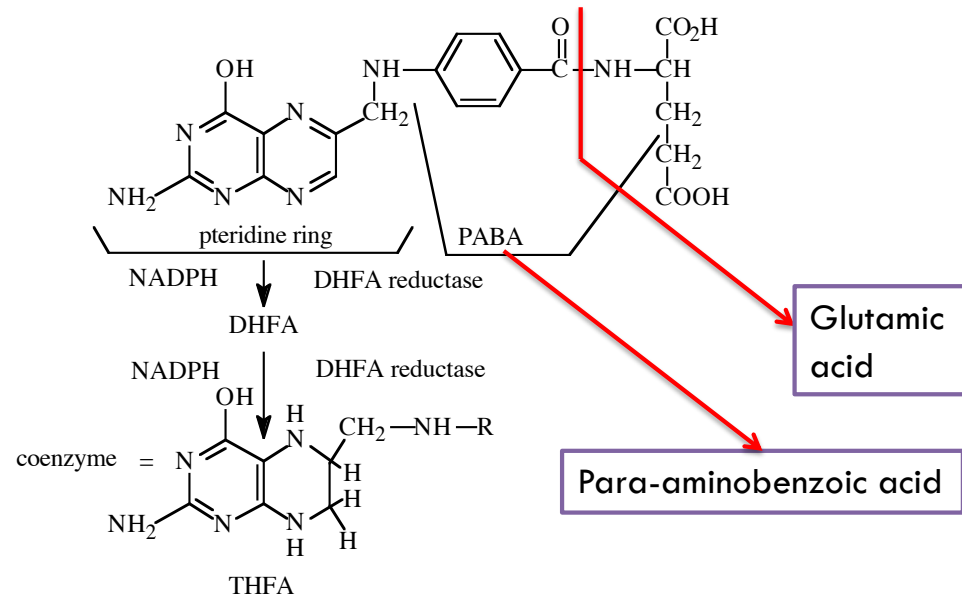


# Folic Acid (B9) - Structure



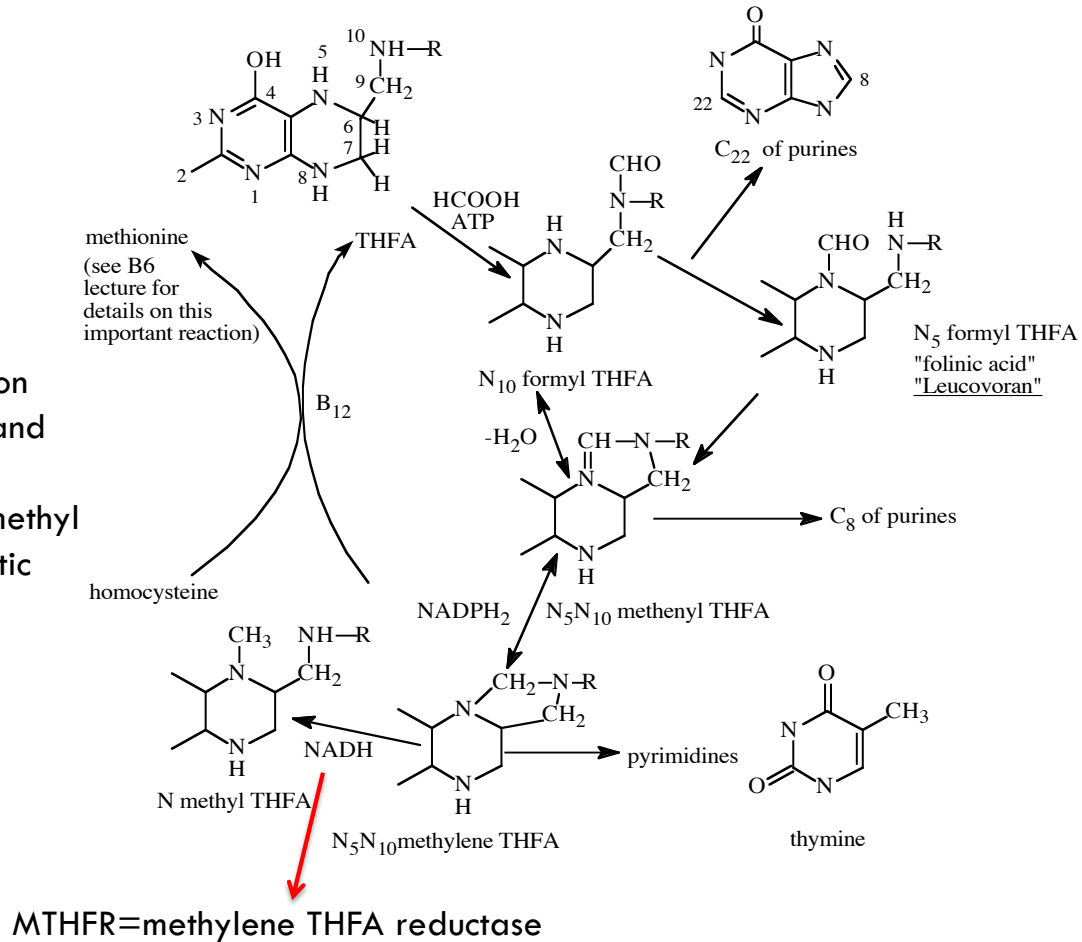
- Other "conjugates" of DHFA and THFA that have more than one glutamic acid exist in foods, but are not as well absorbed as the oxidized monoglutamate.
- Folic acid is pteroyl monoglutamic acid.
- Oxidized form (DHFA) is what is used in supplement. Reduced polyglutamates are found in animal and plant foods.

# Absorption, Transport, Storage

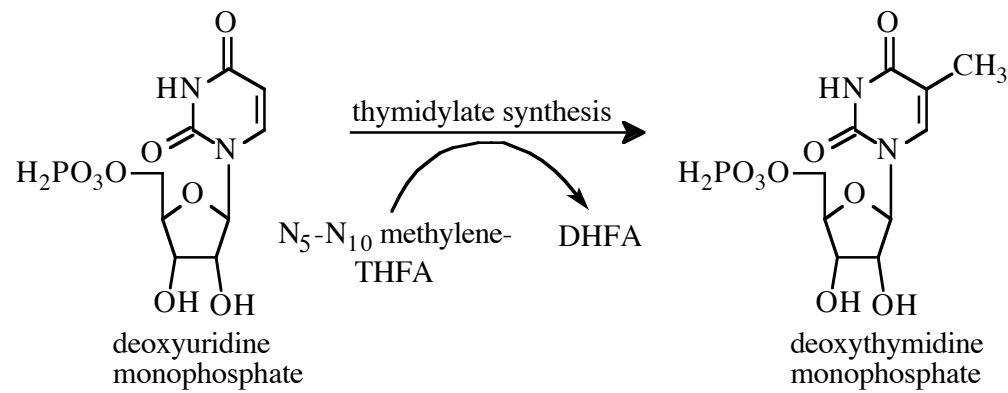
- **Absorption** - dietary folates are enzymatically cleaved by pancreatic and intestinal mucosal peptidases to the **monoglutamate** that is absorbed. Synthetic folic acid is well absorbed with a bioavailability of about 85%. Deficiency in iron and VC can lead to impaired utilization of dietary folate.
- **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 (alcoholics)

# Function – Methylation (related to B12 and B6)

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



# Function – Thymidine Synthesis



DNA methylation (cytosine and adenine) → epigenetic regulation

# Deficiency – Birth Defects

- Folic acid deficiency may result in **teratogenesis** with neural tube defects and possibly orofacial clefts.
- It is important to have adequate folate intake at conception and for the first 3 weeks when the neural tube closes.
- 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 140µg/100g to help decrease risk for birth defects.

# Deficiency – Other Phenotypes

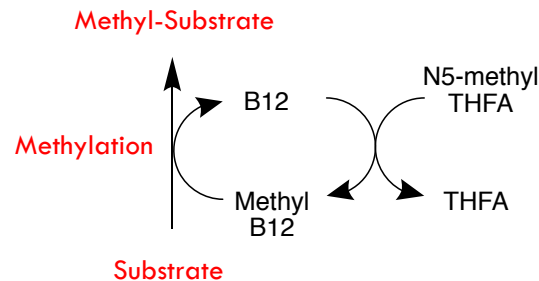
- Deficiency results in megaloblastic anemia.
  - Symptoms include headache, fatigue, weight loss, anemia, nausea, anorexia, diarrhea, insomnia, irritability, forgetfulness. Signs are macrocytic red blood cells and megaloblasts in the bone marrow.
- Deficiency may result in elevated **homocysteine** (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).
- 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-MTHFA.

## Deficiency – Causes and Diagnosis

- Folate deficiencies are seen under conditions of poor nutrition, heavy alcohol ingestion and pregnancy and lactation.
- Alcohol decreases enterohepatic circulation of N5-methyl THFA.
- Folate level in erythrocytes is used to **assess folate status**.

# Relationship with B12 Deficiency

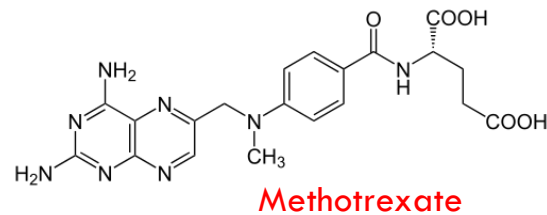
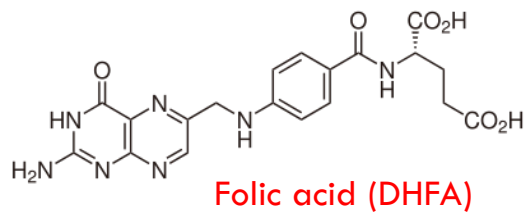
- B12 deficiency results in a folate deficiency because folates are not recycled (see above folate cycle scheme). This is because 5-MeTHFA is not converted back to THFA in the absence of B12. Megaloblastic anemia therefore results from a deficiency of B12.
- In a B12 deficiency, neurological damage is 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.



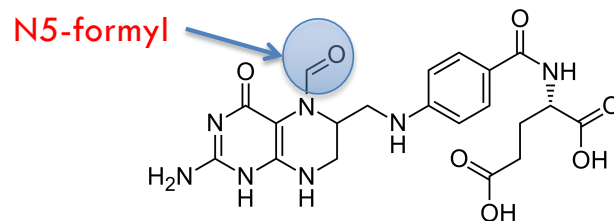


# Folate Drug Antagonists and Interactions

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

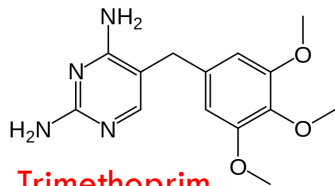


- "Leucovorin rescue" (supplementation of N5-formyl THFA) technique allows ordinarily lethal dose to be administered with consequent increased tumor kill.

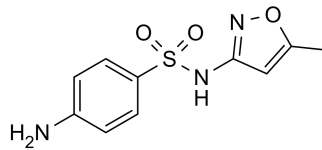


## Folate-Drug Interactions (Antagonists) – Cont'd

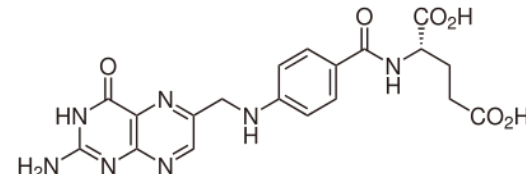
- Trimethoprim – binds to and inhibits bacterial DHFA reductase and, combination with sulfamethoxazole (Bactrim® Roche, Septra® BW), which is a PABA antagonist, forms a "double hit" against bacterial folate metabolism. There is usually very little effect on mammalian DHFA reductase → good antibiotic.



Trimethoprim

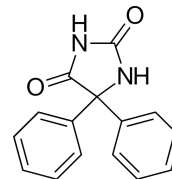


Sulfamethoxazole



Folic acid (DHFA)

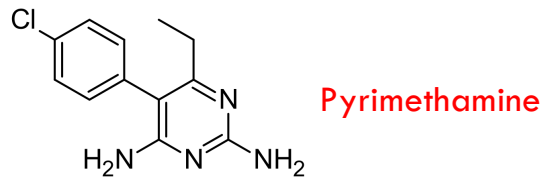
- Phenytoin – anti-seizure drug. Inhibits intestinal conjugases that convert folate polyglutamate to monoglutamate for better absorption. Suboptimal folate levels observed with long term therapy and rare megaloblastic anemia. But, folic acid (in high doses) decreases phenytoin levels with cases of seizures reported. So effects are both ways.



Phenytoin

## Folate-Drug Interactions (Antagonists) – Cont'd

- Pyrimethamine – used for parasite infections (e.g. toxoplasma, malaria) by inhibiting parasite DHFA reductase. Folic acid supplements may reduce effect of drug, but Leucovorin is OK.



Pyrimethamine

- Nitrous oxide – continued frequent inhalation has produced fatal megaloblastic hematopoiesis and a neuropathy similar to pernicious anemia. The N<sub>2</sub>O oxidizes the cobalamin to create a B12 deficiency with resulting folate deficiency.

# Use

- Deficiency - use with oral contraceptives and during pregnancy and lactation.
- To prevent neural tube defects in the unborn: 0.4 mg/day for women of childbearing potential (see previous slides).
- Cervical dysplasia, bronchial squamous dysplasia, and dysplasia of colonic tissue in ulcerative colitis patients – elevated risk is associated with low folate.
- Colon and breast cancer - long term use of B9 associated with lower risk.
- Coronary heart disease and stroke – elevated homocysteine associated with an increased risk. Combination of folic acid, B12 and B6 can lower homocysteine level.

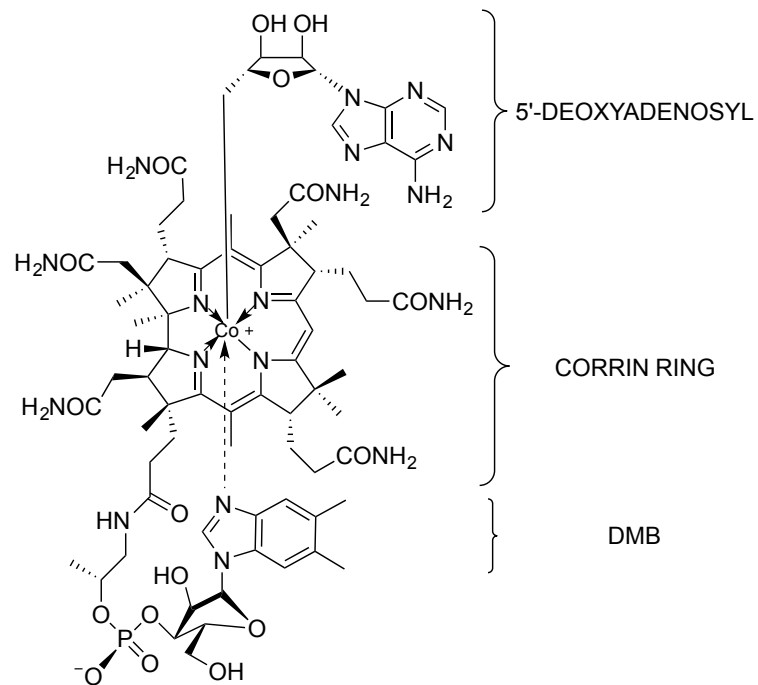
# Dose, Toxicity, Source, Stability

- DV = 0.4 mg of folate
- UL = 1 mg/day (essentially non-toxic)
- Preparations containing greater than 0.8 mg are on Rx only (may mask B12-deficiency induced pernicious anemia).
- Special benefit in alcoholics
- Rarely needed as a single supplement; used as multivitamin;
- Source: leafy vegetables, fruit juices, beans; meats and most fruits are low. "Enriched" flour now contains 140  $\mu\text{g}$  folic acid /100 g. Can be synthesized by **intestinal microflora**.
- Stability: labile to light, heat, and storage



Image from medlineplus.gov

# Vitamin B<sub>12</sub> - Structure

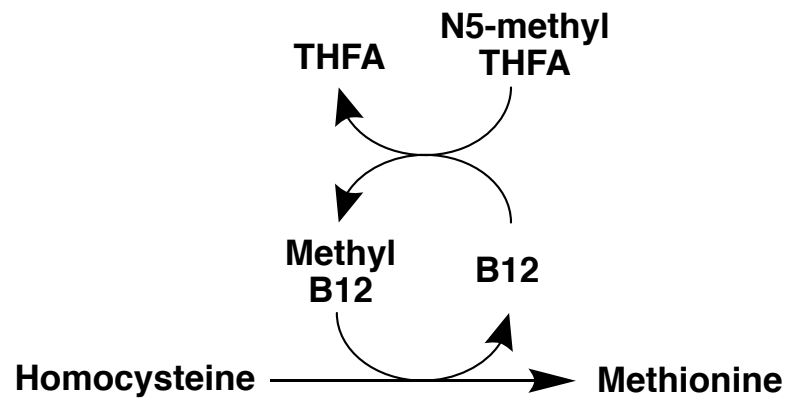


Synthetic material is cyanocobalamin (CN)  
Coenzymes = 5'-deoxyadenosine or methyl group replacing the CN; Hydroxy cobalamin is also active.

# Absorption and distribution

- HCl in stomach splits B12 from peptide links in food → PPIs impair B12 utilization.
- Intrinsic factor (IF; a glycoprotein) secreted by stomach mucosa; required for transport of B12 across ileum wall (also requires  $\text{Ca}^{2+}$  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.
- B12 is the best stored vitamin: liver 60%, muscle 30%

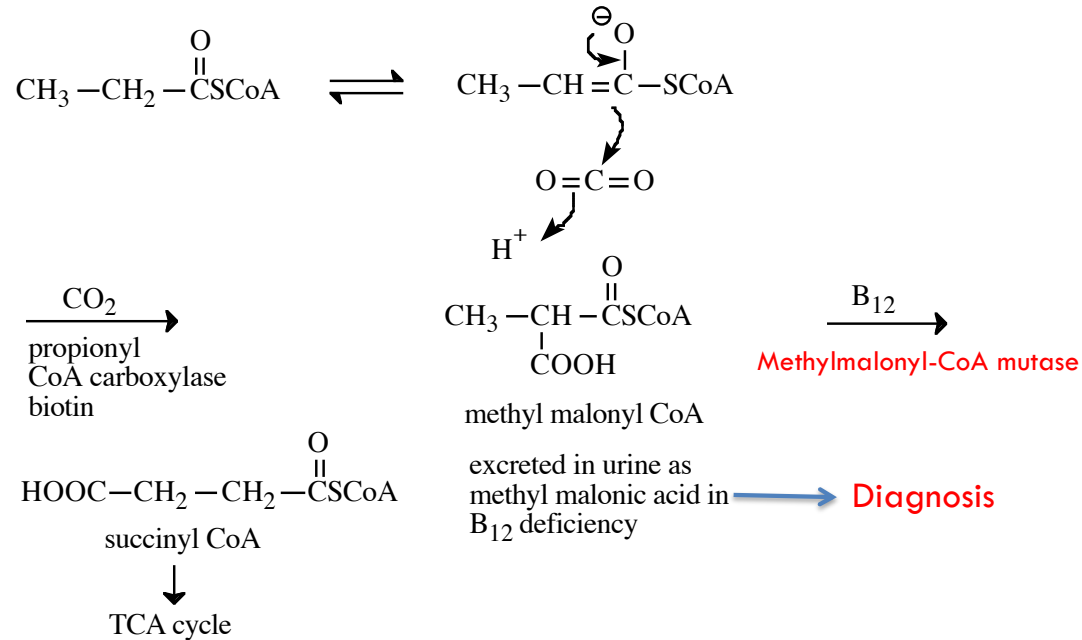
# Function – Methyl Transfer Reactions



Needed for recycling of THFA



## Function – Metabolism of Odd Chain Fatty Acids



5-deoxyadenosyl cobalamin is the coenzyme form of B<sub>12</sub> for this reaction

## Function – Myelin Synthesis

- B12 deficiency leads to buildup of methyl malonic acid (gets incorporated into myelin) and prevent normal fatty acid synthesis, which result in decrease in myelin synthesis → demyelination and neurological damage

# Deficiency

- Leads to pernicious anemia (deficiency in the production of red blood cells), which 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

## Deficiency – Cont'd

- 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
- **Diagnosis**
  - Schillings test (oral or IM radio-labeled B<sub>12</sub>) and then check urine secretion: normally > 10% secreted; if deficient, < 10% secreted.
  - Urinary excretion of methyl malonic acid (MMA) is also diagnostic of B<sub>12</sub> deficiency.
  - Accurate diagnosis is important for rational therapy of anemias.

# Source

- Meats, especially liver and yeast; **microorganisms** (bacteria) are ultimate sources of B<sub>12</sub>. Not found in plants.



Image from medlineplus.gov

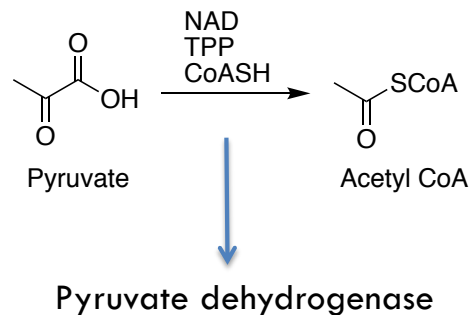
# Use

- DV = 6 µg; labile to light; stable at pH 4-7.
- In pernicious anemia, give 100 ug IM once every 4 weeks; Studies indicate that 1 mg/day P.O. will work also. High oral doses (>500ug/d), sublingual (1mg) or nasal spray (0.5mg/0.1 mL) may obviate the need for IM injections. Long-acting form is hydroxycobalamin (Alpha redisol® MSD) – dose is 100 ug/mo.
  - If problem is lack of HCl for absorption, PO doses are OK.
  - If problem is due to defect in IF, then PO won't work (need IM injection).
- High IM doses are used for methylmalonic acidemia (MMA), an inborn error of metabolism.
- Together with B6 and folic acid, to keep homocysteine 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<sub>12</sub> from foods (if it is caused by intrinsic factor deficiency, then oral dose does not help).
- Toxicity: essentially nontoxic



# Function

- As a thioester bond, a component of coenzyme A, is a high energy bond; 8,800 cal/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.





# Deficiency

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



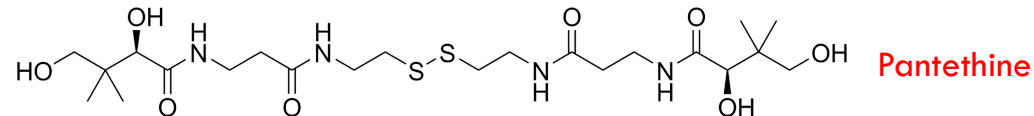
Image from vkool.com

# Use

- DV = 10 mg
- Treatment of deficiency states – typically for ulcers and sores, dry, rough, scaly, itchy skin, e.g. Panthoderm
  - Also used in hair products. It binds to hair shaft and coat and seal hair surface (not related to coenzyme activities)

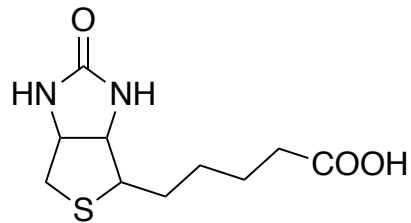


- Alcohol or calcium salt somewhat more stable, therefore used in vitamin preparations, stable at neutral pH, but not in acid or alkali.
- A dimer (P-S-S-P) called pantethine is used in Europe as a drug to lower cholesterol. It is available in the USA as a dietary supplement. Seems safe and has mild effects in decreasing total cholesterol.

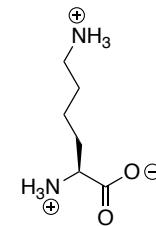


- Essentially nontoxic

# Biotin (B7) - Structure

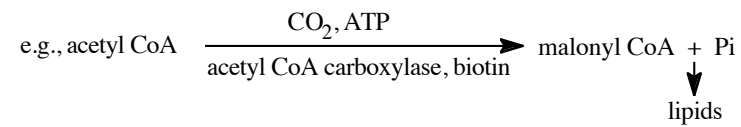


Bound to enzymes through the amino group of lysine; dietary proteins are digested to lysine-biotin (biocytin) which is hydrolyzed by biotinidase to release biotin. An inborn error with a defect in biotinidase is known.



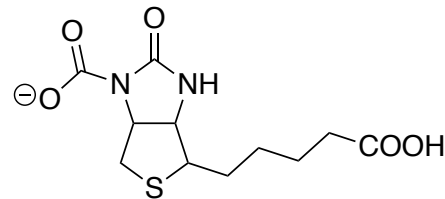
Lysine

# Function – Carboxylation in Lipid and Carbohydrate Synthesis



e.g. pyruvate carboxylase – gluconeogenesis

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



carboxybiotin = active species

# Tissue Distribution and Source

- Appreciable storage in liver, higher in fetus than maternal
- Eggs, meat, nuts are rich sources. Synthesized by intestinal **microflora**. Recycled by biotinidase.



Image from nftips.com

# Deficiency

- Rare; dermatologic lesions; rash, hair loss, brittle nail, fatty deposits on face, depression.
- Avidin (protein from egg whites) can precipitate deficiency state → dermatitis, muscle pain, etc.; called “egg white injury”.
  - This interaction commonly used for protein pull-down experiments in biochemistry
- 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



From <https://vitamins.vitanetonline.com>



From [www.medicalrealm.net/](http://www.medicalrealm.net/)

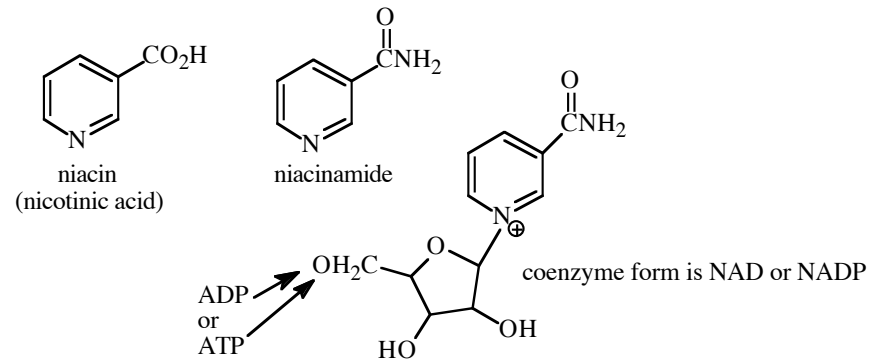


<http://info.spectracell.com>

## Use

- DV = 0.3 mg
- 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)
- Together with chromium (Diachrome®) for improved glucose tolerance in diabetics.
- Stability: normally very stable. Unstable to oxidizing conditions that promote simultaneous lipid oxidation.

# Niacin (B3) - Structure



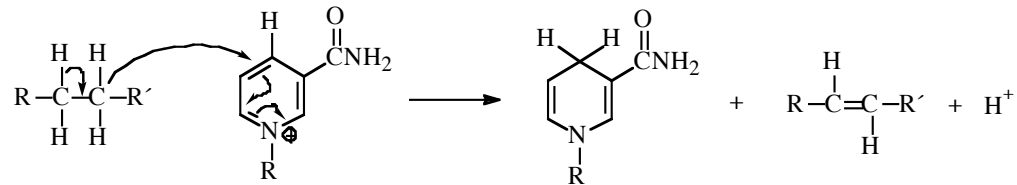


# Transport

- Free in plasma, brain cells have high-affinity system for Nam.  
Receptor: GPCR found in adipose

# Function

- >150 enzymes require NAD or NADP. > 200 reactions in metabolism of carbohydrates, fatty acids ( $\beta$ -oxidation), and amino acids.
- Redox and electron transport



- Ribosylation of proteins in cell signaling and DNA replication and repair:
  - NAD + protein  $\rightarrow$  niacin + ADP-protein

# Deficiency

- 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.



From [medifitbiologicals.com](http://medifitbiologicals.com)

# Biosynthesis

- Can be synthesized from tryptophan by our body (see B6-catalyzed reaction).
- 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 tryptophan to niacin.
- Low Zn also contributes to Pellagra as Zn plays some roles in tryptophan → niacin conversion.

## Use

- For improving serum lipids -- use Niacin (NA); Niacinamide is not effective; NA used in doses up to 10 g/d (usually 3-5g, not supplement level), 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.

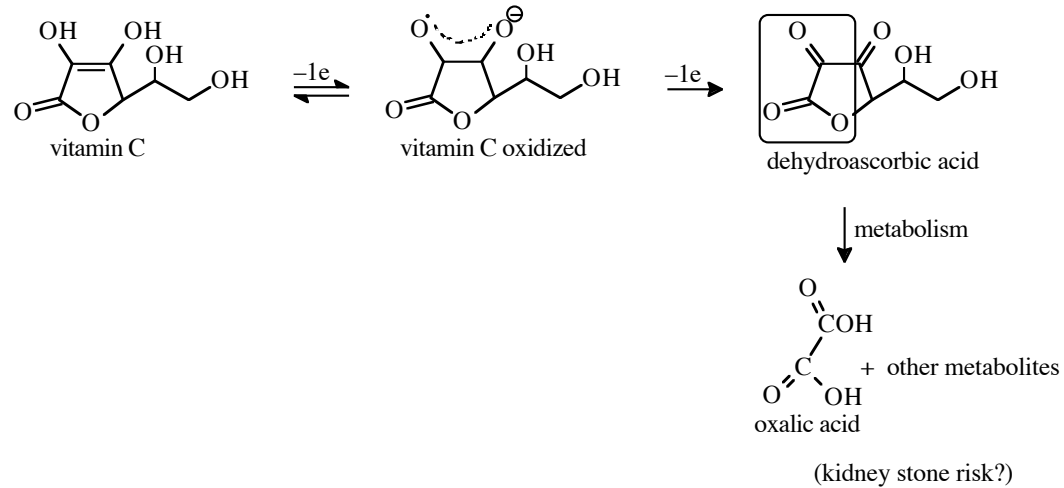
# Source, Dose, Stability, Toxicity

- Stability: very stable, but much is lost if cooking water is discarded.
- DV = 20 mg, but requirement depends on tryptophan intake.
- UL = 35 mg.
- Toxicity observed in doses over UL (gram quantities):
  - Peripheral vasodilation, flushing (due to vasodilation), GI upset, ulcers, diarrhea, impaired glucose tolerance, liver damage, and increased gout.
  - These 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).
  - Aspirin and NSAIDS help with vasodilation and GI upset (Merck drug Cordaptive; Europe only, not FDA approved).
  - 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)
- Source: meat, fish, whole grain cereals, peanuts. Ingested in foods as NAD or NADP then hydrolyzed in the intestinal mucosa.

Image from medlineplus.gov

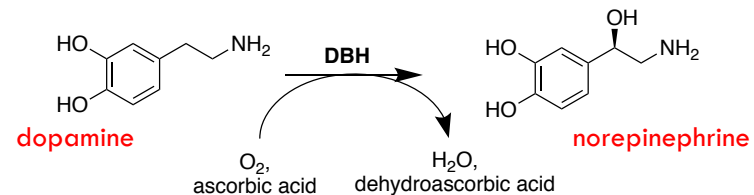


# Vitamin C (Ascorbic Acid) - Structures

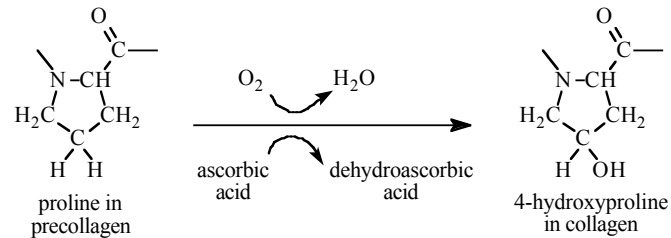


## Function – Electron Donor for Redox Reactions

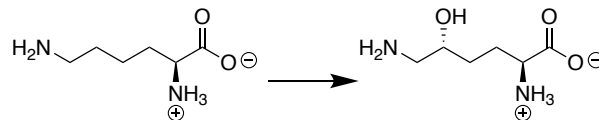
- Dopamine → norepinephrine (dopamine beta-monoxygenase; DBH)



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



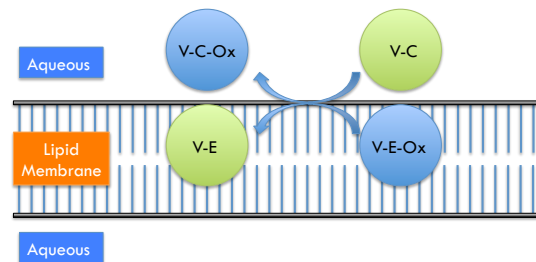
- Lysine hydroxylase → collagen by lysyl hydroxylase





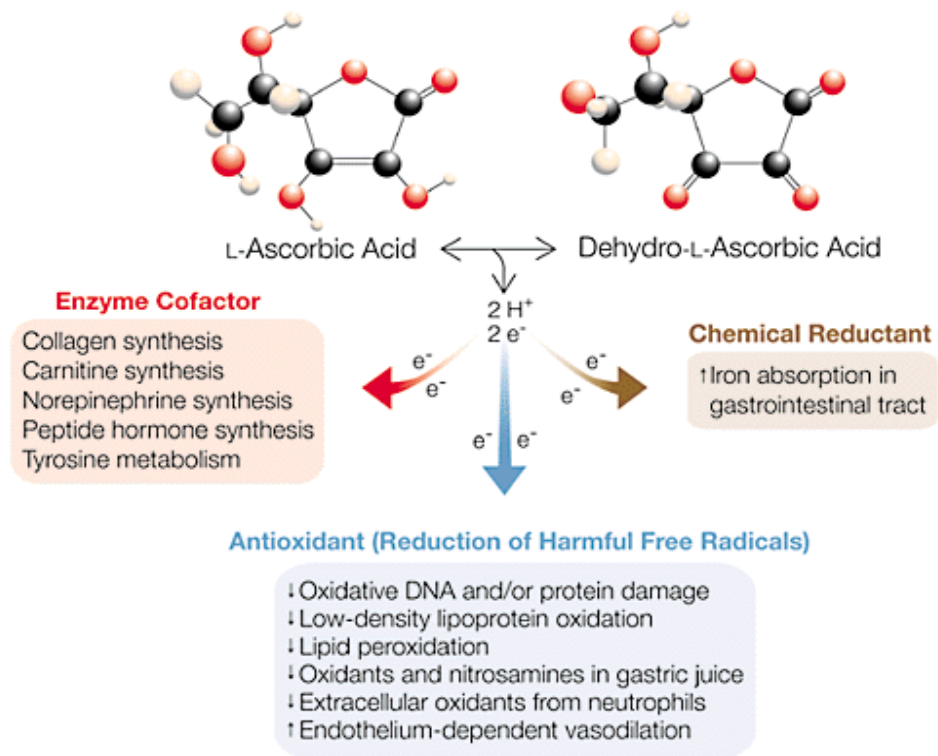
# Function – Cont'd

- Folic acid → THFA via DHFA reductase (this explains the macrocytic anemia seen in scurvy).
- Involved in absorption of iron (keeps in ferrous form for better absorption)
- General water soluble antioxidant/free radical scavenger.
  - Keeps LDL from being oxidized
  - Possible regeneration of reduced vitamin E
  - Prevent generation of mutagenic compounds in gastric juices and elsewhere



- Fatty acid desaturation

# Function - Summary



## Deficiency – Scurvy (Collagen Defect)

- Symptoms – hemorrhages from mucus membranes, lassitude, weight loss, bone weakening, anemia, edema, tooth loss (look up on internet; graphical)
- 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

# Storage and Sources

- Present in most tissues at low levels. No reserve, excesses are quickly excreted. Brain levels are among the last to be affected.
- Sources
  - Richest sources are not citrus juices, but broccoli, brussel sprouts, peppers;
  - Other items high in ascorbate: citrus products, potatoes, and tomatoes.
  - 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

# Bioavailability

- AUC-PO/AUC-IV is true bioavailability (at steady state)
  - 200 mg. = 80% bioavailability
  - 500 mg. = 63% bioavailability
  - 1250 mg. = 46% bioavailability
  - at > 500 mg/d, much less fraction of vitamin C being absorbed
  - Therefore, best dose is 200-500 mg/d
- Timed release products, Ester-C (calcium ascorbate) or esters of vitamin C, are not worthwhile

# Use

- DV = 60 mg. RDA is 75 mg for females and 90 mg for males. UL = 2g/d.
- In surgery and fractures – to increase collagen synthesis.
- 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.
- Heart disease – low dietary levels and low blood levels are associated with an increased risk. Vitamin C supplements can modestly lower blood pressure 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).

## Use – Cont'd

- Cataracts-higher intakes show lower risks (likely due to its antioxidant activity, like B2).
- Neurological function: positive correlation with memory performance (low in schizophrenia).
- Diabetes: reduce glycosylation of plasma proteins → prevent diabetic complications
- Skin health: due to the essential role in collagen synthesis
- Lung health: antioxidant protection → prevent pneumonia and asthma
- Bone health: prevent scurvy
- Most multivitamins provide 60mg/dose, which may be too low for optimum benefit.
- The benefits of taking high doses (>200mg) of vitamin C are not established.

## Toxicity and Interactions

- Essentially nontoxic
- 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 (normally reduced by sugar)
- In doses over 250 mg/d can make false negative haemoccult test for blood in stool (need **oxidation** of substrate to blue color by  $H_2O_2$ ; ascorbate prevents such oxidation)
- Contraindicated when have iron overload (enhance iron absorption → iron toxicity).