -- synthetic material is cyanocobalamin

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





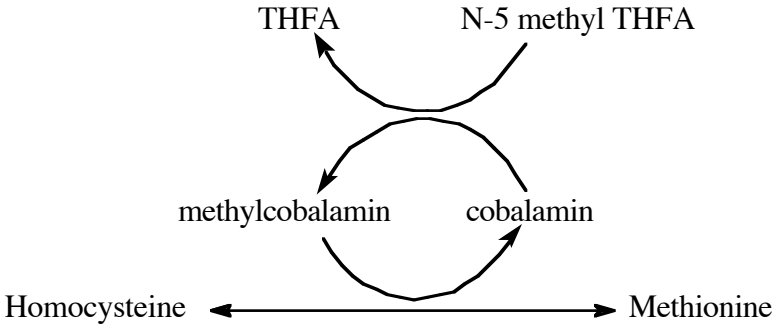
Vitamin B_{12}

2. Absorption and distribution

- HCl in stomach splits B_{12} from peptide links in food → PPIs impair B12 utilization
- Intrinsic factor (a glycoprotein) secreted by stomach mucosa; required for transport of B_{12} 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 B_{12}.
- A simple B_{12} deficiency may be seen in older populations due to decrease in gastric HCl.
- B12 is the best stored vitamin: liver 60%, muscle 30%

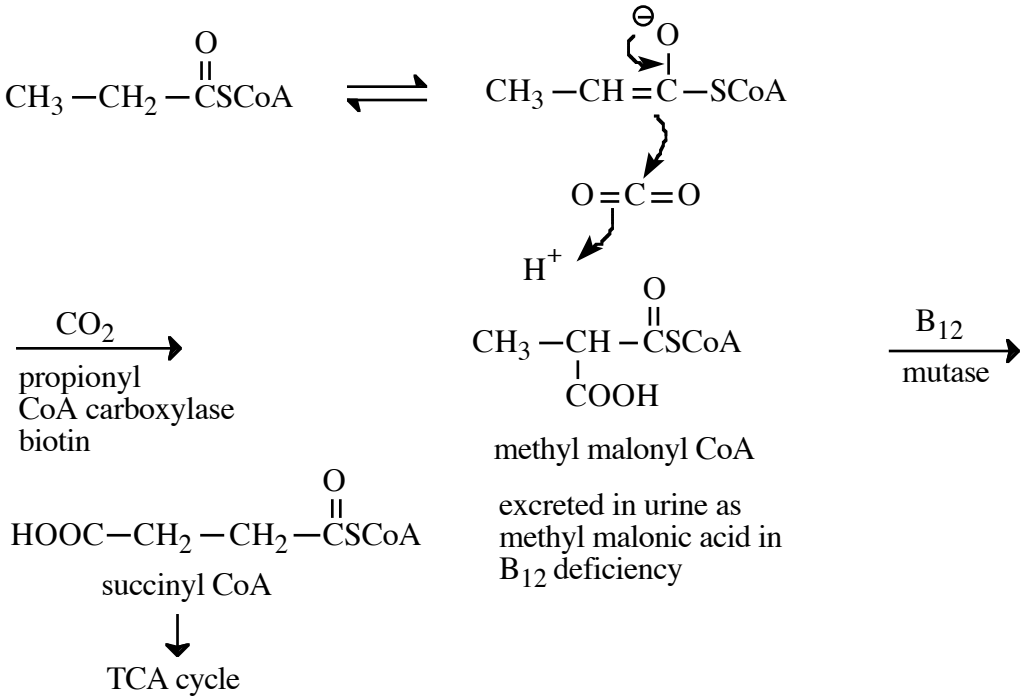
3. Function

a. Methyl transfer reactions



i.e. needed for recycling of THFA

b. Metabolism of odd chain fatty acids



Note: 5-deoxyadenosyl cobalamin is the coenzyme form of B12 for this reaction

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

4. **Deficiency** -- pernicious anemia (deficiency in the production of red blood cells); is a type of megaloblastic anemia

- Similar to folate deficiency in many aspects
- Deficiency leads to methylation defect -> delay or failure of normal cell division -> reduced mitotic rate -> formation of abnormally large, cytoplasm-rich cells -> megaloblastic 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.
- Cardiovascular risk factor: deficiency leads to homocysteine increase.
- Deficiency is rarely diet based, although vegans are at risk (no B12).
- Affects 30-40% of older adults

5. **Source** -- meats, especially liver and yeast; microorganisms (bacteria) are ultimate sources of B_{12}. Not found in plants.

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. Sublingual 1mg "dots"may be better. A nasal solution 0.5mg/0.1ml) is marketed and is convenient. High IM doses are used for methylmalonic acidemia (MMA), an inborn error of metabolism.

Increasing interest in the role of B_{12} 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 (multivitamin) due to decreasing HCl that is needed for absorption of B_{12} from foods.

10. **Diagnosis**: Schillings test (radio-labeled B_{12}), which measures methyl malonic acid in plasma. Urinary excretion of MMA is also diagnostic of B_{12} deficiency. 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. High oral doses (>500ug/d), sublingual or nasal spray may obviate the need for IM injections. If problem is lack of HCl, po doses are OK. Long-acting form is hydroxycobalamin (Alpha redisol® MSD) -- dose is 100 μg/mo.

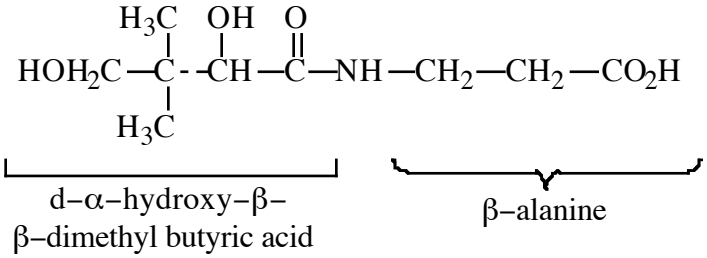
11. **<u>Toxicity</u>** -- essentially nontoxic

12. **Patient Counseling/ patient use issues**

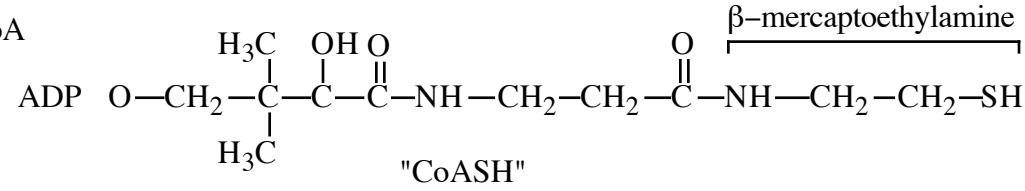
- **a. An important vitamin especially for older adults who may not absorb B12 from foods efficiently due to low stomach acid or have pernicious anemia**
- **b. B12 is important for maintaining adequate levels of folic acid in the body**
- **c. Needed, together with B6 and folic acid, to keep HCS low**
- d. **An injection or special oral dosage forms needed to treat pernicious anemia**
- e. **For most, the amounts contained in a good multivitamin will be sufficient**

G. Pantothenic Acid -- (Vitamin B_5)

1. **<u>Chemistry</u>**



Coenzyme = CoA



Only the d-enantiomer has biological activity

2. **Tissue distribution**
Mostly in mitochondria. High in CSF because CoA is involved in the synthesis of neurotransmitter

3. **<u>Function</u>** -- as a thioester bond, a component of coenzyme A, is a high energy bond; 8,800 ca/mole; more than ATP -- used in transfer of acyl groups. Thus

important in metabolism of fatty acids, amino acids, and carbohydrates. CoA serves as a cofactor of 4% of known enzymes.

pyruvate $\xrightarrow[\text{TPP}]{\text{NAD}}$ acetyl CoA
CoASH

4. **Deficiency** -- rare -- widespread occurrence in foods. Can also be synthesized by intestinal microflora. Reduce lipid synthesis and energy production -> symptoms are fatigue, numbness in extremities, cramps.

5. **Use** -- deficiency states -- topically for ulcers and sores, e.g. Panthoderm.

6. **Requirements** -- 10 mg = DV.

7. **Source** -- widespread in foods; liver, meat, eggs, potatoes are rich sources.

8. **Properties** -- alcohol or calcium salt somewhat more stable, therefore used in vitamin preparations, stable at neutral pH, but not in acid or alkali.

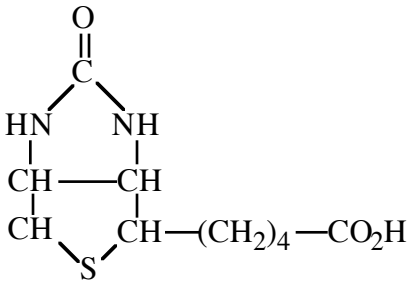
9. **Toxicity** -- essentially nontoxic

10. **Other**—a dimer (P-S-S-P) called pantethine is used in Europe as a drug to lower cholesterol. It is available in the USA as a dietary supplement. Seems safe and has mild effects in decreasing total cholesterol. Controversial use in treating acne.

11. **Patient Counseling/ patient use issues Nothing special; deficiencies are very rare; is a component of most multivitamins**

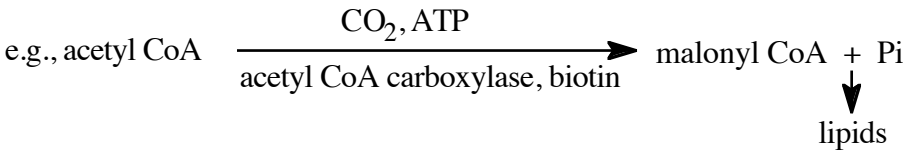
H. Biotin (B7)

1.



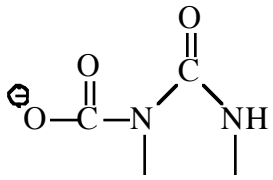
Bound to enzymes through ε amino of lysine; dietary proteins are digested to lysine-biotin (biocytin) which is hydrolyzed by biotinase to release biotin. An inborn error with a defect in biotinase is known.

2. **<u>Function</u>** -- carboxylation reactions in synthesis of lipids and carbohydrates



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

carboxybiotin = active species



3. **Tissue distribution**: appreciable storage in liver, higher in fetus than maternal

4. **<u>Deficiency</u>**
- rare; dermatologic lesions; rash, hair loss, fatty deposits on face, depression.
- avidin (protein from egg whites) can precipitate deficiency state → dermatitis, muscle pain, etc.; called "egg white injury".
- Birth defects due to marginal status
- Low biotin may cause SIDS
- Disorders of biotin metabolism: biotinidase deficiency affects release and recycle of biotin; 1 in 60,000 live birth

5. **<u>Stability</u>** – normally very stable. Unstable to oxidizing conditions that promote simultaneous lipid oxidation.

6. **<u>Source</u>** -- eggs, meat, nuts are rich sources. Synthesized by intestinal microflora. Recycled by biotinidase.

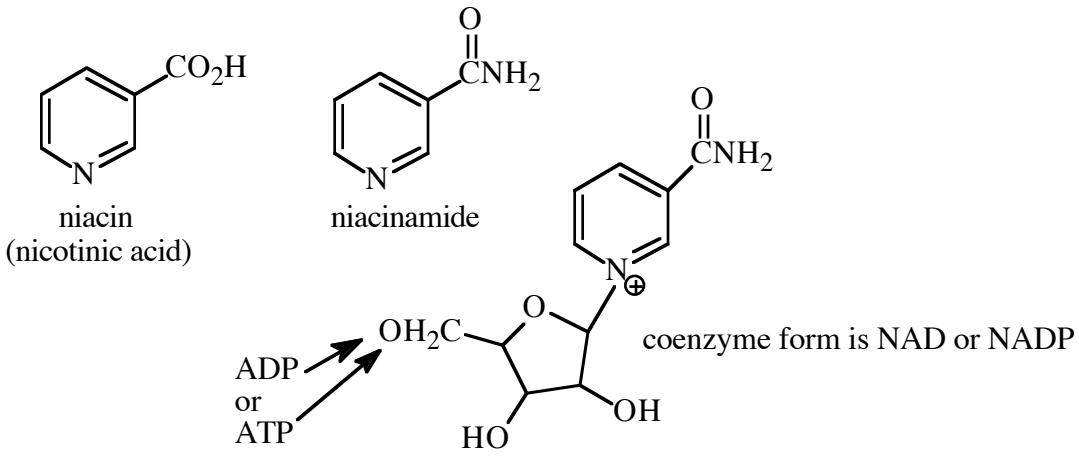
7. **<u>DV</u>** = 0.3 mg.

8. **Use** -- rarely used alone; several biotin responsive inborn errors of metabolism are known, the most common being a defective biotinidase. Some use for brittle nails [mg doses and together with chromium (Diachrome)] for improved glucose tolerance in diabetics

9. **Patient Counseling/ patient use issues - nothing special; deficiencies are very rare; is a component of most multivitamins.**

I. Niacin (Vitamin B_3)

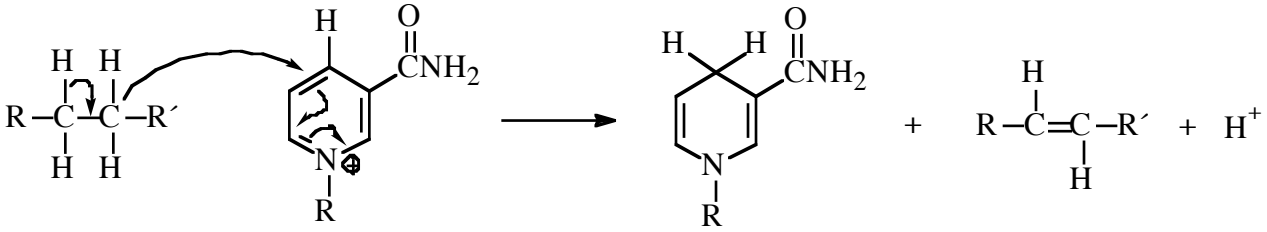
1. Chemistry



2. **Transport**: free in plasma, brain cells have high-affinity system for Nam. Receptor: GPCR found in adipose

3. **Function** -- >150 enzymes require NAD or NADP. > 200 reactions in metabolism of carbohydrates, fatty acids (β-oxidation), and amino acids.

a. redox and electron transport



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

NAD + protein → niacin + ADP-protein

4. **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.

5. **Biosynthesis** -- See B_6 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 tryp to niacin. Low Zn also contributes to Pellagra as Zn plays some roles in tryptophan -> niacin conversion.

6. **Source** -- meat, fish, whole grain cereals, peanuts. Ingested in foods as NAD or NADP then hydrolyzed in the intestinal mucosa.

7. **Stability** -- very stable, but much is lost if cooking water is discarded.

8. **Requirements** -- DV = 20 mg, but requirement will depend on tryptophan intake. UL=35mg

9. **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 g/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: at least in part due to inhibition of lipolysis in adipose tissue -> decreased fatty acid supply -> lower production of VLDL; also activates lipoprotein lipase -> enhance VLDL clearance. 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.

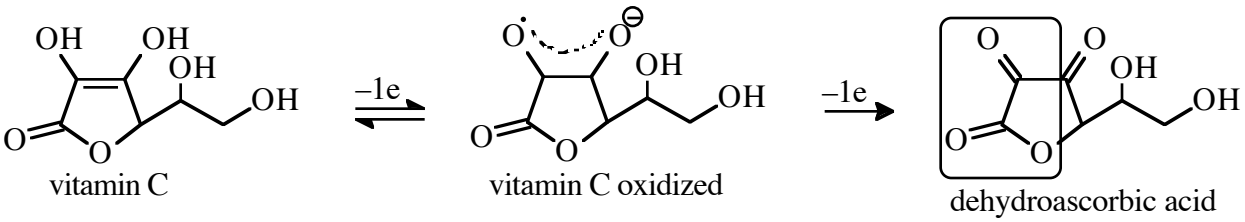
   d. Diabetes—there is interest in niacinamide in high doses to prevent type 1 diabetes in high risk kids and for type 2 adults. Niacinamide may help protect pancreatic beta cells but results showing benefit are preliminary.

10. **Toxicity** (in doses over UL) -- peripheral vasodilation, flushing (due to vasodilation), 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 (deplete methyl groups due to increased demand for methylation to excrete Niacinamide).

11. **Patient counseling Issues**
    a) Single use is not necessary for nutritional purposes. Take a multivitamin.
    b) High dose (gram quantities or Niaspan) are not for self-medication because niacin can cause toxicity at doses needed for favorably affecting blood lipids.

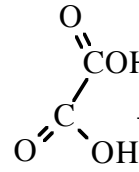
J. Vitamin C (ascorbic acid)

1. Structure



Vitamin C as a free radical scavenger

metabolism



+ other metabolites

oxalic acid

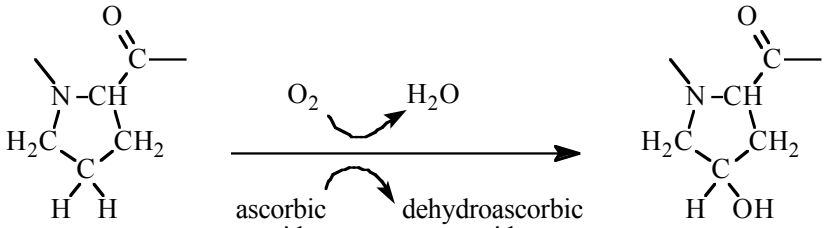
(kidney stone risk?)

2. **Storage** -- is present in most tissues at low levels. No reserve, excesses are quickly excreted. Brain levels are among the last to be affected.

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

a. Dopamine → norepinephrine

b. Proline → hydroxyproline (this is a major component of collagen and many of the signs of scurvy are due to impaired collagen synthesis).



c. Lysine hydroxylase → collagen.

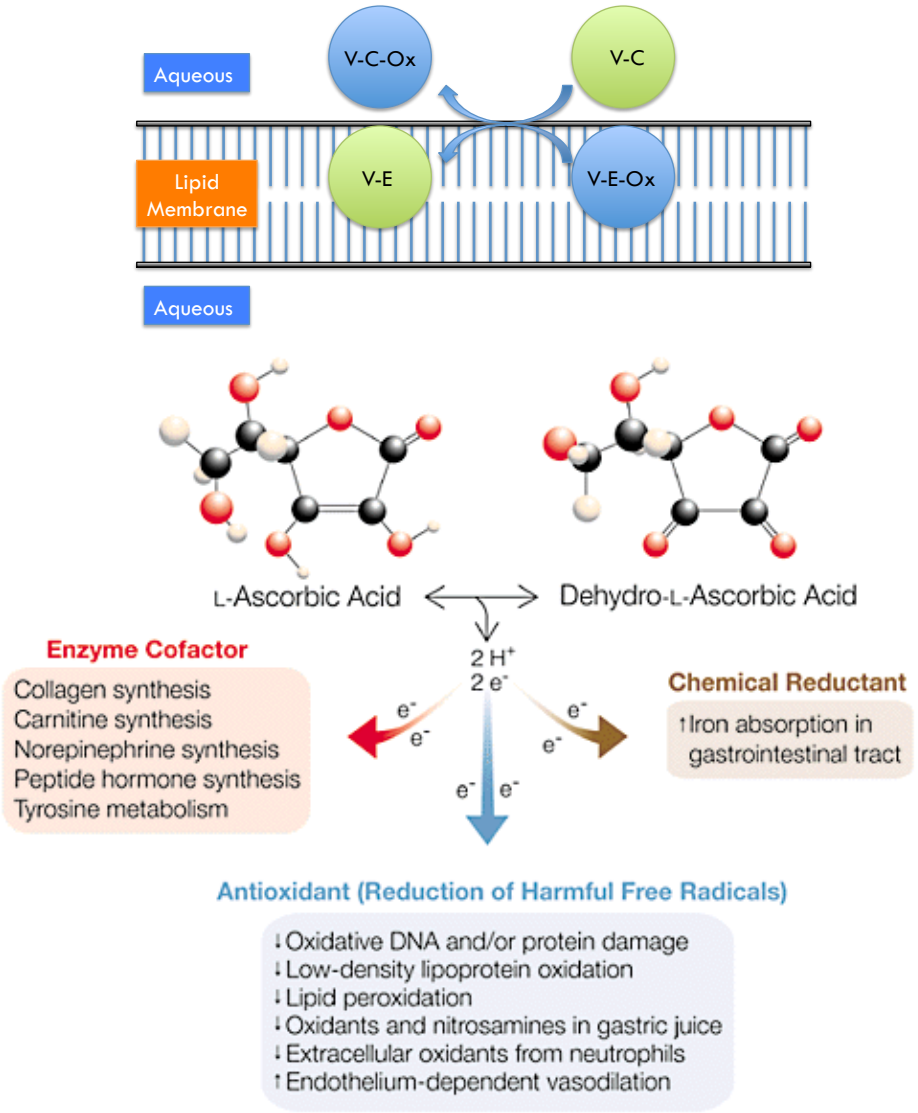
d. Folic acid → THFA (this explains the macrocytic anemia seen in scurvy).

e. Involved in absorption of iron (keeps in ferrous form for better absorption).

e. General water soluble antioxidant/free radical scavenger.

a. Keeps LDL from being oxidized
b. Possible regeneration of reduced vitamin E
c. Prevent generation of mutagenic compounds in gastric juices and elsewhere

f. Fatty acid desaturation



4. **<u>Deficiency state</u>** → Scurvy

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

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

Risk factors: smoking, stress, chronic diseases, diabetes

5. **<u>Utility of C</u>**

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 > 200 mg/d vitamin C are associated with lower cancer risks, especially esophagus, stomach, colon and lung but studies using VC 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. Can reduce LDL oxidation (in combination with vitamin E).

e. Cataracts-higher intakes show lower risks but the benefit of intervention with supplements is not proven.

g. Neurological function: positive correlation with memory performance (low in schizophrenia).
h. Diabetes: reduce glycosylation of plasma proteins -> prevent diabetic complications
i. Skin health: due to the essential role in collagen synthesis
j. Lung health: antioxidant protection -> prevent pneumonia and asthma
k. Bone health: prevent scurvy

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. Most higher animals can synthesize VC from glucose, but not humans, so Scurvy can be considered a congenital metabolic disease, hypoascorbemia.

7. **Stability** -- in solution is relatively unstable being oxidized by air and being photolabile, therefore, much VC 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 haemoccult test for blood in stool; contraindicated when have iron overload.

9. **Requirements** -- DV = 60 mg. But 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

11. Patient Counseling Issues

a) The benefits of taking high doses (>200mg) of vitamin C are not established

b) Most multivitamins provide 60mg/dose, which may be too low for optimum benefit

c) Daily consumption of a glass of rehydrated frozen orange juice will, when taken with a multivitamin, will give saturation levels (~200mg) of C

d) Studies to date indicate the value of eating ample fruits and vegetables to help prevent cancers and heart disease. The use of a multivitamin may also help.

e) Synthetic vitamin C is the same as natural vitamin C

f) Use of high doses to prevent or treat the common cold is unwarranted. Doses of ~200mg may slightly reduce severity of the cold.