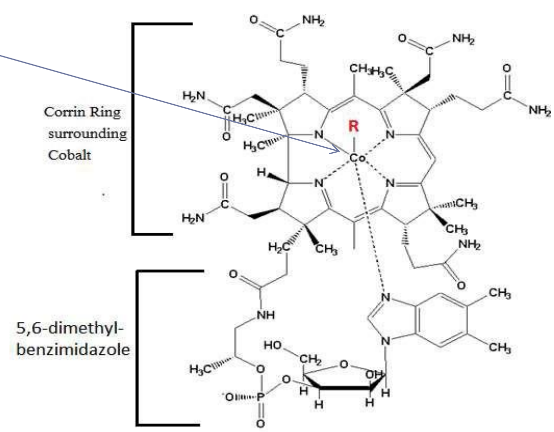
# ATMS B12/Folate

## PART 2 – CHEMISTRY: COBALAMIN & FOLATE

- Vitamin B12 is composed of
  - * Tetrapyrrole ring surrounding Cobalt atom.
    - * 5,6-dimethyl-benzimidazole.
    - * R group.

  Attached to Cobalt




The R group may be either:

- ❑ Cyanide in Cyanocobalamin (common in supplements, not a physiological form, more stable)
- ❑ *Methyl in methyl cobalamin*
- ❑ *5'- deoxyadenosine in adenosylcobalamin*
- ❑ OH in hydroxocobalamin.(natural form, produced by bacteria, used in supplements and injections)

## Chemistry

B12 is the **most chemically complex of all vitamins**.

- Chemical name **cobalamin is derived from its central cobalt atom** (which is positively charged) (Vitamin B12: Cobalamin 2013)
- It's impressive formula $C_{63}H_{88}N_{14}O_{14}PCo$ reflects the intricate molecular structure (Types of Vitamin B12 2018)
- Vitamin B12 is the **only metal-containing vitamin**. It is also **watersoluble** and **stored in the liver** (Vitamin B12: Cobalamin 2013)
- For anaerobic bacteria to synthesize B12, cobalt must be in the soil.



## Chemistry

B12 is the collective term for a group of cobalt-containing compounds known as **corrinoids,** which when assembled with 5th and 6th position ligands are known as **cobalamins** (Krautler 2012) (Vitamin B12: Cobalamin 2013).

- The principal cobalamins are:
  - **cyanocobalamin** & **hydroxocobalamin** (Types of Vitamin B12 2018).
- The two co-enzyme forms are:
  - **methylcobalamin** & **5-deoxyadenosylcobalamin (adenosylcobalamin)** (Types of Vitamin B12 2018).

## Chemistry

***The cobalamins are the only known substances to naturally contain cobalt***

- Vitamin B12 is often thus treated as synonymous with cobalamin.
- It is an odourless, intensely **dark-red coloured**, crystallized, heat and light sensitive substance (Vitamin B12: Cobalamin 2013).
- Cobalamin is almost never found in its chemically pure form as it is usually bound to other molecules.

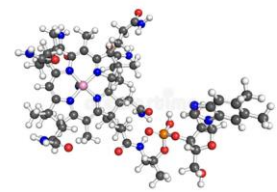


## Epidemiology of B12 Deficiency

Overall prevalence of B12 deficiency is unknown.

- **Framingham Offspring Study** found up to **39% of US adults** were at risk of **B12 deficiency** (serum B12 <258pmol/L) (Tucker et al. 2000).
- **Globally, B12 deficiency is likely to be higher**, especially in **developing countries** (Stabler & Allen 2004).

## Aetiology



Vitamin B12 is an **essential vitamin obtained only from diet or supplementation**.

- **Stores** of vitamin B12 in the **liver** remain in the body for **years** (O'Leary & Samman 2010).
- Vitamin B12 deficiency normally results from **chronic**, **long-term deficiency** (O'Leary & Samman 2010).



## Aetiology

- Decreased intake or absorption of vitamin B12 increases risk of deficiency.
- Increasing incidence of **gastric bypass** surgeries in Western countries, infers potential for continued increase in the prevalence of B12 deficiency (Majumder, Soriano, Cruz & Dasanu 2013).

## Aetiology

Generally, aetiologies of B12 deficiency can be categorised as:

- **Decreased dietary intake**
- **Food-bound cobalamin malabsorption (FBCM)** (Shipton & Thachil 2015)
- **Malabsorption via GIT or methylation** (Linus Pauling Institute 2014).

Patients at high risk of vitamin B12 deficiency may include:

- strict **vegans**
- history of **gastric or intestinal surgery**
- history of **atrophic gastritis**
- the **elderly** (Linus Pauling Institute 2014).



## Aetiology

Other predisposing factors include:

- Particular **medications** (Shipton & Thachil 2015).
- Conditions causing **malabsorption** (Shipton & Thachil 2015).
  - **IBD:** particularly **Crohn's disease**
  - **Coeliac disease**
- **Bacterial overgrowth syndromes (SIBO)**
- ***Helicobacter pylori*** may be related to atrophic gastritis diminishing the ability to break down vitamin B12 from food

# Pathophysiology

Specifically, B12 is an **important cofactor** in 2 specific biochemical processes involving

  - **methylmalonic acid (MMA)** and **homocysteine (Hcy)** as precursors (Office of Dietary Supplements - Vitamin B12 2018).

- B12 deficiency impairs the conversion of **MMA to succinyl co-A**.
- B12/folate deficiency impairs the conversion of **Hcy to methionine**.
- Methionine is critical in the production of S-adenosylmethionine (SAMe) (Office of Dietary Supplements - Vitamin B12 2018).



# Pathophysiology

- B12 and folate are integral for normal **haematopoiesis** and bone marrow function.
- Prolonged severe deficiency of B12 may eventuate in neurological and haematologic disorders.
- A relationship between neurological diseases (dementia, depression, cognitive impairment) and B12 deficiency has also been established in clinical literature (Soysal 2018, Kalita 2008).

http://bestpractice.bmj.com.ezproxy.une.edu.au/topics/en-us/822

## What To Expect When Treating B12 Deficiency

**Expected Duration**

(Harvard Health Publishing 2018)

With adequate supplementation, symptoms begin to improve within a few days.

- In vegans and those whose deficiency is diet-related, oral B12 supplementation should be adequate.
- Those with PA or absorption issues require B12 injections every 1-3 months (or more), indefinitely.



## What To Expect When Treating B12 Deficiency

**Treatment**

(Harvard Health Publishing 2018)

- People who cannot absorb B12 need regular injections. Initially, severe cases may need 5-7 injections in the first week.
- Injection response usually expected within 48-72 hours, with increased production of RBC's.
- Once B12 reserves reach normal levels, injections every 1-3 months prevents symptomatic return

## What To Expect When Treating B12 Deficiency

- In SIBO, appropriate treatment may stop bacterial overgrowth and allow B12 absorption to normalise.
- **B12 deficiency resulting from inadequate dietary intake is easily reversed via diet and oral supplementation.**
- In severe anaemia (very low RCC), blood transfusions may initially help until B12 injections kick in.



## Pernicious Anaemia (Destructive Anaemia)

B12 deficiency is the cause of **pernicious anaemia**, which was historically fatal with an unclear aetiology. First described in medicine in 1835.

## Mrs Lincoln

Mary Todd Lincoln was diagnosed post humously with **pernicious anaemia** (PA).
Whilst deadly at the time, it is now know to be caused by B12 deficiency (Scientist, 2018).

- Lincoln's suffered moodiness, hallucinations, the fact that she was a hypochondriac and often bedridden, overcome by melancholy. She was hospitalised for psychiatric problems (Scientist, 2018).
- Dr John Sotos, pursuing Lincoln's ailments for years, has concluded after researching >100 historical documents and 678 surviving letters, that Mrs Lincoln suffered PA in its worst manifestations (Scientist, 2018).
- Lincoln died in 1882 at age 63. "In the 19th century there was no treatment, so the disease progressed to its fatal endpoint.
- **A complete description and cure of pernicious anaemia were discovered in 1926**

(Scientist, 2018).



## Marie Curie (D. 1934)

## The Cobalamin Mystery

**1920** (approx.) whilst experimentally **inducing anaemia in dogs**, Whipple discovered that ingesting **large amounts of raw liver** resolved the condition (Sinclair, 2008).

**1926**, Minot & Murphy **trialled raw liver as tx for pernicious anaemia** in 45 adults (120–240 g of liver and 120g of muscle meat/day).

- They observed rapid symptomatic improvement and increased red cell count (RCC). Within 4-10 days the formation of new young RBC's (reticulocyte count) had increased from 1% to approx. 8%, jaundice lessened (less RBC destruction), and Hb concentration and RCC increased (Sinclair, 2008).

Minot & Murphy published their results in the *Journal of the American Medical Association* in 1926 (Sinclair, 2008).

- Eating **raw liver** or drinking liver juice became tx for the previously fatal condition.

**Whipple, Minot, and Murphy shared the 1934 Nobel Prize in Physiology or Medicine**.

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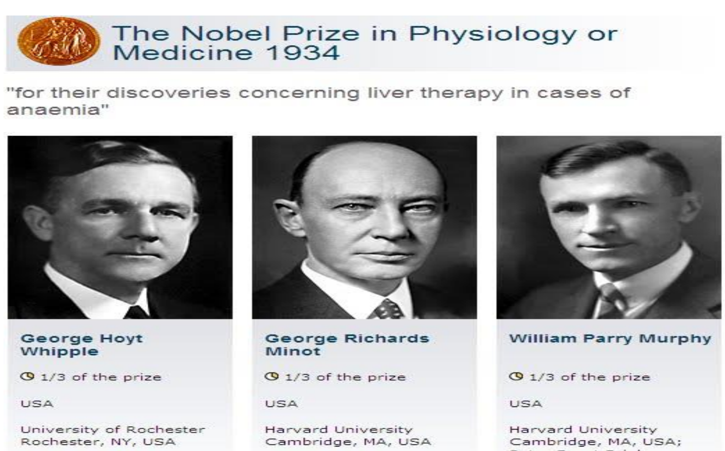


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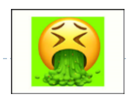
# Raw Liver Smoothie… mmmmm

**Ingredients**

1/4 cup yogurt/kefir/milk
Small handful berries
1/2 banana
1 Tbsp frozen **raw** liver
2 raw egg yolks
1 tsp lemon/lime juice
1 tsp cinnamon
Pinch of sea salt
Honey/maple syrup

**Method**

1. Blend all ingredients (except egg yolks) until smooth
2. Add egg yolks and pulse to incorporate
3. Enjoy….!!!



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# The Cobalamin Mystery

- **1928**, chemist Edwin Cohn prepared a **liver extract 50-100 times more potent** than the natural liver products. The extract was the first workable treatment for the disease.
- These events led to **discovery of the soluble vitamin B12, from bacterial broths**.
- 1947 - Shorb & Folkers were given a US$400 grant to develop the so-called **"LLD (*Lactobacillus lactis)* assay"** for B12. Dorner, a bacterial strain requiring "LLD factor" for growth was eventually identified as B12 (Scott & Molloy 2012).
- Shorb used the LLD assay to rapidly extract the **anti-pernicious anaemia factor from liver extracts,** and **pure B12 was isolated** by **1948**.
- **1956**, **chemical structure** of the molecule was determined by Dorothy Crowfoot Hodgkin (Scott & Molloy 2012).
- **1950's, methods of producing the vitamin in large quantities from bacteria cultures were developed**, leading to the modern treatment form.
- **1980's**, MIT and Harvard researchers discovered the final piece of the synthesis pathway of B12, the **only vitamin synthesised exclusively by microorganisms**.

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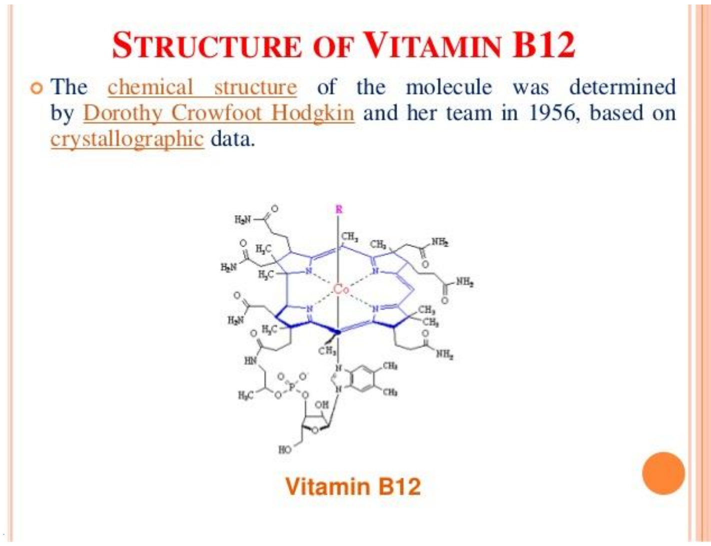
## The Cobalamin Mystery

- The biosynthesis of this essential nutrient is intricate, involved and **confined to certain members of the prokaryotic world**, seemingly never having made the eukaryotic transition (Martens, Barg, Warren, Jah, 2002)
  - Prokaryote = a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.
- **Humans require only trace amounts of B12 (approx. 1µg/day),** to assist the functions of only two enzymes, **methionine synthase** and **(R)-methylmalonyl-CoA mutase** (Martens, Barg, Warren, Jah, 2002).



**Vitamin B12 is produced by soil microbes that live in symbiotic relationships with plant roots** (MIT News, 2018).

## 1956 – Dorothy Hodgkin

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## “ The Mount Everest of Biosynthetic Problems'.

- B12 is pieced together as an elaborate molecular jigsaw involving around 30 individual components.
- Unique amongst the vitamins as it is exclusively made by bacteria.
- In the early 1990's it was discovered that two pathways exist in nature for the de novo biosynthesis of adenosylcobalamin; the coenzyme form of vitamin B12, to allow its construction - one requiring oxygen and the other not

(Moore et al., 2013).

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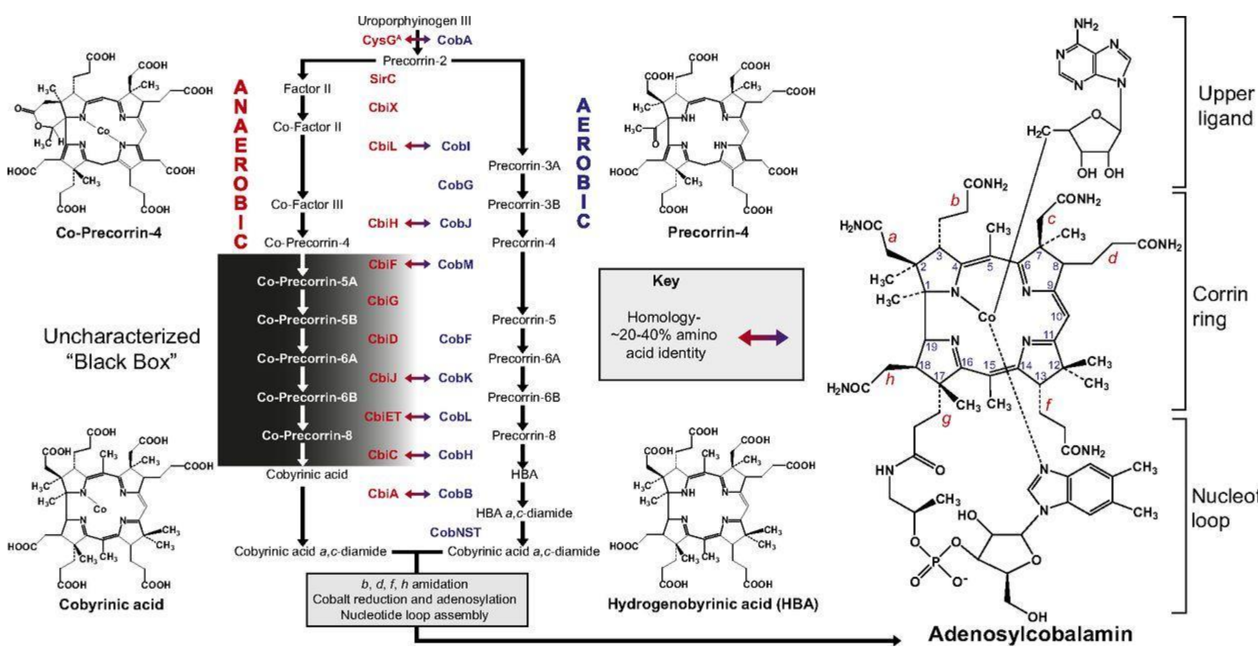
# Anaerobic Pathway

The **anaerobic** (more common) pathway has yet to be fully characterised, due to the instability of its oxygen-sensitive intermediates which are very unstable and rapidly degrade (Moore & Warren 2012)

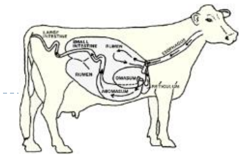
- Bioscientists at the University of Kent have trained a friendly bacterium; *Bacillus megaterium* to produce all of the components of the anaerobic B12 pathway (Moore & Warren 2012).
- This can be used to help persuade bacteria to make the vitamin in larger quantities, thereby contributing to its use in supplements, livestock feed and health and medical research.






Moore, Simon J., Andrew D. Lawrence, Rebekka Biedendieck, Evelyne Deery, Stefanie Frank, Mark J. Howard, Stephen E. J. Rigby, and Martin J. Warren. “Elucidation of the Anaerobic Pathway for the Corrin Component of Cobalamin (Vitamin B12).” *Proceedings of the National Academy of Sciences* 110, no. 37 (September 10, 2013): 14906–11. https://doi.org/10.1073/pnas.1308098110.

## Cobalamin - Dietary Sources & Requirements




**To confirm: Cobalamin is synthesised solely by microorganisms** (Linus Pauling Institute, 2014).

- Ruminants obtain cobalamin from the foregut, whereas the **only source for humans is food of animal origin**, (meat, fish, and dairy products).
- Vegetables, fruits, and other foods of non-animal origin are free from cobalamin unless contaminated with bacteria.



## Cobalamin - Dietary Sources & Requirements - Summary

A **normal Western diet contains 5–30µg of cobalamin daily**.

Adult daily requirements are only approx. 2-2.8µg (Linus Pauling Institute, 2014).

Adult daily losses (excreted via urine and faeces) are 1–3µg (~0.1% of body stores).

**Body stores are approx.2–3mg, sufficient for 3–4 years if supplies are completely cut off**.

## Natural Sources of B12

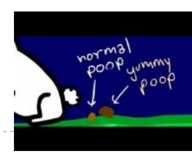
- Eat animal products, particularly liver & meat
- Eat insects/worms
- Eat vegetables with soil still attached

**Or more adventurously…..**

- Eat contents of ruminant stomachs and intestines
- Eat fermented and part-digested moss from reindeer gut (Inuits evenused to eat the feaces)
- Eat molluscs
- Eat poop



## Rabbit Food!




- Faeces are a rich source of vitamin B12 and many species, including dogs, cats, and rabbits eat faeces.
- Species within the Lagomorpha (rabbits, hares, and pikas) produce two types of feacal pellets: hard ones, and soft ones called cecotropes.
- Animals in these species **re-ingest their own cecotropes**, which consist of chewed plant material that was metabolised by bacteria in the cecum, a chamber between the small and large intestines.
- Cecotropes contain digestible carbohydrates and B vitamins synthesised by the resident bacteria (Intestinal Bacteria as a Vitamin B12 Source 2018).

## Insects, a Potential B12 Food Source

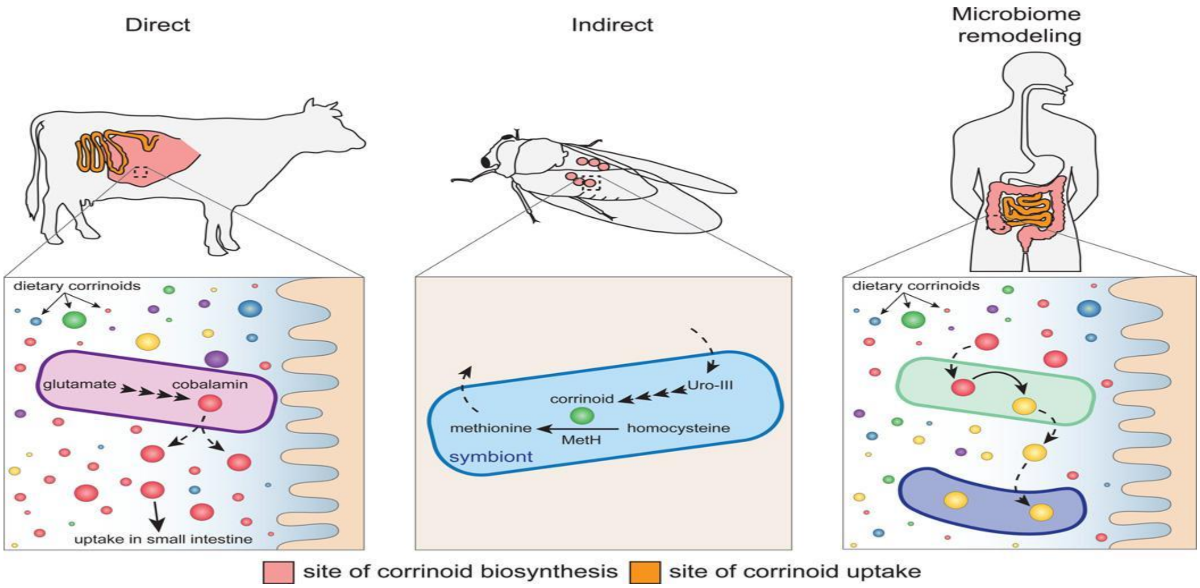
- Insects containing B12 include:House crickets (*Acheta domesticus)* (5.4µg/100g in adults and 8.7µg/100g in nymphs)(Anankware, Fening, Osekre, Obeng-Ofori, 2015).
- Mealworm larvae (*Tenebrio molitor*) 0.47 µg per 100 g (Anankware, Fening, Osekre, Obeng-Ofori, 2015).

More research is needed to identify edible insects rich in B vitamins, and the breakdown and utilisation from human consumption of insects.



## Microbial corrinoid metabolism in the gut

- In ruminants, gut microbes provide a direct source of cobalamin (direct effect)(Degnan, Taga, Goodman 2014).
- In insects, corrinoids are essential cofactors for obligate symbionts that provide key nutrients to the host (indirect effect) (Degnan, Taga, Goodman 2014).
- Competition and exchange of corrinoids likely shape gut microbiota composition and expressed functions in humans and other animals (microbiome remodeling) (Degnan, Taga, Goodman 2014).

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## Cobalt Deficiency in Stock

- **Presence of cobalt in soils markedly improves the health of grazing animals** (Huwait, Kumosani, Moselhy, Mosaoa & Yaghmoor 2015). An uptake of 0.20 mg/kg a day is recommended because they have no other source of vitamin B12.
- In the early 20th century during the development of farming on the North Island Volcanic Plateau of New Zealand, cattle suffered from what was termed "bush sickness". It was discovered that the volcanic soils lacked the cobalt salts essential for the cattle food chain.
- The "coast disease" of sheep in the Ninety Mile Desert of the Southeast of South Australia in the 1930s was found to originate in nutritional deficiencies of trace elements cobalt and copper (AgricWA, 2018).

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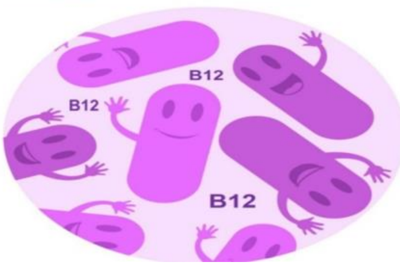
## Capping it off!

"**B12** is the **largest** and most structurally **complicated** vitamin. **Neither fungi, plants, nor animals are capable of producing vitamin B12**. **Only bacteria** have the **enzymes** required for its synthesis. Many foods are a natural source of B12 because of bacterial symbiosis. Thus, **B12 can be produced industrially only through bacterial fermentation-synthesis**."







## Summary

- B12 is **only** produced by some **prokaryotes** (certain bacteria and archaea) (Martens, Barg, Warren, Jah, 2002).
- B12 is synthesised by some gut bacteria in the human colon, however cannot be absorbed as the colon is too far from the small intestine, where B12 absorption occurs.
- **For gut bacteria of ruminants to produce B12,** the animal **must consume sufficient amounts of cobalt** (Huwait, Kumosani, Moselhy, Mosaoa & Yaghmoor 2015).
- **Grazing animals pick up B12 and bacteria** that produce it from the **soil** at the roots of the plants they eat (Huwait, Kumosani, Moselhy, Mosaoa & Yaghmoor 2015).

## Methylation Pathways - Methionine Synthase

- **Methylcobalamin** is required for the function of the folate-dependent enzyme **methionine synthase** (Linus Pauling Institute, 2014).
- This enzyme is required for the synthesis of the amino acid methionine from homocysteine (Linus Pauling Institute, 2014).
- **Methionine** is required for the synthesis of **S-adenosylmethionine (SAMe)**, a **methyl group donor** used in many biological methylation reactions, including the methylation of a number of sites within DNA and RNA (Linus Pauling Institute, 2014).
- Inadequate function of **methionine synthase** can lead to an accumulation of **Hcy**, which has been associated with increased risk of cardiovascular disease (Linus Pauling Institute, 2014).



## Methylation Pathway - Methylmalonyl-CoA Mutase

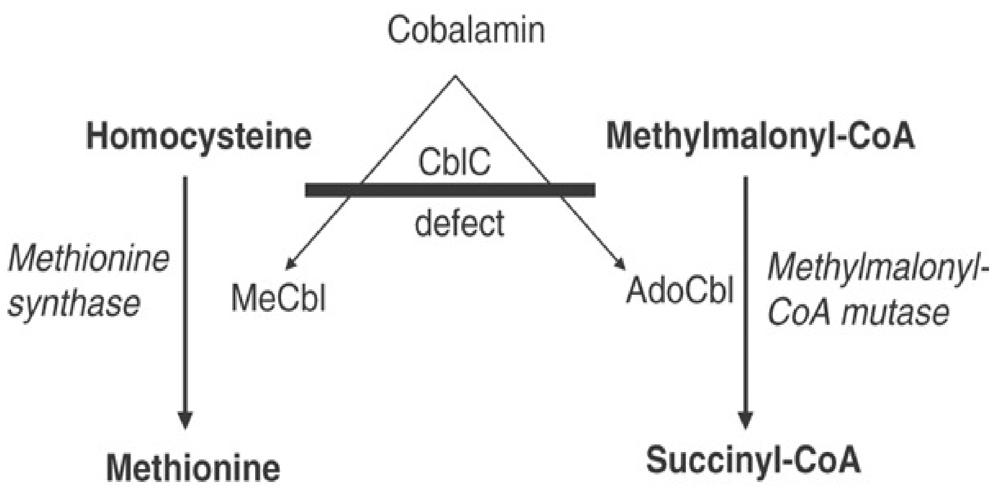
**Methyl or Adenosylcobalamin** are required by the enzyme that catalyses the **conversion of Methylmalonyl-CoA to Succinyl CoA** (BMJ, 2018)

- **Succinyl CoA** then enters the citric acid cycle (BMJ, 2018).

**Succinyl CoA** functions include:

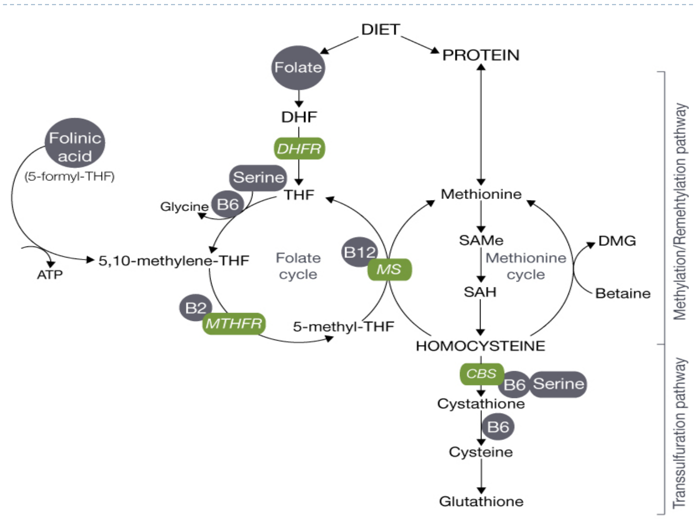
- an important role in the production of energy from lipids and proteins (BMJ, 2018).
- an essential role in the synthesis of **Hb** (oxygen-carrying component of RBCs) (BMJ, 2018).

## Genetic Defect





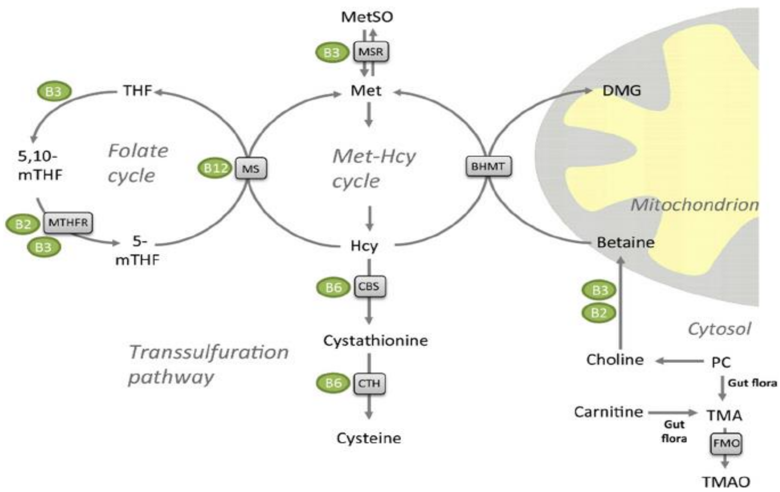

## Transsulfuration/Methylation/Re-methylation Pathway




Higdon. 2003

## Transsulfuration Pathway – Hello B6☺

- The transsulfuration pathway is the major route for the metabolism of the sulphur-containing amino acids.




Higdon. 2003

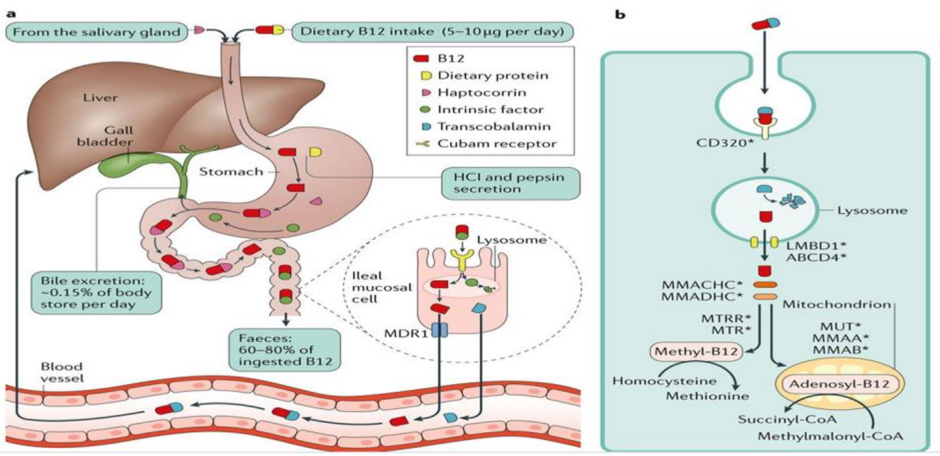


## Absorption of Vitamin B12

- **Passive** (Linus Pauling Institute, 2014)
  - via buccal duodenal & ileal mucosa
  - rapid but inefficient with under 1% absorbed by this process
- **Active** (Linus Pauling Institute, 2014)
  - occurs through the ileum
  - mediated by gastric intrinsic factor (gene at chromosome 11q13) (IF)

Figure 4 : Absorption, enterohepatic circulation and intracellular metabolism of vitamin $B_{12}$.

From: Vitamin $B_{12}$ deficiency



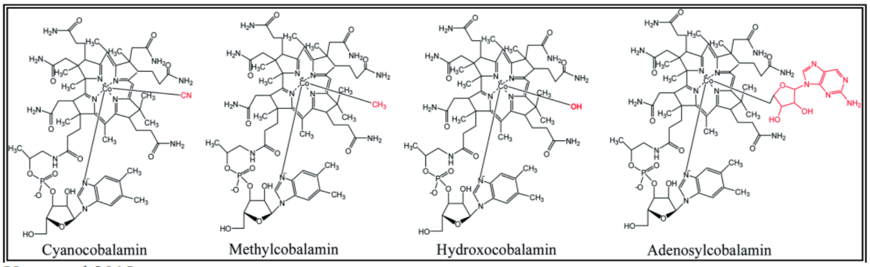



## Proteins associated with vitamin B12 metabolism

- ❑ R protein (aka Haptocorrin or TCN I )
- ❑ Intrinsic factors
- ❑ Cubilin receptors
- ❑ Transcobalamin II
- ❑ Cell surface receptors for TCNII-B12 complex
- ❑ Enzymes involved in formation of- adenosyl and methyl cobalamin forms (Neale 1990).

## Forms of B12 by Structure

There are a variety of Cbl forms, which share the core structure of Cbl but contain different upper ligands. Also the physiological forms of cobalamin (hydoxycobalamin, adenosylcobalamin and methylcobalamin) are available as supplements with different routes of administrations (Obeid et al 2015) as:




Yang et al 2015

*The corrin ring of vitamin B12 is similar but not identical to the porphyrin ring found in heme-containing proteins. Vitamin B12 is not used as a source of heme.



## Cyanocobalamin (CNCbl)

**Synthetically manufactured** form of vitamin B12 (Paul & Brady 2017).

- It is the **most stable** form of B12, due to the presence of a cyanide molecule (Paul & Brady 2017).
- While the amount of cyanide is not dangerous, it does require the body to expend energy to convert and remove it.
- CNCbl is a stable and **inexpensive** synthetic form commonly used for **food fortification** and **oral supplements** (Paul & Brady 2017).

## Methylcobalamin (MeCbl)

This is the **most active form in the human body** (Paul & Brady 2017). **Converts homocysteine to methionine**, which helps protect the cardiovascular system.

- Offers overall protection to the nervous system.
- Can also cross the blood-brain barrier (without assistance) to protect brain cells.
- It **contributes essential methyl groups** needed for detoxification and to start the body's biochemical reactions (Paul & Brady 2017).
- In mammalian cells, MeCbl is a cofactor for the cytosolic methionine synthase (Paul & Brady 2017).



## Hydroxocobalamin

An **abundant** and **physiologically relevant intermediate form** (Paul & Brady 2017).

- **Bacteria naturally create this form** of vitamin B12, making it the **main type found in most foods**.
- It **easily converts into methylcobalamin in the body** (Paul & Brady 2017).
- Hydroxocobalamin is **commonly used via injection** as a treatment for B12 deficiency as well as a treatment for cyanide poisoning (Thakkar, Billa 2015; Zhang, Ning 2008).

## Adenosylcobalamin (AdoCbl)

- AdoCbl is a **cofactor for the mitochondrial methylmalonyl-CoA (MM-CoA) mutase** (Thakkar, Billa 2015, Paul & Brady 2017).
- The **energy formation** that occurs during the Citric Acid cycle requires this form of B12 (Thakkar, Billa 2015).
- Although **naturally occurring**, it is the **least stable** of the four types of B12 outside the human body and does not translate well into a tablet-based supplement (Thakkar, Billa 2015, Paul & Brady 2017).
- **Harder to find in supplement form.**



## Supplementation

- When supplemented, **CNCbl needs to be converted into MeCbl and AdoCbl** in order to exert its anticipated biological effect on the cell (Thakkar, Billa 2015, Paul & Brady 2017).
- The concept of replacing CNCbl/HOCbl with the coenzyme forms as ready-to-use sources of the cofactors has emerged.
- Supplementation of MeCbl and AdoCbl is postulated to be preferable to HOCbl and especially CNCbl/, depending on factors including genetics, environment, age and GIT function (Thakkar, Billa 2015, Paul & Brady 2017)

## Example Dosages Found in Common Supplements

- Sublingual Cyanocobalamin Spray: 500ug per spray
- Sublingual Cyanocobalamin Tablet: 1000ug per tablet
- Chewable Hydroxocobalamin Tablet: 2000ug per tablet
- Liposomal Hydroxocobalamin Liquid: 500ug per 0.5ml dose
- Liposomal Methylcobalamin: 200ug per Spray
- Sublingual Methylcobalamin tablet: 5000ug (HIGH DOSE!!)

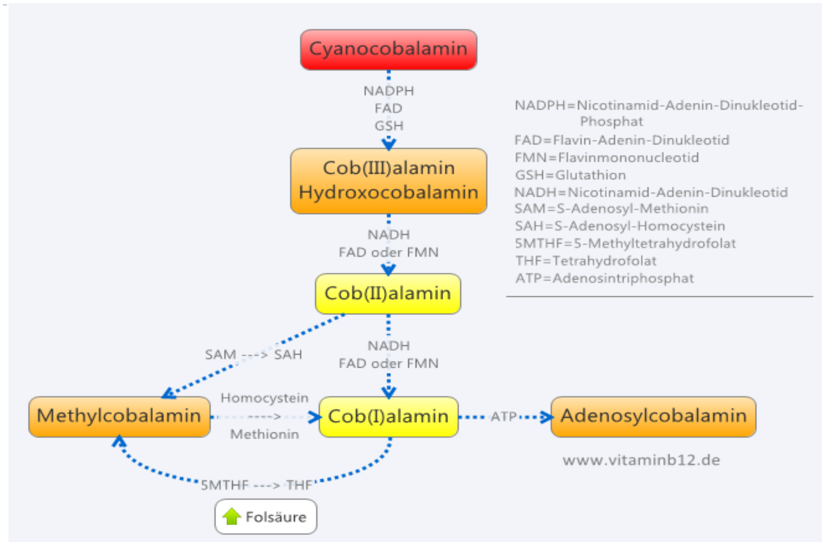


## Forms of Cobalamin

Types of Vitamin B12 Compared

| Cobalamin | Natural Form? | Bioactive Coenzyme? | Conversion steps necessary | Sustained Release | Special Effect |
|---|---|---|---|---|---|
| **Cyanocobalamin** 'the synthetic B12' | no | no | 4 | average to poor | No particular effect |
| **Hydroxocobalamin** 'the long lasting B12' | yes | no | 3 | very good | Detoxification of cyanide & NO |
| **Methylcobalamin** 'the DNA & nerves B12' | yes | yes | 0 | average | DNA, brain, nerves, blood, detoxification |
| **Adenosylcobalamin** 'the energy B12' | yes | yes | 0 | average | Energy, muscles, brain, DNA |

## B12 Forms and absorption

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## Vitamin B12 Lab Values & Deficiency Signs

- ❑ **While severe deficiency causes permanent neurological damage, earlier manifestations are generally subtle or asymptomatic**
- ❑ Vitamin B12 deficiency is a common condition that can manifest with Neurologic signs & sx
- ❑ Psychiatric signs & sx
- ❑ Hematologic disorders

(BMJ Best Practice2018)

The lab guidelines for B12 deficiency can be vague.

**In Australia :**

**<147 pmol/L** indicates **probable deficiency**

**148-258 pmol/L** indicates **possible deficiency**

**>258 pmol/L** indicates that **deficiency is unlikely??**

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## Screening Factors

- ❑ Paraesthesias
- ❑ Vegan/vegetarian
- ❑ Medication use
- ❑ Positive Romberg test - Ataxia
- ❑ Decreased vibration sense
- ❑ Pallor, Fatigue
- ❑ Petechiae
- ❑ Glossitis
- ❑ Angular cheilitis
- ❑ Cognitive impairment
- ❑ Swollen tongue, bleeding gums
- ❑ decreased appetite.

**B12 is required to make the protective coating (myelin sheath) surrounding nerves, so inadequate B12 can expose nerves to damage**
(Shipton & Thachil 2015).

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## Screening Factors

**Vegans**

- ❑ Vegans not supplementing with B12 are potentially at an increased risk of vitamin B12 deficiency.
- ❑ Up to 88% of vegans were found to have evidence of vitamin B12 deficiency in one study.

**Age >65 years**

- ❑ Prevalence of B12 deficiency increases with advancing age (Clarke et al. 2004).
- ❑ 12% to 15% of people aged >65 years have biochemical evidence of vitamin B12 deficiency
- ❑ MOA = **Poorer GI absorption**, and higher prevalence of atrophic gastritis

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## Risk factors (Medications)

Metformin

- Chronic metformin use may cause low serum vitamin B12 levels and place patients at risk of vitamin B12 deficiency (Gupta, Jain, & Rohatgi 2018).
- Patients using metformin for 4.3 years have an increased risk of vitamin B12 deficiency.

H2 receptor antagonist or proton-pump inhibitors (PPIs)

- Vitamin B12 bound to food must be freed by peptic acid secreted from the stomach.
- Taking H2 receptor antagonists or PPIs may increase risk of deficiency
- >2 years' use of PPI or H2 receptor antagonists increase the risk of B12 deficiency (Lam 2013)



## Risk Factors (Health Conditions)

*Helicobacter pylori* infection

- Studies reveal a potential association between *H pylori* infection and vitamin B12 deficiency.
- Unclear whether it is the organism or atrophic gastritis that causes vitamin B12 deficiency (Serin et al. 2002, Avcu et al. 2001).

## Risk Factors (Health Conditions)

**Terminal ileum disease**

- The majority of patients with terminal ileum resection were found to have evidence of vitamin B12 deficiency in an early study (Skidmore 1989).

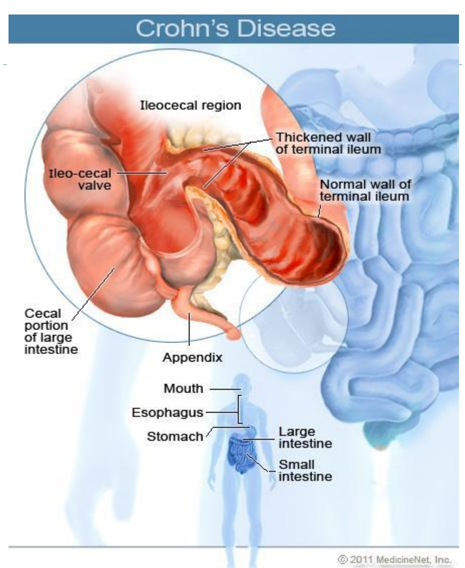
**Crohns Disease**

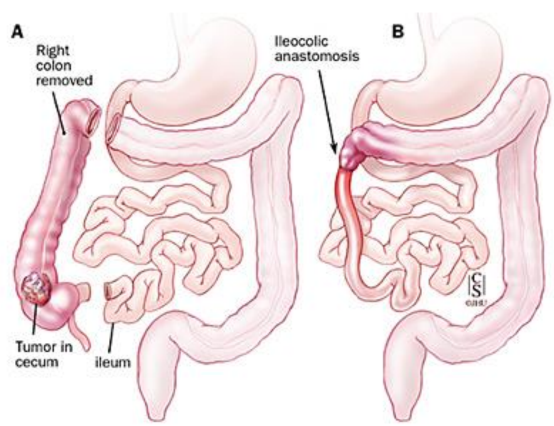
- 50% of patients with Crohns disease who have had >20 cm of terminal ileum removed have evidence of vitamin B12 deficiency.

**Gastric Surgery**

- Gastric surgery causes inadequate B12 absorption via decreased IF (Kapadia 1995, Rhode 1995, Sumner 1996).





## Diabetes

- Neuropathy in patients with diabetes is common (Gupta 2018, Wang 2017).
- This means that concomitant vitamin B12 deficiency may be easily overlooked (Tavares 2017, Pflipsen 2009, Wang 2017, Metaxis 2018).
- B12 status should therefore be part of diabetic health screening.



## Pregnancy

- B12 deficiency is common during pregnancy as levels of vitamin B12 decrease from the first to the third trimester

(Rogne et al. 2017, Lai 2017, Dayaldasani 2014).

## Investigations: Full Blood Count (FBC/CBC/FBE)

Provides important information about the types, populations, and health of blood cells.

- Aids diagnosis and assessment of **anaemia**, nutritional deficiencies, blood disorders, infection, and many other disorders.

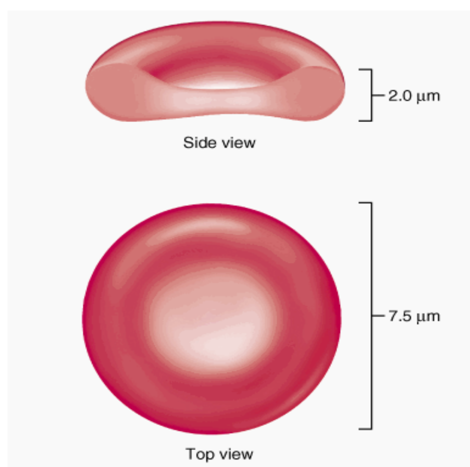
An FBC consists of:

- **RBC** (including red cell indices)
- **WBC** (including white cell indices)
- **Haemoglobin (Hb)**
- **Haematocrit (Hct)**
- **Platelets**



## Microscopy

A **peripheral blood smear** (a way of looking at blood cells under the microscope) may also provide useful information.
In a normal peripheral blood smear, RBC appear regular & round with a pale centre.
Variations in the size or shape of these cells, otherwise known as **anisocytosis**, may suggest a blood disorder.



# Primary Pathological Investigations

**FBC – *(Screening tool not diagnostic)***

- To determine baseline RDW (not always included in an FBC) as well as MCV, Hb and Hct.
- Many individuals with documented vitamin B12 deficiency do not have macrocytosis or anaemia (Chui et al. 2001).
- Not useful for diagnosing early vitamin B12 deficiency (except increased RDW)
- Not useful to rule out vitamin B12 deficiency.
- **B12 deficiency may cause:**
- Increased RDW, increased MCV and increased Hct, depending on the stage of deficiency.



# Serum Vitamin B12

**<147 pmol/L** indicates **probable deficiency**
**148-258 pmol/L** indicates **possible deficiency**
**>258 pmol/L** indicates that **deficiency is unlikely????**

**Optimal serum vitamin B12 levels for hematologic and neurologic function are still undetermined.**

# Primary testing

**Methylmalonic Acid (MMA)**

- Produced in very small amounts, used in metabolism and energy production (Methylmalonic Acid 2018).
- In one step of metabolism, B12 **promotes the conversion of methylmalonyl CoA to succinyl CoA** (Methylmalonic Acid 2018).
- If there is not enough B12 available, then the MMA concentration begins to rise, resulting in an increase of MMA in the blood and urine.
- **Elevated urinary MMA is a sensitive and early indicator of B12 deficiency** (Sun et al. 2014).



# MMA

- **Test**
  - Potential differential diagnosis = renal disease, in which increased MMA levels also occur.
  - Increased MMA may be misleading and requires follow-up to determine whether MMA normalises with adequate treatment (Oh 2018).
- **Result**
  - Increased (lab-specific)

## Homocysteine (Hcy)

**Test**

**Not as specific as MMA for vitamin B12 deficiency**

(Vashi, Edwin, Popiel, Lammersfeld & Gupta 2016).

- Differential diagnosis = folate deficiency and hypothyroidism, in which Hcy is also increased.
- More readily available than MMA
  - **Result**
    - Increased (lab-specific)



## Intrinsic Factor (IF) antibody

- **Once vitamin B12 deficiency is confirmed, IF antibody can determine whether pernicious anaemia is the cause.**
  - Only 50% sensitive, but highly specific for pernicious anaemia.
- **Result**
  - Positive if pernicious anaemia is the cause

## Antiparietal Cell (APC) antibody

- Parietal cell antibodies are proteins produced by the immune system that mistakenly target a type of specialised cells that line the stomach wall.

This test **detects these antibodies in the blood to help diagnose pernicious anaemia.**

**So….. Wrap your head around this…….**



If both parietal cell antibody and intrinsic factor antibody are positive

= Immunological evidence of Pernicious Anaemia.

If both parietal cell antibody and intrinsic factor antibody are negative

= No immunological evidence of Pernicious Anaemia.

If parietal cell antibody is positive but intrinsic factor antibody is negative

= Gastric Parietal cell antibody is associated with > 90% of patients with Autoimmune Gastritis, the end result of which may be Pernicious Anaemia.

In 20-30% of patients, relatives of patients with pernicious anaemia, autoimmune thyroiditis a small percentage of healthy persons may be positive and run an increased long term risk of pernicious anaemia.

- **A negative Intrinsic Factor antibody result does not exclude the diagnosis of Pernicious anaemia, as only 60% of patients with pernicious anaemia will have this antibody.**

## Intrinsic Factor Antibody

**Two types:**

- Type 1(blocking) antibody prevents the attachment of vitamin B12 to intrinsic factor: present in 50-60% of patients with pernicious anaemia (RCPA 2018).
- Type 2 (precipitating) antibody prevents attachment of the vitamin B12-intrinsic factor complex to ileal receptors: present in 30% of patients with pernicious anaemia (RCPA 2018).



## Active B12

**Holotranscobalamin (Active B12)**

**Test**

- Measures B12 bound to transcobalamin.
- Decreased values along with low normal serum vitamin B12 suggest inadequate absorption (Nexo & Hoffman-Lucke 2011).
- Used more frequently

**Result**

***<35pmol/L may be diagnostic of deficiency***

## Reticulocyte Count

- **Test**
  - Decreased reticulocyte index indicates decreased production of reticulocytes
  - Differential diagnosis = haemolytic anaemia, in which reticulocyte index would be increased.
- **Result**
  - Decreased corrected reticulocyte index.



## Serum Gastrin & Schilling Test

- **Serum Gastrin (Fasting) Test**
  - Gastrin levels rise in gastric achlorhydria and can signify pernicious anaemia (Slingerland, Cardarelli, Burrows & Miller 1984).
- **Result** = Increased if pernicious anaemia is the cause

- **Schilling test** ***(rarely available)***
  - Performed with an initial test flushing dose of 1000ug of cyanocobalamin, intramuscularly.
  - Generally unavailable and rarely used.
- **Result**: Differs for different stages

## Pathophysiology

Two major pathophysiologic processes contribute to the anaemia resulting from B12 deficiency:

Resulting **haemolysis** is associated with a 30% to 50% reduction in RBC lifespan (Green 2017).

- **Ineffective erythropoiesis**, caused by intramedullary apoptosis of megaloblastic erythroid precursors (Green 2017).
- Increased rigidity of produced erythrocytes, associated with abnormal RBC membrane proteins, leading to **shortened RBC survival** (Green 2017).

http://www.bloodjournal.org/content/bloodjournal/129/19/2603.full.pdf



## Pathophysiology

- Increased plasma bilirubin
- Increased serum lactate dehydrogenase (LDH)
- LDH-1 predominating over LDH-2.
- Serum AST levels are however, often normal.
- Moderately increased serum EPO levels.
- Ineffective erythropoiesis causes decreased Fe utilisation, resulting in **increased serum Fe and ferritin levels.**
- Increased levels of soluble serum transferrin receptor, presumably resulting from increased haemolysis.
- Corresponding to the increase in LDH, there may be an increase in serum muramidase, caused by increased granulocyte turnover.

(Barcellini & Fattizzo 2015)
http://www.bloodjournal.org/content/bloodjournal/129/19/2603.full.pdf?sso-checked=true.

# Toxicity – Upper level limits

**No toxic or adverse effects have been associated with large intakes of vitamin B12 from food or supplements in healthy people.**

- There is insufficient data upon which to set any upper daily limit for B12 (Nutrient Reference Values 2017).
- When high doses of vitamin B12 are given orally, only a small percentage can be absorbed, which may explain the low toxicity Because of the low toxicity of vitamin B12, no tolerable upper intake level (UL) has been set by the US Food and Nutrition Board.



# The B12 – Folate Story

Holding Hands

# B12/Folate Methylation

- **Homocysteine (Hcy)**
  - Vitamin B12 cannot fulfil its role in the deconstruction of Hcy without folate – the two vitamins are dependent on one another (Vitamin B12 And Folic Acid 2018).
  - Before we explore this relationship in detail, it is important first to touch upon the most important information regarding the role of folate (Vitamin B12 And Folic Acid 2018).
- **Methionine Synthase**
  - Both **B12 & folate** play important roles in the **conversion of dangerous Hcy to methionine** (Vitamin B12 And Folic Acid 2018).
- **Tetrahydrofolate**
  - B12 is responsible for re-activating folic acid, by converting it through various reactions back into tetrahydrofolate, (the form of folate the body can use). A B12 deficiency thus leads to an indirect folate deficiency: even if enough folate is provided for the body, it cannot be used unless a supply of B12 is present; its simply sits in its inactive form (Vitamin B12 And Folic Acid 2018).



# B12/Folate Methylation

**Cobalamin deficiency leads to Folate deficiency**

- Since a lack of B12 also leads to a folate deficiency, vitamin B12 has a doubly important position in cell metabolism.

A reminder that folate is responsible for the following tasks:

- Cell division
- Haematopoiesis (blood formation)
- Mucosal structure
- DNA synthesis
- Protein metabolism
- Fat metabolism

## Folic versus Folate

**Folic acid** is a **synthetic** compound which **cannot be directly used by our body** (Vitamin B12 And Folic Acid 2018).

**Folate** is the **natural** and **bioactive form** of folic acid, which is used in various chemical forms in the body (Vitamin B12 And Folic Acid 2018).

- Normally, the body is capable of absorbing folic acid and converting it into folate. However, since this isn't always the case, supplements which contain bioactive folate instead of folic acid are now also available (Vitamin B12 And Folic Acid 2018).



## Functional Folate Deficiency

Two hypotheses have been developed to explain how cobalamin-deficiency anaemia is in fact caused by functional folate deficiency (Castellanos-Sinco et al. 2015):

- **Methyltetrahydrofolate trapping, or the "folate trap".**
- **Formate deficiency**

## Formate deficiency

- When **tetrahydrofolate** is depleted, stored **methyltetrahydrofolate** cannot be converted into formate-mediated **formyltetrahydrofolate**, (another functional form of folate used in purine synthesis) (Castellanos-Sinco et al. 2015).
- Plasma formate concentration and urinary formate excretion are equally sensitive indicators of folate deficiency, as is plasma Hcy (Castellanos-Sinco et al. 2015).
- Cobalamin and folate metabolism share another common feature: they both require methylenetetrahydrofolate (a product of tetrahydrofolate) and dUMP to form thymidylate synthase-mediated thymidylate and dihydrofolate (Castellanos-Sinco et al. 2015).



## Summary

- **Diminished activity of methionine synthase in B12 deficiency inhibits the regeneration of tetrahydrofolate** (THF), **trapping folate** in a form that is not usable, **resulting in folate deficiency**, even in the presence of adequate folate levels (Castellanos-Sinco et al. 2015).
- Thus, **in both folate and vitamin B12 deficiencies, folate is unavailable to participate in DNA synthesis** (Castellanos-Sinco et al. 2015).
- This **impairment of DNA synthesis** affects the rapidly dividing cells of the bone marrow earlier than other cells, resulting in the production of large, immature, Hb-poor red blood cells causing **megaloblastic anaemia** which is the symptom for which the disease, pernicious anaemia was named (Castellanos-Sinco et al. 2015).

# Timeline of Nutrient Discovery Isolation and Synthesis

| Vitamin | Alternative name | Discovery | Isolation | Structure | Synthesis |
|---|---|---|---|---|---|
| Vitamin A | Retinol | 1909 | 1931 | 1931 | 1947 |
| Provitamin A | β-Carotene | 1831 | 1831 | 1930 | 1950 |
| Vitamin D | Calciferol | 1918 | 1932 | 1936 | 1959 |
| Vitamin E | Tocopherol | 1922 | 1936 | 1938 | 1938 |
| Vitamin K | Phylloquinone | 1929 | 1939 | 1939 | 1939 |
| Vitamin B1 | Thiamin | 1897 | 1926 | 1936 | 1936 |
| Vitamin B2 | Riboflavin | 1920 | 1933 | 1935 | 1935 |
| Vitamin B3 | Niacin | 1936 | 1936 | 1937 | 1994 |
| Vitamin B5 | Pantothenic acid | 1931 | 1938 | 1940 | 1940 |
| Vitamin B6 | Pyridoxine | 1934 | 1938 | 1938 | 1939 |
| Vitamin B7 | Biotin | 1931 | 1935 | 1942 | 1943 |
| Vitamin B9 | Folic acid | 1941 | 1941 | 1946 | 1946 |
| Vitamin B12 | Cobalamin | 1926 | 1948 | 1956 | 1972 |
| Vitamin C | Ascorbic acid | 1912 | 1928 | 1933 | 1933 |