E. Folic Acid

1. Chemistry

![Folic Acid Structure]

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.

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

- **ATP**
- **C22 of purines**
- **N10 formyl THFA**
- **N5 formyl THFA** "folinic acid" "Leucovoran"
- **N10 methenyl THFA**
- **N3N10 methylene THFA**

- **methionine** (see B6 lecture for details on this important reaction)

- **homocysteine**

- **N methyl THFA**

- **NADH**

- **NADPH2**

- **pyrimidines**

- **thymine**

- **thymidylate synthesis**

- **deoxyuridine monophosphate**

- **deoxythymidine monophosphate**
4. **Folate deficiency**

- Deficiency results in megabloblastic 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)(see B6 discussion). 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 Km 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. Megabloblastic 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**


      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 hematopoisis and a neuropathy similar to pernicious anemia. The N\textsubscript{2}O oxidizes the cobalamin to create a B\textsubscript{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.

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 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, brochial squamous dysplasia, and dysplasia of colonic tissue in ulcerative colitis patients -- studies show elevated risk is associated with low folate. A decrease risk of progression is seen with intake of folic acid from diet or supplements. 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. Dietary folic acid reduced risk of both colon and breast cancer, especially women with a family history of these.

   e) Alzheimer’s disease. Preliminary evidence shows low folate levels associated with increased risk.

   f) End stage renal disease. Hyperhomocysteinemia is commonly seen and folic acid can lower HCS. Give with B6 and B12. Use 1-15mg/d to try and get HCS below 12 micromoles/L

   g) Hyperhomocysteinemia- 0.8-5mg/d will lower HCS about 30% unless HCS is very high (genetic polymorphism in methylene THFA reductase) in which cases up to 25mg/d is needed. The higher the baseline HCS, the more effect of folic acid in lowering. Supplementation of folic acid in foods has helped. Multivitamins help. The question is: does elevated HCS cause coronary artery disease (CAD) or does CAD cause high HCS? Secondary intervention studies with folic acid, B6 and B12 and heart disease have been disappointing.
8. **Source**

Especially leafy vegetables. Some fruits (bananas, lemons) and fruit juices (orange, tomatoe; meats are low. "Enriched" flour now contains $140 \text{ \mu g}/100 \text{ g}$ 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=1ug of food folate; 1ug of supplement folic acid =1.7DFE. On an empty stomach it is 2DFE.

11. **Toxicity**: essentially non-toxic except for the issue of masking pernicious anemia.

UL=1000ug 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.
2. **Function**

a. Methyl transfer reactions

![Diagram of methyl transfer reactions]

<table>
<thead>
<tr>
<th>THFA</th>
<th>N-5 methyl THFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>methylcobalamin</td>
<td>cobalamin</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Methionine</td>
</tr>
</tbody>
</table>

i.e. needed for recycling of THFA

b. Metabolism of odd chain fatty acids

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CSCoA} & \rightleftharpoons \text{CH}_3\text{CH}=-\text{CSCoA} \\
\text{CO}_2 & \rightarrow \text{CH}_3\text{CH}=-\text{CSCoA} \rightarrow \text{CH}_3\text{CH}=-\text{CSCoA} \rightarrow \text{CH}_3\text{CH}=-\text{CSCoA}
\end{align*}
\]

propionyl CoA carboxylase
biotin

\[
\begin{align*}
\text{HOOCCH}_2\text{CH}_2\text{CSCoA} & \rightarrow \text{HOOCCH}_2\text{CH}_2\text{CSCoA} \\
\text{TCA cycle} & \rightarrow \text{HOOCCH}_2\text{CH}_2\text{CSCoA} \\
\text{B}_{12} \text{mutase} & \rightarrow \text{B}_{12} \text{mutase}
\end{align*}
\]

methyl malonyl CoA
excreted in urine as methyl malonic acid in B\textsubscript{12} deficiency

TCA cycle

3. **Deficiency** -- pernicious anemia

Symptoms related to inadequate myelin synthesis and megabloblastic 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** -- $B_{12}$ absorption

HCL in stomach splits $B_{12}$ from peptide links in food; 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. Those on H2 blockers or proton pump inhibitors may also lack HCl needed to slit $B_{12}$ from food.

5. **Source** -- meats, especially liver and yeast; microorganisms are ultimate sources of $B_{12}$.

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 sublingual 1mg “dot” product may be better. A 1mg nasal solution (0.5mg/0.1ml) is marketed and is convenient. High IM doses are used for methylmalonic acidurea, 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 $B_{12}$ 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. High PO doses (1mg) are OK due to absorption by passive diffusion.

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

11. **Toxicity** -- essentially nontoxic
G. Pantothenic Acid -- (Vitamin B₅)

1. **Chemistry**

![Chemical structure of Pantothenic Acid]

\[
\text{Coenzyme } = \text{CoA} \quad \begin{array}{c}
\text{H}_3\text{C} \\
\text{OH} \\
\text{O}
\end{array} \quad \begin{array}{c}
\text{H}_3\text{C} \\
\text{d-α-hydroxy-β-} \\
\text{β-dimethyl butyric acid}
\end{array} \quad \begin{array}{c}
\text{H}_3\text{C} \\
\text{β-alanine}
\end{array}
\]

2. **Function** -- as a thioester bond –A component of coenzyme A.- high energy bond; 8,800 ca/mole; more than ATP -- used in transfer of acyl groups.

\[
\text{pyruvate} \quad \rightarrow \quad \text{acetyl CoA}
\]

\[
\text{NAD} \\
\text{TPP} \\
\text{CoASH}
\]

3. **Deficiency** -- rare -- intestinal synthesis probably important, as well as, widespread occurrence in foods. Symptoms are fatigue, numbness in extremities, cramps.

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

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

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

7. **Properties** -- The calcium salt somewhat more stable, therefore used in vitamin preparations. Dexpantenol is the d-enantiomer of the alcohol. It is used topically for minor skin problems. An IM form is used to decrease the risk of paralytic ileus following intestinal surgery. The idea is to improve levels of acetylcholine (from acetylCoA).

8. **Toxicity** -- essentially nontoxic

9. **Other** — a dimer (P-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.
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

\[
\begin{align*}
\text{e.g., acetyl CoA} & \xrightarrow{\text{CO}_2, \text{ATP}} \text{malonyl CoA} + \text{Pi} \\
& \text{acetyl CoA carboxylase, biotin} \\
& \text{lipids}
\end{align*}
\]

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. Biotin is sometimes used to correct brittle nails. There is some limited evidence for this use.
I. Niacin (Vitamin B₃)

1. Chemistry

\[ \text{niacin (nicotinic acid)} \]

\[ \text{niacinamide} \]

coenzyme form is NAD or NADP

2. Function -- >150 enzymes require NAD or NADP.
   
   a. redox and electron transport

   \[ \text{NAD} + \text{protein} \rightarrow \text{niacin} + \text{ADP-protein} \]

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

3. 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.

4. 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 tryp to niacin.

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

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

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

8. Use -- Component of multivitamin preps also used in high doses for its pharmacological effects as described below.
a. For improving serum lipids -- use Niacin; Niacinamide is not effective; Niacin is used in doses up to 10 q/d (usually 1-5g), 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 1g does lower production of VLDLP and activates lipoprotein lipase. Niacin and some sustained release products are OTC. The extended release product (Niaspan) is Rx. Advicor (KOS) is extended release niacin (500mg) plus lovastatin (20mg) to be taken at HS. Higher doses are available.

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 (decreased uric acid excretion via kidney). 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**

   ![Chemical structures of vitamin C, dehydroascorbic acid, oxalate, and metabolites](image)

   Vitamin C as a free radical scavenger

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).

   ![Chemical structures of proline, 4-hydroxyproline](image)

   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. **Use**

   a. In surgery and fractures -- to increase collagen synthesis. Probably mildly 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. increase iron absorption. 200mg will increase absorption by 40%
f. cataracts-higher intakes show lower risks but the benefit of intervention with
supplements is not proven.
g. Age related macular degeneration. An antioxidant combination (C, Zn, E, and beta
carotene) reduced rate of vision decline but the role of each ingredient is uncertain.
More on this later.
h. Progression of atherosclerosis. Modest doses of C (500mg) and E (270 IU) slowed
progression in older men but not older women. More later.
i. Heart disease and cancer. Modest doses of antioxidant vitamins (including 120mg
C) for 7yrs decreased the incidence of cancer and heart disease events in healthy
men but not healthy women. Conflicting results using antioxidant vitamins in other
studies. More later.
j. Skin. Vitamin C applied topically can help protect skin from damaging UV light and
improve elasticity (better collagen??). 10% creams are available.

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. Ready to drink orange juice is
mostly oxidized vitamin C which is poorly absorbed. Frozen is OK and is about
200mg/8oz.

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 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% bioavailability
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