B12 and Folate

Active Forms

- The only 2 **active** forms of B12 in humans:
  - **Methylcobalamin** (MeCbl)
  - **Adenosylcobalamin** (AdoCbl)

Active Forms

- Typically **pharmaceutical** forms given are hydroxocobalamin (OHCbl) or cyanocobalamin (CNCbl). **Neither are active without conversion**.
- CNCbl does not exist as a normal source in nature.

Active Forms

- MeCbl and AdoCbl are normally made in most cells by enzymes.
- **MeCbl is made in the folate cycle**.

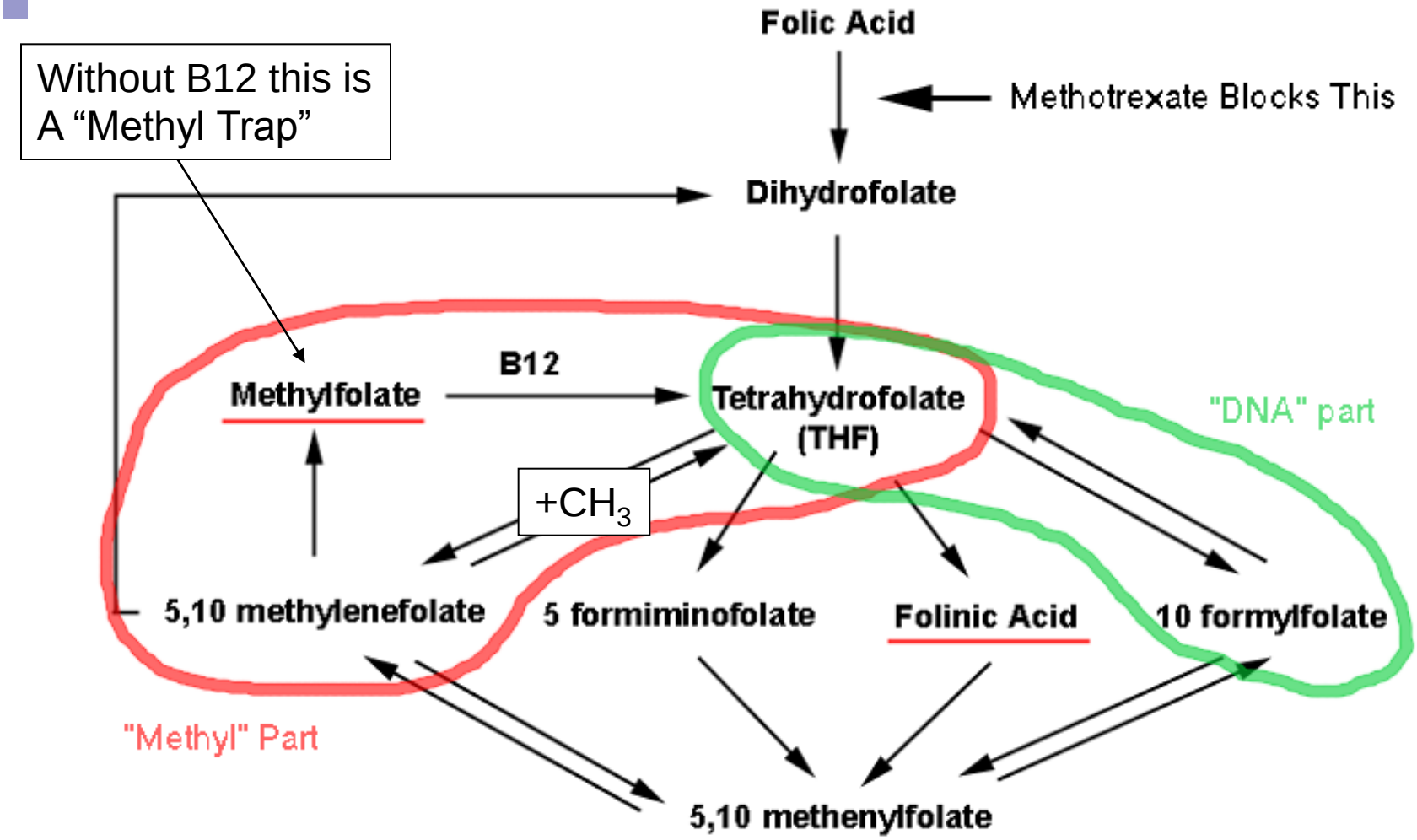
Active Forms

- Reduced MeCbl levels produce ALL the ***neurological* consequences** of B12 deficiency (inc. pernicious anaemia).
- Reduced AdoCbl levels lead to raised methylmalonic acid (MMA) and inability to burn Ile, Met and Val in the Kreb's cycle.
- Deficiency of AdoCbl Reduces succinyl co-A production & Kreb's cycle does not function properly.

Active Forms

- The 2 typical pharmaceutical forms of folate are **Folic Acid** and **Folinic Acid**. *Neither are active*.

- The folate cycle has several functions, but two major ones are the “methyl part” and the “DNA part”.



Simplified Folate Cycle

Active Forms

- The folate cycle is a *one-carbon transfer system* (transfers methyl groups) and needs constant recycling of tetrahydrofolate (THF).
- The methyl group used to make MeCbl and recycle tetrahydrofolate comes from Serine. (Serine > Glycine)

Active Forms

- MeCbl is a *methyl carrier*, it does not matter whether it comes from the folate cycle or is given as exogenous MeCbl.

- MeCbl forms about 60-80% of all B12 in plasma and about 90% of all B12 in CSF.
- AdoCbl accounts for over 70% of all cobalamin in the entire brain.

Active Forms

- **MeCbl is the normal principal storage form of CH_3**, it is a large buffer against acute decreases in methylation capacity.

Active Forms

- Without B12/Folate, Hcy piles up.
- Hcy is also cleared by *cystathionine synthase* (CS), an enzyme that converts Hcy to cystahionine. CS requires B6.
- Further enzymes convert cystathionine to cysteine (Cys) which drives GSH production and keeps the GSH/GSSG ratio normal.

Warning!

- Both B12 and/or folate deficiency cause impairment of the folate cycle leading to megaloblastic anaemia.
- Of these only B12 (MeCbl) deficiency causes neurological degeneration.

Warning!

- Giving Folic Acid does NOT restore methylation impairment due to B12 deficiency – degeneration can continue despite haematological response.

Efficacy

Efficacy

- Much of this info comes from **cases**.
- There are a few published cases/studies using high dose MeCbl or folate. These studies are generally designed to demonstrate control of markers, such as Hcy, but do demonstrate lack of toxicity for high doses.

Efficacy

- There is a Double-Blind Placebo Controlled, Cross-Over Trial of Subcutaneous MeCbl on Behavioral and Metabolic Measures in Children With Autism running in the USA which is currently recruiting. (NIH, funded UC Davis)

- This is in response to the widespread clinical use of MeCbl in autistic spectrum disorders.

Efficacy

- The study hypothesis is “Methylcobalamin injections will improve measures of executive function, speech, and socialization in children with autism, and will be associated with metabolic improvement.” (Phase II and III)
- The use of parenteral MeCbl in autism is based largely on clinical experience.

Efficacy

- As a background to this is an emerging understanding of the role of methylation and methyl donors in various neurological states.
- There is also emerging a body of evidence showing that CSF B12 levels can be low in various neurological conditions despite normal plasma B12.

Efficacy

- A nice approachable review of this can be found from Schuitemaker and Hoogland:

  Schuitemaker GE, Hoogland AJ; Cobalamin deficiency, methylation and neurological disorders. JOM 1996: 11(4):190

- Biological Therapies has also issued a newsletter on this issue (recommended):

  Vitamin B12 Brief Communication: Low B12 despite normal serum levels

Efficacy

- MeCbl by injection has been traditionally used in Japan, and is registered in Japan and France to treat neurological disorders; Peripheral neuropathies and neuritis/polyneuritis. Most studies are Japanese
- Typical positive studies include:

Efficacy

- Kuwabara S, Nakazawa R, Azuma N, Suzuki M, Miyajima K, Fukutake T, Hattori T. Intravenous methylcobalamin treatment for uremic and diabetic neuropathy in chronic hemodialysis patients. Intern Med. 1999 Jun;38(6):472-5. PubMed PMID: 10411351

Efficacy

- Kaji R, Kodama M, Imamura A, Hashida T, Kohara N, Ishizu M, Inui K, Kimura J. Effect of ultrahigh-dose methylcobalamin on compound muscle action potentials in amyotrophic lateral sclerosis: a double-blind controlled study. Muscle Nerve. 1998 Dec;21(12):1775-8. PMID: 9843082
- Watanabe T, Kaji R, Oka N, Bara W, Kimura J. Ultra-high dose methylcobalamin promotes nerve regeneration in experimental acrylamide neuropathy. J Neurol Sci. 1994 Apr;122(2):140-3. PMID: 8021696

Efficacy

- Okada et al.; Effect of methylcobalamin on diminished motor nerve conduction velocity in the tibial nerve of poorly controlled diabetics. Clin Trials J. 1985. 22(6);534
- Ikeda T, Yamamoto K, Takahashi K, Kaku Y, Uchiyama M, Sugiyama K, Yamada M. Treatment of Alzheimer-type dementia with intravenous mecobalamin. Clin Ther. 1992 May-Jun;14(3):426-37. PMID: 1638584

Efficacy

- Because the folate cycle and methylation is run by enzymes, polymorphisms in the genes coding for these enzymes can have big impacts on methylation capacity and susceptibility to deficiencies.
- There is a HUGE amount of research on this, both for various B12 enzyme genes and various folate enzyme genes.

Efficacy

- Paul et al have published an approachable review on folate enzyme genes, folate and depression:

Paul RT, McDonnell AP, Kelly CB. Folic acid: neurochemistry, metabolism and relationship to depression. Hum Psychopharmacol. 2004 Oct;19(7):477-88.

Efficacy

- There are several reviews of B12 status in various neurological states, including Alzheimer's disease:

McCaddon A, Hudson P, Abrahamsson L, Olofsson H, Regland B. Analogues, ageing and aberrant assimilation of vitamin B12 in Alzheimer's disease. Dement Geriatr Cogn Disord. 2001 Mar-Apr;12(2):133-7.

Why Injections?

Why Injections?

- There is a lot of debate to be had about the relative effectiveness of oral vs. injectable (IM) delivery systems for B12. IV of course delivers bang for your buck, but for B12/folate this is not the most common route of administration.

Why Injections?

- The advantages of injected B12 and/or folate over oral are not as clear cut as they are for Vit C.
- IM injection *may* produce similar plasma pharmacokinetics to oral dosing. This has NOT been studied for a large range of disease states.

Why Injections?

- Above normal dietary levels, B12 can bypass intrinsic factor mechanisms and be absorbed passively (~1% of dose is absorbed).
- Again, do not have studies for a large range of disease states.

Why Injections?

- Essentially, a preference for IM B12 over oral B12 cannot be based in pharmacokinetics (unlike Vit C) but must be **based on patient response**.

Why Injections?

- Choosing injections (B12 and folate) then often comes down to formulation or medical decisions:
  - Formulation, purity, quality.
  - Severe GIT problems.
  - Dementia, alcoholics, patients with poor compliance for whatever reason.
  - Inborn errors of metabolism
  - 100% sure the patient got the dose.

Safety

Safety

- There are very few ADR reports to injectable B Vitamins in general, Very few indeed to B12 or folate.
- Published ADR reports identify benzyl alcohol (phenol – a preservative) as a culprit.

Turvey SE, Cronin B, Arnold AD, Twarog FJ, Dioun AF. Adverse reactions to vitamin B12 injections due to benzyl alcohol sensitivity: successful treatment with intranasal cyanocobalamin. Allergy. 2004 Sep;59(9):1023-4.

Safety

- Despite this there are a very few ADR reports to Cytamen or Neo-Cytamen (no benzyl alcohol). Usually allergic symptoms – anaphylaxis extremely rare.
- Martindale – patients with multiple doses of Neo -Cytamen can develop antibodies to the transcobalamin complex (rare).
- Same with Folic Acid (injected). Allergic reactions – very rare.

Safety

- There is some high dose literature for both MeCbl and Folate – the safety profile is well established.
- Major precaution for Folic Acid has already been covered – do not prescribe for megaloblastic anaemia caused by B12 deficiency.

Safety

- Analgesics, hydantoin anticonvulsants (Dilantin), carbamezapine (Tegretol), oestrogens, oral contraceptives, phenobarbital, primidone (Mysoline) have all been reported to increase requirements for **folic acid**.
- **Folic acid** may decrease the effectiveness of hydantoin anticonvulsants.

Safety

- B12 therapy for megaloblastic anaemia may lower serum K^+. There are no recent reports, however this should be monitored as a general precaution.

Lawson DH, Murray RM, Parker JL, Hay G. Hypokalaemia in megaloblastic anaemias. Lancet. 1970 Sep 19;2(7673):588-90.

Protocols

Protocols

- Both MeCbl and Folic Acid are usually delivered IM.
- Folic Acid can be given IV by diluting 15mg/mL in 149mL 0.9 % normal saline for injection or 149mL 5% glucose for injection. OR
  by diluting 5mg/mL in 49mL 0.9 % normal saline for injection or 49mL 5% glucose for injection.

Protocols

- For treatment of *megaloblastic anaemia*, the usual dose of Folic Acid is 1-5 mg per day until improvement.

- It may be wise to include B12 in case there is a B12 deficiency associated with the megaloblastic anaemia.

Protocols

- MeCbl - Warm to body temperature. 2 mL (10mg) by slow I.M. (deep intragluteal) injection. ALWAYS PROTECT FROM LIGHT!
- Can be given IV with appropriate dilution.
- Smaller amounts can be given SC to aid the treatment of autism.

Protocols

- B12 Not suitable for megaloblastic anaemia from folate deficiency (not dangerous, just won’t work).

Protocols

- Neuropathies:
  Usual dose is 10mg (1 vial). Can be given daily until response (French).
  N.B. Doses reported in papers vary from 500mcg 3X per week i.m. to 25mg daily i.v. (Japanese).

Protocols

- MeCbl is commonly used in a variety of typical disorders (see cases next session):
  - Autism
  - Neurological – degenerative/other
  - Dementia – Alzheimer's etc.
  - CFS
  - Diabetes, "homocysteine" patients.

Protocols

- Blood tests for B12 can be misleading. Plasma/CSF levels *may* be mismatched in several clinical conditions (e.g. alcoholism, Alzheimer's – see BT newsletter).
- Urine MMA is reflective of AdoCbl levels, may not always reflect MeCbl in CSF.
- Dosing is non-toxic and cheaper than tests.

Protocols

- The reliability and diagnostic value of B12 tests is an area open to debate.

Solomon LR. Cobalamin-responsive disorders in the ambulatory care setting: unreliability of cobalamin, methylmalonic acid, and homocysteine testing. Blood. 2005 Feb 1;105(3):978-85. Epub 2004 Oct 5.

Protocols

- B12/folate would not be expected to distort any lab test results, however some antibiotics may give false Folic Acid test levels.
- Folic acid is incompatible with oxidising and reducing agents and ions of heavy metals. **Best to give it separately**.

Protocols

- Common to combine B complex with Vit C and Mg, additionally Ca and GSH in the form of the Myer's Cocktail outlined earlier in this lecture.

Concerns

Concerns – Recent Publications

- House AA, Eliasziw M, Cattran DC, Churchill DN, Oliver MJ, Fine A, Dresser GK, Spence JD. Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. JAMA. 2010 Apr 28;303(16):1603-9. PubMed PMID: 20424250

Concerns – Recent Publications

- “High doses of B vitamins compared with placebo resulted in a greater decrease in GFR and an increase in vascular events”
- BUT – B Vit group had ++ periph vasc disease pre study
- 2.5 mg folate per day 3 yrs – huge – unbalanced supplementation

Concerns – Recent Publications

- Very small numbers represent CVD risk
- Findings are at odds with other research
- Research is scattered – wide range of results

Concerns – Recent Publications

- Ebbing M, Bønaa KH, Nygård O, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njølstad I, Refsum H, Nilsen DW, Tverdal A, Meyer K, Vollset SE. Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA. 2009 Nov 18;302(19):2119-26. PubMed PMID: 19920236

Concerns – Recent Publications

- Found inc risk of cancer (esp lung) and all cause mortality with folic acid (800 mcg per day for approx 3 years)
- Risk did not show up until 2-3 years **after stopping** supplements
- Supplemetation unbalanced

Concerns – Recent Publications

- Doses over 2-3- years do not have massive effect on Hcy, do not have massive effect on lifetime of developing chronic disease
- Results of these studies are all over the place – no consistent picture
- Fairly consistent though – no demonstrated benefit

Concerns

- There has been some recent research that has opened the suggestion that folate supplementation may induce epigenetic changes. I.e., the mother's diet may influence the expression of genes in the infant at germ level.
- Much of the epigenetic regulation of gene expression has been linked to methylation changes.

Concerns

- Such methylation changes have been studied in mice and can be as dramatic as to determine coat colour, i.e. a mother eating a methylating diet during conception/pregnancy will produce offspring with a different coat colour.
- Such medical conditions as schizophrenia, CVD and stroke susceptibility may be inherited this way.

Concerns

- **There is very limited data at all about this in humans.** Epigenetic changes in mice have produced both good and bad outcomes. "Fat yellow" mice fed a methyl rich diet (folate, B12, choline and betaine) produced offspring that were mottled brown, lean and healthy and *stayed that way*. Otherwise, fat yellow mice beget more fat yellow mice.

Concerns

- Epigenetic research so far has been performed on special mice, i.e. mice with identifiable epigenetically sensitive genes that can be switched on or off (such as the fat yellow mice).
- The epigenetic effects in real populations, though definitely occurring, are also likely to be much more complex.

Concerns

- So far, most mouse research has produced “good” epigenetic outcomes in offspring.
- There has been some research however, on different mutant mice, that has produced “fat” offspring via epigenetic changes.

Concerns

- Clearly, this effect has been studied only in pregnancy (in special mice) and the combination of effectors is unknown.
- The combinations of effectors in humans is unclear.
- It has little bearing on usual treatment with B12 etc.

Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr. 2002 Aug;132(8 Suppl):2393S-2400S.

Wilkins JF. Genomic imprinting and methylation: epigenetic canalization and conflict. Trends Genet. 2005 Jun;21(6):356-65.

Zeisel SH. Epigenetic mechanisms for nutrition determinants of later health outcomes. Am J Clin Nutr. 2009 May;89(5):1488S-1493S. Epub 2009 Mar 4. PubMed PMID: 19261726; PubMed Central PMCID: PMC2677001

Further Reading

- Inada M, Toyoshima M, Kameyama M. Cobalamin contents of the brains in some clinical and pathologic states. Int J Vitam Nutr Res. 1982;52(4):423-9.
- Van Tiggelen, CJM: Alzheimers disease - alcohol dementia - Association with zinc deficicncy and cerebral vitamin B12 deficiency. J Orthomolecular Psych 1984: 13:97-104
- Kanazawa S, Herbert V. Total corrinoid, cobalamin (vitamin B12), and cobalamin analogue levels may be normal in serum despite cobalamin in liver depletion in patients with alcoholism. Lab Invest. 1985 Jul;53(1):108-10.
- Nijst TQ, Wevers RA, Schoonderwaldt HC, Hommes OR, de Haan AF. Vitamin B12 and folate concentrations in serum and cerebrospinal fluid of neurological patients with special reference to multiple sclerosis and dementia. J Neurol Neurosurg Psychiatry. 1990 Nov;53(11):951-4.