UNIT OVERVIEW:
HYPMETHYLATION, FOLIC ACID
METABOLISM AND VITAMIN B12

15. Folic acid and deficiency
16. Synthesis and metabolism of tetrahydrofolate (THF)
17. Biochemical actions and physiologic roles of THF (dNTPs)
18. Folic acid and hyperhomocysteinemia
19. Vitamin B$_{12}$ metabolism and deficiency (pernicious anemia)

For further reference on folic acid, see Stryer (5th ed) pp.216-7.
Folic Acid (Folate, Folacin):

- Latin: folin - “leaf” Yellow-orange, slightly soluble in water

- Animals can’t synthesize folic acid

- Dietary requirements:
  RDI: 400 µg/day (preg. 800 µg/day)
  (>1000 µg/day may cause toxicity)

-Folic acid is widely distributed in leafy green vegetables, mushrooms, asparagus or liver, kidney, steak, yeast but is destroyed by cooking and exposure to reducing agents.
Folic acid is the most common form of vitamin deficiency. On a worldwide basis, deficiency of folic acid is believed to be the most common form of vitamin under-nutrition.

Particularly prevalent in underdeveloped countries in the tropics, where most of the population is believed to have at least marginal folic acid deficiency.

In the U.S. many indigents and elderly people suffer from deficiency of folic acid, manifested as anemia, weight loss, and weakness.

Pregnant women and infants are particularly vulnerable.

Folic acid deficiency is a major feature of tropical sprue, in which there is a general deficiency in absorption of many nutrients from the small intestine. Folate deficiency is also frequently involved in Megaloblastic anemia (B12).
Folate deficiency, effects:

- Neural tube defects in fetus (e.g. spinobifida)
- Premature atherosclerosis and thromboembolism - due to ↑ plasma homocysteine (increased plasma homocysteine found in 40% of all atherosclerosis patients)
- Suppression of DNA synthesis (GI, mucosa, b.m.)
- Malabsorption can lead to megaloblastic (macrocytic) anemia (immature erythrocytes).
- Depression, schizoid psychosis - 10-50% of psychiatric patients are folic acid deficient (due to treatment regimes).
- Increased risk of colorectal cancer (especially in drinkers) - due to ↓ DNA methylation
- Neurological - peripheral neuropathy, myelopathy, spinal cord syndromes, restless legs

Low serum RBC folate has been shown to be associated with the following conditions:

Drug induced:
- Antiepileptic drugs
- Oral contraceptives, NSAIDS
- Smoking
- Alcohol
- Methotrexate (antifolate chemotherapy)
- Nitrous oxide anesthesia
- Methionine therapy

Genetic:
- Hyperhomocysteinemia patients
- Ulcerative cholitis (↑ Colon cancer)
The structure of dietary folyl / pteroyl glutamic acid is indicated below, illustrating the unique γ-glutamyl-linkage between PABA and glutamate residues.

Humans cannot make pteridine, PABA, or make γ-glutamyl linkage.
FOLATE POLYGLUTAMATE in diet (unstable when cooked or stored)

- hydrolysis is Zn dependent (conjugase is in intestinal lumen)

FOLATE MONOGLUTAMATE (metabolized by intestinal lumen)

- Transport to cells

5-Methyl THF
(Taken up by liver and bone marrow via ATP dependent transporter)

- homocysteine
- methionine (1C units)

\[ \text{homocysteine} \xrightarrow{\text{methyl synthase}} \text{methionine} \]

TETRAHYDROFOLATE
(active form of F.A.)

- TRANSFERS: - CH₃ methyl
- CH₂ methylene
- CHO formyl
- CH=NH formimino

- CELL STORAGE FORM
- POLYGLUTAMATES
  (better enzyme substrates)

- 5-Methyl THF

- TRANSPORT TO CELLS
Oxidation state of 1C groups carried by THF:

<table>
<thead>
<tr>
<th>Oxidation Level</th>
<th>Group Carried:</th>
<th>THF Derivative(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formate</td>
<td>Formyl (-CH=O) (folinic acid, leucovorin)</td>
<td>N⁵-Formyl-THF N¹⁰-formyl-THF</td>
</tr>
<tr>
<td></td>
<td>Methenyl (-CH=)</td>
<td>N⁵,N¹⁰-Methenyl-THF</td>
</tr>
<tr>
<td></td>
<td>Formimino (-CH=NH)</td>
<td>N⁵-Formimino-THF</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Methylene (-CH₂-)</td>
<td>N⁵,N¹⁰-Methylene-THF</td>
</tr>
<tr>
<td>Methanol</td>
<td>Methyl (-CH₃)</td>
<td>N⁵-Methyl-THF</td>
</tr>
</tbody>
</table>
Generation of folate derivatives:

Red.

\[
\begin{align*}
\text{5,10 Methylene THF reductase} & \quad \text{NAD}^+ & \quad \text{NADH} \\
\text{5 methyl THF} & \quad \text{5,10 Methylene THF} \\
\text{Glycine} & \quad \text{Serine} \\
\text{de novo 1C path} & \quad \text{5 formyl THF} \\
\text{5,10 methylene THF} & \quad \text{10 formyl THF} \\
\text{5,10 methenyl THF} & \quad \text{5 formyl THF} \\
\text{H}_2\text{O} & \quad \text{NADPH} \quad \text{NADP}^+ \\
\text{Methenyl-THF cyclohydrolase} \quad \text{H}_2\text{O} \\
\text{ADP} + \text{P}_i + \text{H}_2\text{O} & \quad \text{Formate} + \text{ATP} \\
\text{10 Formyl THF synthase} & \quad \text{Methanol} \\
\text{Formalin (blindness)} & \quad \text{Methionine synthase (B12)} \\
\text{THF} & \quad \text{Devlin P.}
\end{align*}
\]
Utilization of 1C units: Purine synthesis

Because of requirement for 1C fragments, conditions which enhance GMP/AMP synthesis also reduce the toxicity of methanol.
One carbon pathway / drug interactions:

5 Formyl THF (Folinic acid - leucovorin) synthetase

ATP ADP

5,10 methylene THF

Dihydrofolate reductase

NADPH

GSH

NADP

Dihydrofolate

Thymidylate synthase

DNA synthesis and repair

folate

Aminopterin Methotrexate C.I.

S.I. + 5,10

Methylene THF reductase

NADH NAD

Phenytoin (antiepileptic)

methionine synthase (homocys. methyltrans.) (B12)

methionine

SAM

Homocysteine

PLASMA

5 methyl THF

5,10 remethylation cycle

Adapted from Stryer (6th ed)
Methotrexate is a (10 methyl) THF analog which inhibits dihydrofolate reductase competitively. Systemic treatment with methotrexate has been used for leukemia, lymphoma, bladder and breast cancer.

Side effects of methotrexate therapy include myelosuppression and mucositis. These effects result from the fall in THF levels and can be ameliorated using folinic acid (leucovorin) if given promptly.

Methotrexate prevents one-carbon transfer reactions which are required for DNA, RNA and purine biosynthesis, in particular thymidylate synthase.

\[
\text{pteridine} - CH_2N - etc \quad \text{pteridine} - CH_2N - etc
\]

\[
\text{Folic Acid} \quad \text{Methotrexate}
\]
Antimetabolite therapy:

**Methotrexate**

**Aminopterin**

**5-Fluorouracil**
Remethylation cycle, details:

- S-adenosyl homocysteine methyl transferase
- Active -CH₃ methylation of DRUGS, DNA, phospholipids, proteins

Remethylation Cycle:
- Methionine synthase (homocysteine methyl transferase)
- Methionine adenosyl transferase
- S-adenosyl methionine (SAM) (highly reactive)
- Methyl transferase
- Active -CH₃
- S-adenosyl homocysteine hydrolase
- GSH
- ATP
- PPi + Pi
- Tetrahydrofolate
- 5,10 methyl. THF
- 5 methyl THF
- Plasma
- Homocysteine
- S-adenosyl homocysteine
- Methylation of DRUGS, DNA, phospholipids, proteins

* Inhibited by NO anesthetic
Chronic alcoholism causes inactivation of methionine adenosyltransferase so that methionine is not converted to SAM. This causes methylation deficiency as well as cysteine deficiency, resulting in GSH deficiency.

Hypoxia, viral liver cirrhosis, septic shock also form oxygen radicals which inactivate methionine adenosyltransferase.

Thus a deficiency in folic acid via diet or secondary to vitamin B12 deficiency can induce hyperhomocysteinemia.
Individuals with hyperhomocysteinemia (5-7% of general population) can develop atherosclerosis (premature coronary artery disease) and/or atherothrombosis by 30-40 years of age.

- **Causes:** vitamin deficiencies of pyridoxine (B6), folic acid (B9), or B12 can result in elevated homocysteine levels. Elevated homocysteine may also occur as a result of genetic abnormalities (MTHFR, methionine synthase, cystathione beta-synthase).

- **Homocystinuria** due to CBS deficiency is a special sub-type of hyperhomocysteinemia. Major organ systems typically affected in this autosomal recessive disorder include ocular (dislocation of lens a common presenting symptom), CNS and skeletal abnormalities.

- The minimum required folic acid intake to prevent defects in the fetus is 200 ug/day (400). Recommended folic acid intake to prevent homocysteine induced vascular disease is 350 ug/day.

- In 1998 cereal flours in the US were required to be enriched with folic acid to a higher level. Multivitamin dose can be 800 ug/day.
Homocystinuria, suggested mechanisms:

- Homocysteine thiolactone
- LDL
- LDL-homocysteine thiolactone aggregate
- Homocysteine
- Foam Cells
- Proliferation of vascular smooth muscle cells
- Reactive Oxygen Species
- Lipid Peroxidation
- Oxidation of LDL
- Endoplasmic reticulum oxidative stress (copper)
- Unfolded proteins synthesised
- Damaged endothelial cells
- Atherothrombosis

References:
Cystathionine beta-synthase overexpression:

- Downs syndrome represents most common genetic cause of mental retardation. Associated with trisomic duplication on chromosome 21.

- CBS gene located in trisomic region of 21. Analysis of individuals with Downs syndrome demonstrate significant elevation of CBS activity, resulting in lowered levels of homocysteine and elevated cystathionine levels in blood plasma. Leukocyte DNA samples exhibit hypermethylation in DS patients compared to normal siblings.
Vitamin B12, general properties:

- Name given to cobalt-containing compounds possessing a corrin ring

- B12 is a water soluble vitamin produced in bacteria, and is obtained from foods of animal origin (i.e. meat 2.7- liver 95, kidney, and dairy products). Vitamin B12 may be present, but is not available to humans from plant sources (lacto-ovo vegetarians usually OK).

- Vitamin B12 (cyanocobalamin) USDA requirements are 5 ug per day (8 ug per day for pregnancy, elderly) (RNI is actually 1.5 ug/day. B12 is required for maintenance of the hematopoietic and nervous systems.

- Three enzymatic reactions depend upon B12 : methylmalonyl CoA mutase, leucine aminomutase, and methionine synthase.
Cobalamins, Vitamin B12:

Cobalamin (vitamin B12) (hydroxycobalamin)

[Chemical structure diagram]

- corrin ring system
- Cyano
- 5'-deoxyadenosine
- cobalt

5,6-di-methylbenzimidazole ribonucleotide
### Vitamin B12, ligands:

<table>
<thead>
<tr>
<th>Derivative:</th>
<th>Application:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(III)-CN cyanocobalamin (B12)</td>
<td>Commercial product</td>
</tr>
<tr>
<td>Co(III)-OH hydroxocobalamin</td>
<td>Bacterial form, Cyanide &amp; hydrogen sulfide antidote</td>
</tr>
<tr>
<td>Co(III)-Ado 5’ deoxyadenosylcobalamin</td>
<td>Body reserves, a.a. metabol.</td>
</tr>
<tr>
<td>Co(III)-CH3 methylcobalamin</td>
<td>Methylation reactions homocysteine →methionine</td>
</tr>
</tbody>
</table>
Vitamin B12, uptake and distribution:

Protein digestion in the stomach makes vitamin B12 available to bind to cobalophilin (saliva).

The cobalophilin/B12 complex is hydrolyzed in the duodenum, releasing B12 for binding to intrinsic factor (a glycoprotein made by parietal cells of the gastric mucosa).

The B12/IF complex (but neither alone), can then be absorbed from the distal third of the ileum.

In the blood, vitamin B12 is bound to transcobalamin II. In cells, vitamin B12 is stored bound to transcobalamin I and III.

Frequently, the defect in cases of vitamin B12 deficiency is that binding to intrinsic factor is impaired. Such is the case in pernicious anemia (both genetic and autoimmune forms are known) and in Crohn’s disease.
Succinyl CoA is thus the point of entry of some carbon atoms of methionine, isoleucine and valine. Propionyl CoA and then methylmalonyl CoA are intermediates in the breakdown of these three non-polar amino acids.

Vitamin B12 and methionine synthase:

Remethylation cycle

\[
\begin{align*}
\text{(CO}_2\text{)} & \quad \text{SAM} \\
\text{S-adenosyl homocysteine} & \quad \text{Homocysteine} \\
\text{Methionine} & \quad \text{Tetrahydrofolate} \\
\end{align*}
\]

\[
\begin{align*}
5\text{-Methyl-THF} & \quad \text{Methionine synthase} \\
\text{MS Co (II)} & \quad \text{5,10-Methyl-tetrahydrofolate} \\
\text{MS Co (I)} & \quad \text{MS Co (III)} \\
\end{align*}
\]

Diet provides only 50% of methionine requirement (synthesis of proteins, SAM, polyamines)

Biochem. 36, 8082-91, JBC 276, 27296
Vitamin B12 deficiency:

**Symptoms:**
- \( \uparrow \uparrow \) plasma methymalony CoA and homocysteine
- pale, shiny tongue \( \rightarrow \) red, sore, glossitis
- vegans, gastrectomy patients, crohns disease, 6-8% elderly
- genetic mutation, \( \text{N}_2\text{O} \) anaesthetic (oxidises Co(I) of Co(II)), oral contraceptives, hormone replacement therapy

**Consequences:**
- pernicious anemia
- diseases of bone marrow, intestinal tract, CNS
- increased methyl malonyl CoA causes growth retardation
- increased plasma homocysteine \( \rightarrow \) premature atherosclerosis, thromboembolism
- depression and dementia in geriatrics
- cognitive impairment in the elderly, peripheral neuropathy
- multiple sclerosis (demyelination disorder)
- morbidity in transplant patients

Ref. [Nut. Rev. 54, 382-9 (1996)].
Pernicious anemia:

- Pernicious anemia arises under conditions of vitamin B12 or intrinsic factor deficiency.

- Reduction in B12 levels blocks the metabolism of folic acid, resulting in secondary folate deficiency.

- Folate deficiency results in impaired erythropoiesis, resulting in the premature release of immature erythrocyte precursors (megaloblastic anemia)

- The most common cause of this form of anemia is dietary deficiency of vitamin B12, however it can also arise from defects in “intrinsic factor” (autoimmune).
Pernicious anemia, therapy:

Blood smear from anaemic patient, showing a number of megaloblasts.

Blood smear from patient 72 hours after treatment with 25 μg of vitamin B12. Note the reduction in megaloblast numbers.
Summary of material covered to date:

1. Hemoglobin, myoglobin, role of allosteric modulators in $O_2$ and $CO_2$ transport
2. Role of glycolysis in hemoglobin function
3. Drugs, toxins, mutations which affect erythrocyte function
4. Role of Glutathione
5. Pentose Phosphate Pathway
6. Genetic polymorphisms / Diseases and Malaria
7. Iron Homeostasis and treatment of anemia
8. Iron homeostasis and metabolism
   - Iron deficiency anaemia and iron therapy
   - Iron overload and Genetic disease: hemochromatosis
   - Erythropoietin Therapy
9. Folic acid metabolism, hyperhomocysteinemia and folate deficiency
10. Vitamin B12 metabolism and deficiency (pernicious anemia)